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Review Article

Hypertensive Disorders in Pregnant Women Receiving Fertility Treatments

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Abstract

Hypertensive disorders (HDs) as the most prevalent medical problem during pregnancy, predispose the patient to a lot of comorbidities and may even cause maternal or fetal death. The rate of infertility has been increasing in recent decades. So, we collected and summarized data about the co-existence of these two entities and found that HDs are somewhat more common in women receiving fertility treatments regardless of pathophysiologic correlation of infertility and hypertension or older age and chance of multiple pregnancies.

Keywords: Gestational Hypertension, Hypertension, Infertility, Preeclampsia, Pregnancy

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Introduction

Hypertensive disorders (HDs) are the most prevalent medical problem during pregnancy. It is estimated that HDs involve up to 6-8% of all pregnancies (1). HDs account for about 25% of all pre-birth hospital admissions (2). It has been well-documented that hypertension plays an important role in development of atherosclerosis consequently leading to nonfatal or fatal myocardial infarction and cerebrovascular accidents. It has also been shown that hypertension is the main cause of perinatal and maternal morbidity and mortality including intrauterine growth retardation (IUGR), Hemolysis, Elevated Liver enzymes, and Low Platelet count (HELLP) Syndrome, renal impairment, premature labor, neonatal intensive-care-unit admission, caesarean section, placental abruption, perinatal death and maternal convulsion (3-5).

In a recent retrospective study done in Ethiopia, Seyom et al. (6) reported rate of dead fetus, low birth weight and low APGAR score, abortion, preterm delivery and HELLP syndrome as 10.2, 30.5, 18.5, 10.7, 31.4 and 12.4%, respectively in 55,860 pregnant women with HDs.

Zibaeenezhad et al. (7) found a prevalence of 2.32% for HDs in pregnant women in south of Iran including a prevalence of 2.13% for chronic hypertension. Moreover, Khosravi et al. (8) reported a prevalence of 9.8% for HDs among pregnant women who were admitted to a tertiary center in Tehran for delivery. So, the disease is also prevalent in Iran.

Infertility is also a common condition and physicians are deeply concerned about it, because it involves a couple, rather than a single individual. It is defined as inability of a couple to conceive after one year of regular intercourse without using any form of contraception (9).

The prevalence of infertility is markedly high in Eastern Europe, North Africa, Oceania and sub Saharan Africa (10). The main causes of infertility include male factors, decreased ovarian reserve, ovulatory factors, tubal factors, uterine factors, pelvic factors, and unexplained reasons (11).

Once the pathologic basis of infertility is recognized, therapy is directed toward curing reversible causes and modifying irreversible etiologies. Therapeutic interventions for both male and female infertility includes drug therapy (12) and surgery (13), with or without procedures like intra uterine insemination (IUI) or *in vitro* fertilization (IVF) (14, 15).

Methodology

We searched PubMed and Google search engines for incidence of hypertension and also history of infertility in pregnant women. We also checked the internet for causes of female infertility and their association with hypertension, all kinds of treatments and medications that are applied for female infertility and the chance and the mechanisms by which they changing blood pressure (BP). Then we quested for general considerations, treatment modalities and follow-up in pregnant cases with HDs, with or without a history of infertility.

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Physiological blood pressure changes during pregnancy Normotensive women usually experience about 5 to 10 mmHg fall in their BP starting from the first trimester which may be continued up to the third trimester; after that, BP is restored to its preconception level (16). This is due to marked vasodilation which can overcome the increment of blood volume in this period. This phenomenon can also induce normal BP in cases with mild chronic hypertension which results in reduction in dose or discontinuation of antihypertensive medications or even masking previously undiagnosed cases.

Diagnosis and risk of hypertension

Hypertension is generally labeled when systolic BP is ≥140 mmHg and/or diastolic BP is ≥90 mmHg, according to the mean of at least two measurements, checked using the same arm with at least fifteen minutes intervals, in clinic or in hospital (17). Although the definition of HDs is somewhat different in some references and defined only when diastolic BP is greater than 90 mmHg on two sessions with more than 4 hours interval or when a single diastolic BP >110 mmHg was recorded (18). BP should be measured in the sitting position while the arm is at the level of the heart, using a cuff of appropriate size. Mild hypertension is defined as a diastolic BP of 90-99 mmHg and/ or a systolic BP of 140-149 mmHg. Severe hypertension is defined as a systolic BP of ≥160 mmHg or a diastolic BP of ≥110 mmHg. Obviously, moderate hypertension ranges between mild and moderate values (Table 1) (17, 19, 20).

Table 1: Grading of severity of hypertension and the need for antihypertensive treatment

Grade of hypertension	Blood pressure levels (mm Hg)	Treat	Grade of treatment
Mild	Diastolic: 90-99 Systolic: 140-149	No*	Not applicable*
Moderate	Diastolic: 100-109 Systolic: 150-159	Yes	<150 systolic* <100 diastolic*
Severe hypertension	Diastolic: ≥110 Systolic: ≥160	Yes	<150 systolic* <100 diastolic*

*; Except for women with chronic hypertension with end-organ damage who should be treated even if blood pressure is mild and the goal is to normalizing their blood pressure. Modified from: hypertension in pregnancy: the NICE guidelines (20).

HDs are classified into four major groups according to working group of National Institutes of Health (NIH) report on high BP in pregnancy (3): i. Chronic or preexisting hypertension diagnosed either before pregnancy or earlier than 20 gestational weeks, ii. Preeclampsia-eclampsia. Preeclampsia described as the presence of hypertension, along with new-onset of significant proteinuria of >0.3 g/24 hours. However, there are some other definitions in other references which are more precise and complete, iii. Preeclampsia superimposed on chronic hypertension, and iv. Gestational hypertension is defined as a hypertension beginning at later than 20 gestational weeks and can persist for up to 42 days postpartum.

It has also been mentioned that gestational hypertension usually resolves within up to 12 weeks post-partum however this is not applicable for cases with chronic hypertension (21).

Risk factors for chronic hypertension are: early middle age or about age 45, black race, using tobacco, too much salt (sodium) in diet, too little potassium, calcium or vitamin D in diet, drinking too much alcohol, high levels of stress, being overweight or obese, little or no exercise, history of high BP in the family (22). Certain chronic conditions also may increase the risk of high BP, such as kidney disease, diabetes and sleep apnea.

Risk factors for preeclampsia include: prior history of preeclampsia, family history of preeclampsia, intervals of more than 10 years between pregnancies, nulliparity, preexisting medical conditions like antiphospholipid syndrome, type 1 or 2 diabetes mellitus, chronic kidney disease, chronic hypertension, chronic autoimmune diseases like systemic lupus erythematous (SLE), mother age more than 40 years, BMI >35 kg/m^2, multiple pregnancy, high BP in the first visit, gestational trophoblastic disease, fetal triploidy (23, 24).

Thus, infertility by itself is neither a major risk factor for HDs during pregnancy nor a major risk factor for preeclampsia.

Hypertension and infertility

Higher rates of HDs in women who underwent infertility treatment might be due to higher age and/or increased risk of multiple pregnancies. Also, pathologic basis of infertility like polycystic ovaries and endometriosis, must be considered as a cause of or in correlation with hypertension. In these conditions, hypertension may simply occur due to associated obesity or insulin resistance, androgen excess, sympathetic nerve over activity and chronic use of oral contraceptives (25, 26).

Furthermore, chronic hypertension can cause poor egg quality; also, many hypertensive women suffer from obesity which is mostly a result of excessive estrogen production which can lead to infertility. Antihypertensive medications like angiotensin receptor inhibitors (ARBs) and calcium channel blockers typically affect male fertility rather than female ones.

The most common medications which are used for treatment of female infertility are clomiphene, metformin, aromatase inhibitors like letrozole, human chorionic gonadotropins (hCG) like menotropin, dopamine agonists like bromocriptine and gonadotropin-releasing hormone (GnRH) agonists like leuprolide which is used in GnRH protocol and consists of progesterone and estradiol. Among these, letrozole, leuprolide and estradiol can induce hypertension with a prevalence rate of 5-8% (27-29), 8% (30) and 3-7% (31), respectively. Although bromocriptine usually causes vasodilatation and specially edema thereafter, there are some case reports on bromocriptine-induced hypertension (32).

It is well known that estrogen-containing medications can induce hypertension in premenopausal women, but the mechanisms are not fully understood. Supraphysiologic concentrations of estrogen and its effect on increment of angiotensinogen and insulin-like growth factor I production by liver, increased sympathetic activity and increased expression of angiotensin subtype 1 (AT1) receptor in the kidneys, are the possible mechanisms (33).

In recently published meta-analysis, it was shown that metformin decreases BP specially systolic type, particularly in nondiabetic cases (34).

Farland et al. (35) reported relative risk of hypertension in infertile women receiving different kinds of treatments, as follows: clomiphene: relative risk (RR)=0.97, confidence interval (CI): 0.90-1.04; gonadotropin alone: RR=0.97, CI: 0.87-1.08, IUI: RR=0.86, CI: 0.71-1.03, IVF: RR=0.86, CI: 0.73-1.01.

We could not find any correlation between risk of hypertension and hCG administration after adjustment by higher chance of multiple pregnancy and other prevalent factors.

In a meta- analytic study which was done in Germany, oocyte donation was also reported as a risk factor for HDs in pregnancy and this effect was possibly mediated through immunological processes and ovarian dysfunction (36).

In 1994, Sealey et al. (37) revealed that renin and urinary aldosterone excretion had 5-fold increases during the luteal phase (day 7) in patients who underwent ovarian stimulation. Alternatively, Tollan et al. (38) showed a statistically significant decrement in both systolic and diastolic BP during ovarian stimulation for IVF, most probably due to decreased level of adrenalin.

In a retrospective observational cohort, Hernández-Díaz et al. (39) interviewed 5151 women within six months of delivery and stated that the incidence of gestational hypertension was significantly greater in women with a history of infertility who were treated for this problem (15.8%) than among those infertile cases who did not receive such managements (8.9%). Results were the same for patients with preeclampsia and also after adjustment for age, twin pregnancy, parity and body mass index. There were some cases without a history of infertility treatments who mentioned some difficulties in fertility in past or untreated sub-fertility in the present pregnancy. Surprisingly, these women were not at increased risk of hypertension and this finding demonstrates the direct role of infertility treatment in induction of hypertension. All kinds of infertility treatment approaches or drugs were associated with a similar increased risk, although not unexpectedly, treatments with the greatest chance of multiple gestations, were associated with higher risk of HDs.

Alternatively, in a prospective cohort study, Farland et al. (35) included 116,430 women and followed them for 20 years to assess the risk of development of hypertension in later life. Among them, 12,183 received some kinds of infertility treatment. During follow-up, approximately 20,066 women were diagnosed with hypertension. The authors emphasized that only infertility due to tubal causes was accompanied by greater risk of hypertension as these cases had 15% greater risk of hypertension than women without a history of infertility. But among other causes of infertility, no clear relation was detected between receiving fertility treatment and subsequent hypertension.

Meanwhile, Toshimitsu et al. (40) showed that the incidence of HDs was significantly higher in infertile couples with IVF/intracytoplasmic sperm injection conception than the spontaneous conception group in both the women aged ≥40 years (20.5 vs. 7.9%) and those aged 30-34 years (14.3 vs. 2.6%). However, gestational diabetes mellitus, premature birth, or low birth weight were not different between these two groups.

So, it seems that we should be concerned about hypertension in all pregnancies particularly in women underwent infertility treatments.

General considerations

BP measurement should be performed in all routine prenatal visits and more frequently in high risk patients. According to the last version of guideline of prenatal care, pregnant women should be visited every 4 weeks up to week 28, every 2 weeks thereafter until week 36 and then weekly up to time of delivery (41). If HDs were diagnosed, then BP should be checked weekly in milder forms and no association with preeclampsia, or twice and four times a week in moderate and severe forms, respectively (24).

Low salt diet is not advised, and consumption of calcium or magnesium supplements, fish oil derivatives, vitamins, antioxidants and garlic are also ineffective. No data supports using heparin and nitric oxide (18, 42). Weight reduction is not recommended. Moderate activity is suggested for patients with well-controlled chronic hypertension and it seems that there is no increase in incidence of preeclampsia in this group. If preeclampsia occurs, some physical activity restrictions may be required although it does not change maternal or fetal outcome (43). Bedtime low-dose aspirin (75-100 mg/day) should be started and continued until delivery to prevent preeclampsia although it has neutral effect on perinatal and maternal morbidity (17, 44). No evidence supports administration of dipyridamole (18).

Drug therapy

In the first half of pregnancy, selected patients with preexisting hypertension may need to discontinue their antihypertensive medications due to physiological drop in BP during this period, however, close monitoring is mandatory. New-onset hypertension during pregnancy is an indication for assessing proteinuria for early diagnosis of preeclamp-

sia and should be repeated weekly. Fetal growth should be regularly monitored by ultrasonography (24).

Most of the patients with pre-existing hypertension have mild-to-moderate increment in BP, thus physicians are not much worried about cardiovascular complications in this group. There are still scanty evidence about clinical benefits of administration of drugs to subjects with mild hypertension during pregnancy (Table 1) (45). Sometimes these patients should be hospitalized for at least a short period of time for confirmation of diagnosis and risk stratification specially for ruling out or ruling in preeclampsia for which the only effective treatment is termination of pregnancy.

Last European Society of Hypertension/ European Society of Cardiology (ESH/ESC) guidelines approved a systolic BP of 140 mmHg or a diastolic BP of 90 mmHg as the thresholds for antihypertensive treatment in pregnant women with one of the following criteria: gestational hypertension (with or without proteinuria), pre-existing hypertension superimposed by gestational hypertension, symptomatic hypertension and subclinical hypertension associated with end-organ damage.

The ESH/ESC thresholds are a systolic BP of 150 mmHg and a diastolic BP of 95 mmHg in any other conditions (Table 2) (43, 46).

Unfortunately, none of the antihypertensive agents could significantly reduce perinatal mortality (45). Continuation of current medication except for angiotensin-converting enzyme (ACE) inhibitors, ARBs, and direct renin inhibitors may be the best strategy in cases with pre-existing hypertension. Drug of choice is α-Methyldopa and labetalol shows comparable efficacy. Calcium channel blockers like nifedipine are drugs of second choice (17, 47). Dosages are stated in Table 3. Generally, diuretics should be avoided in all kinds of HDs as they may theoretically decrease placental blood flow (46); however, thiazides are mentioned as the second line therapy in some references (43, 48) as their teratogenicity or adverse effects have not been extensively studied (45). Mineralocorticoid receptor antagonists should never be prescribed (43). Nowadays, hydralazine is used only in hypertensive urgencies in intravenous form, although its chronic oral use showed no adverse effect. Prazosin and atenolol are no more recommended during pregnancy (17).

Table 2: Recommendations for the management of hypertension

Recommendations	Class of recommendation	Level of evidence
Non-pharmacological management for pregnant women with systolic BP of 140-150 mmHg or diastolic BP of 90-99 mmHg is recommended.	I	C
In women with gestational hypertension or pre-existing hypertension superimposed by gestational hypertension or with hypertension and subclinical organ damage or symptoms at any time during pregnancy, initiation of drug treatment is recommended at a BP of 140/90 mmHg. In any other circumstances, initiation of drug treatment is recommended if SBP ≥150 mmHg or DBP ≥95 mmHg.	I	C
Systolic BP ≥170 mmHg or diastolic BP ≥110 mmHg in a pregnant woman is an emergency, and hospitalization is recommended.	I	C
Induction of delivery is recommended in gestational hypertension with proteinuria with adverse conditions such as visual disturbances, coagulation abnormalities, or fetal distress	I	C
In preeclampsia associated with pulmonary edema, nitroglycerine given as an intravenous infusion, is recommended.	I	C
In severe hypertension, drug treatment with intravenous labetalol or oral methyldopa or nifedipine is recommended.	I	C
Women with pre-existing hypertension should be considered to continue their current medication except for ACE inhibitors, ARBs, and direct renin inhibitors under close BP-monitoring	IIa	C

From: ESC Guidelines on the management of cardiovascular diseases during pregnancy (46).
BP; Blood pressure, SBP; Systolic blood pressure, DBP; Diastolic blood pressure, ACE; Angiotensin converting enzyme, and ARB; Angiotensin receptor blocker.

Table 3: Oral antihypertensive drugs commonly used in pregnancy

Drug	Indication	FDA category	Initial dose	Maximum dose	Potential side effects
Methyldopa	Often used as first line	B	125-250 mg BD	500 mg QID	Lethargy
Labetalol	Often used as first line	C	100 mg BD	200-400 mg QID	Exacerbation of asthma
Nifedipine (immediate release)	Second line or alternative first line	C	10-20 mg BD	40 mg BD	Concern for synergy with magnesium sulfate for neuromuscular depression

Modified from: Queensland Clinical Guideline: Hypertensive disorders of pregnancy (23) and Chronic Hypertension in Pregnancy (16). Category B; Animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women, Category C; Animal reproduction studies have shown an adverse effect on the fetus and there are no adequate and well-controlled studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks, BD; Twice a day, and QID; Four times a day.

Severe hypertension is defined as BP ≥160/110 mmHg or systolic BP ≥170 mmHg and diastolic BP ≥110 mmHg. It is a medical emergency which requires hospital admission, rapid management and monitoring every 10 to 20 minutes depending on management strategy. In a systematic review of cases with very high BP, published from Cochrane library in 2013, 15 different antihypertensive medications in 35 trials were compared and the results showed that less persistent hypertension was seen with calcium channel blockers than with hydralazine and they also had less side effects compared to labetalol (50). The most popular plan is to start treatment with intravenous labetalol or oral nifedipine as soon as possible (Table 4) (49). Intravenous hydralazine is not the first choice according to some references (46) while there are references suggesting it as medication of first choice (48, 49). The optimal drug in hypertensive crises can be sodium nitroprusside. Intravenous magnesium sulfate is the preferable drug only for management of seizures and/or preventing eclampsia in selective cases of severe preeclampsia (46). If recurrent seizures occurred, alternative anticonvulsants like benzodiazepines, such as lorazepam and diazepam, phenytoin (Dilantin), and levetiracetam can be started (51).

Table 4: Management of severe hypertension during pregnancy

Type of medication	Strategy
Hydralazine (IV)	5 mg IV bolus, then 10 mg every 20-30 minutes to a maximum of 25 mg, repeat in several hours as necessary
Labetalol (IV)	20 mg IV bolus over 2 minutes, then 40 mg 10 minutes later, 80 mg every 10 minutes for two additional doses to a maximum of 220 mg
Nifedipine (oral) (controversial)	10 mg po, repeat every 20 minutes to a maximum of 30 mg
Sodium nitroprusside (rarely used, usually when others fail)	0.25 µg/kg/minutes to a maximum of 5 µg/kg/minutes IV infusion Fetal cyanide poisoning may occur if used for more than 4 hours

Modified from: The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure and Emergent therapy for acute-onset, severe hypertension during pregnancy and the postpartum period. Committee Opinion No. 623. American College of Obstetricians and Gynecologists (48, 49).

Blood pressure goals in pregnancy

As a general agreement, diastolic BP should be kept above 80 mmHg (19). The American College of Obstetrics and Gynecology (ACOG) recommended to maintain BP between 120/80 and 160/105 mm Hg in pregnant women with HDs with no complications (52). Clinical practice guidelines of the Society of Obstetricians and Gynecologists of Canada emphasize that systolic BP of 130-155 mmHg and diastolic BP of 80-105 mmHg should be achieved in patients without any comorbidities and systolic BP of 130-139 mmHg and diastolic pressure of 80-89 mmHg are the goal BPs in cases with comorbidities like diabetes mellitus (53). According to a recent systematic review of patients with non -severe hypertension, target BP is variable and obviously dependent on the type of HDs in pregnancy and presence or absence of comorbidities (1). So, if any form of end-organ dysfunction like left ventricular hypertrophy, chronic kidney disease and hypertensive retinopathy exists, the target BP would be 140/90 mmHg (5, 17, 20). In cases with no end-organ damages, target BP would be different. Accordingly in any form of HDs, the target is BP of 150/80-100 mmHg (5, 20), 130-159/80-105 mmHg (17), or, 160/110 mmHg, in patients with chronic hypertension, it would be120-159/80-104 mmHg and at last in women with gestational hypertension or non-severe preeclampsia, the goal of BP is defined as 160/110 mmHg (54).

Time and mode of delivery

Uncontrolled severe hypertension is the most common maternal reason for preterm childbirth. There are no definite data for time of termination of pregnancy in patients with chronic hypertension specially when BP is well controlled. Delivery at term is suggested for cases with gestational hypertension. Patients with milder forms of preeclampsia can be observed up to gestational week 37 (17). Although the amount of proteinuria is not an important factor (43), severe preeclampsia and eclampsia necessitate urgent termination of pregnancy. Some cases with severe preeclampsia at low gestational age, can be closely observed for up to 34 weeks of pregnancy for better prognosis for child (23). In a recent study, adverse perinatal outcome of postponing delivery to later than 39 weeks of gestation (55) was shown; probably the best time for delivery in gestational hypertension is between weeks 38 and 39 (56).

Rout of delivery should be determined as obstetric indication, so vaginal delivery is the first choice (43). If vaginal delivery is planned, then active managing of the third stage of labor with oxytocin is recommended (17).

Breast feeding

Although all antihypertensive medications are secreted in milk at very low levels, breast feeding is safe in hypertensive mothers. Lower dosages of captopril and enalapril are harmless. No adverse effect following administration of calcium channel blockers has been seen, although there are some controversies about nifedipine. Some references also recommend not to use amlodipine. Diuretics are best avoided as they potentially decrease milk production. Beta blockers other than atenolol can be used safely (16, 17, 23, 24, 43).

Methyldopa should not be used in this period due to some reports about the risk of post-natal depression (23), so must be discontinued within 2 days postpartum if has been prescribed during pregnancy (19, 24).

Short and long-term cardiovascular complications

BP commonly rises immediately over the first 5 days after delivery (18.5% in a recent report) (57). Patients with HDs during pregnancy may become normotensive after labor but then becomes hypertensive in the first week.

Women with a history of HDs during pregnancy, par-

ticularly when associated with early-onset pre-eclampsia, premature labor before 32 weeks of gestation, stillbirth, or IUGR are at high risk for developing cardiovascular complications such as hypertension, stroke and ischemic heart disease in later life. So, lifestyle modifications and close screening for sign and symptoms of cardiovascular diseases and also early detection of other risk factors are highly suggested in these groups. Notably, risk of subsequent hypertension or prehypertension in previously normotensive women with a history of HDs in pregnancy, is higher than others regardless of coincidence with gestational diabetes, even during the first year postpartum (58).

Recurrence risk in a subsequent pregnancy

In a subsequent pregnancy, mothers who experienced hypertension in their first pregnancy are at greater risk specially if it was early onset or associated with HELLP syndrome. Although some other predictors like pre-pregnancy plasma volume have been suggested for risk assessment (59), they are not clinically relevant (60).

Conclusion

HDs of pregnancy are highly prevalent, so do infertility treatments. It seems that elevated BP is somehow more common in women who received infertility treatments. Since hypertension is associated with many short and also long-term comorbidities and even perinatal mortality, this group of patients should be particularly screened, treated and followed up for high BP, although the hypertension treatment is not markedly different from those given to fertile mothers.

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Author's Contributions

M.B.; Was responsible for study design, data collection, and edition of main parts of the article specially those sections which focused on hypertension and BP control. S.A.; Contributed in writing some parts including topics with infertility base. Both authors read and approved the final manuscript.

References

1. Gillon TE, Pels A, von Dadelszen P, MacDonell K, Magee LA. Hypertensive disorders of pregnancy: a systematic review of international clinical practice guidelines. PLoS One. 2014; 9(12): e113715.
2. James PR, Nelson-Piercy C. Management of hypertension before, during, and after pregnancy. Heart. 2004; 90(12): 1499-1504.
3. Report of the national high blood pressure education program working group on high blood pressure in pregnancy. Am J Obstet Gynecol. 2000; 183(1): S1-S22.
4. Bramham K, Parnell B, Nelson-Piercy C, Seed PT, Poston L, Chappell LC. Chronic hypertension and pregnancy outcomes: systematic review and meta-analysis. BMJ. 2014; 348: g2301.
5. Hypertension in pregnancy: National Institute for Health and Care Excellence (NICE) guidance. Available from: https://www.nice.org.uk/guidance/qs35/resources/hypertension-in-pregnancy-2098607923141. 2013.
6. Seyom E, Abera M, Tesfaye M, Fentahun N. Maternal and fetal outcome of pregnancy related hypertension in Mettu Karl Referral Hospital, Ethiopia. J Ovarian Res. 2015; 8: 10.
7. Zibaeenezhad M, Ghodsi M, Arab P, Gholzom N. The prevalence of hypertensive disorders of pregnancy in Shiraz, Southern Iran. Iranian Cardiovascular Research Journal. 2010; 4(4): 169-172.
8. Khosravi S, Dabiran S, Lotfi M, Asnavandy M. Study of the prevalence of hypertension and complications of hypertensive disorders in pregnancy. Open J Prev Med. 2014; 4(11): 860-867.
9. Practice Committee of American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss. Fertil Steril. 2008; 90(5 Suppl): S60.
10. Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. PLoS Med. 2012; 9(12): e1001356.
11. Karimzadeh MA, Ahmadi S, Oskouian H, Rahmani E. Comparison of mild stimulation and conventional stimulation in ART outcome. Arch Gynecol Obstet. 2010; 281(4): 741-746.
12. Rahmani E, Ahmadi S, Motamed N, Maneshi HO. Dosage optimization for letrozole treatment in clomiphene-resistant patients with polycystic ovary syndrome: a prospective interventional study. Obstet Gynecol Int. 2012; 2012: 758508.
13. Hasanzadeh M, Vahid Roodsari F, Ahmadi S, Gavedan Mehr M, Azadeh T. Fertility sparing surgery in gestational trophoblastic neoplasia: A report of 4 cases. Int J Reprod Biomed (Yazd). 2016; 14(9): 603-606.
14. Firouzabadi RD, Ahmadi S, Oskouian H, Davar R. Comparing GnRH agonist long protocol and GnRH antagonist protocol in outcome the first cycle of ART. Arch Gynecol Obstet. 2010; 281(1): 81-85.
15. Davar R, Oskouian H, Ahmadi S, Firouzabadi RD. GnRH antagonist/letrozole versus microdose GnRH agonist flare protocol in poor responders undergoing in vitro fertilization. Taiwan J Obstet Gynecol. 2010; 49(3): 297-301.
16. Seely EW, Ecker J. Chronic hypertension in pregnancy. Circulation. 2014; 129(11): 1254-1261.
17. Magee LA, Pels A, Helewa M, Rey E, von Dadelszen P; Canadian Hypertensive Disorders of Pregnancy Working Group. Diagnosis, evaluation, and management of the hypertensive disorders of pregnancy: executive summary. J Obstet Gynaecol Can. 2014; 36(5): 416-441.
18. Hypertension in pregnancy: the management of hypertensive disorders during pregnancy. National Institute for Health and Clinical Excellence: Guidance. Available from: https://www.ncbi.nlm.nih.gov/books/NBK62652/pdf/Bookshelf_NBK62652.pdf. 2010.
19. Hypertension in pregnancy: diagnosis and management, Clinical guideline. nice.org.uk/guidance/cg107. Available from: https://www.nice.org.uk/guidance/cg107/resources/hypertension-in-pregnancy-diagnosis-and-management-35109334009285. 2011.
20. Redman CW. Hypertension in pregnancy: the NICE guidelines. Heart. 2011; 97(23): 1967-1969.
21. Mammaro A, Carrara S, Cavaliere A, Ermito S, Dinatale A, Pappalardo EM, et al. Hypertensive disorders of pregnancy. J Prenat Med. 2009; 3(1): 1-5.
22. Kasper D, Fauci A, Hauser S, Longo D, Jameson J. Harrison's Principles of Internal Medicine. 19th ed. New York: McGraw-Hill Education; 2015.
23. Queensland Clinical Guideline: Hypertensive disorders of pregnancy [Internet]. Queensland Health. Available from: https://www.health.qld.gov.au/__data/assets/pdf_file/0034/139948/g-hdp.pdf. 2015.
24. Weetch J. BJ. Guideline No: 15 Hypertension in Pregnancy: NHS foundation trust. Available from: http://mm.wirral.nhs.uk/document_uploads/guidelines/15-HypertensioninPregnancyGuidelinev1.pdf. 2014 updated 2017.
25. Bentley-Lewis R, Seely E, Dunaif A. Ovarian hypertension: polycystic ovary syndrome. Endocrinol Metab Clin North Am. 2011; 40(2): 433-449.
26. Mu F, Rich-Edwards J, Rimm EB, Spiegelman D, Forman JP, Missmer SA. Association between endometriosis and hypercholesterolemia or hypertension. Hypertension. 2017; 70(1): 59-65.
27. Novartis Pharmaceuticals Corporation East Hanover, New Jersey. Femara. Available from: https://www.rxlist.com/femara-drug.htm. Last reviewed: 1/17/2017.
28. Letrozole Side Effects. Available from: https://www.drugs.com/sfx/

letrozole-side-effects.html.
29. Letrozole: drug information. Available from: https://www.uptodate.com/contents/letrozole-drug-information?source=search_result&search=letrozole%20side%20effects&selectedTitle=1~59. 2017.
30. Leuprolide: drug information. Available from: https://www.uptodate.com/contents/leuprolide-drug-information?source=see_link. 2017.
31. Estradiol (systemic): Drug information Available from: https://www.uptodate.com/ contents/estradiol-systemic-drug-information?source=search_result&search=estradiol&selectedTitle=1~150. 2017.
32. Bromocriptine: drug information. Available from: https://www.uptodate.com/contents/bromocriptine-drug-information? source=search_result&search=bromocriptin&selectedTitle=1~67. 2017.
33. Ashraf MS, Vongpatanasin W. Estrogen and hypertension. Curr Hypertens Rep. 2006; 8(5): 368-376.
34. Zhou L, Liu H, Wen X, Peng Y, Tian Y, Zhao L. Effects of metformin on blood pressure in nondiabetic patients: a meta-analysis of randomized controlled trials. J Hypertens. 2017; 35(1): 18-26.
35. Farland LV, Grodstein F, Srouji SS, Forman JP, Rich-Edwards J, Chavarro JE, et al. Infertility, fertility treatment, and risk of hypertension. Fertil Steril. 2015; 104(2): 391-397.
36. Pecks U, Maass N, Neulen J. Oocyte donation: a risk factor for pregnancy-induced hypertension: a meta-analysis and case series. Dtsch Arztebl Int. 2011; 108(3): 23-31.
37. Sealey JE, Itskovitz-Eldor J, Rubattu S, James GD, August P, Thaler I, et al. Estradiol-and progesterone-related increases in the renin-aldosterone system: studies during ovarian stimulation and early pregnancy. J Clin Endocrinol Metab. 1994; 79(1): 258-264.
38. Tollan A, Oian P, Kjeldsen SE, Holst N, Eide I. Effects of ovarian stimulation on blood pressure and plasma catecholamine levels. Scand J Clin Lab Invest. 1993; 53(4): 353-358.
39. Hernández-Díaz S, Werler MM, Mitchell AA. Gestational hypertension in pregnancies supported by infertility treatments: role of infertility, treatments, and multiple gestations. Fertil steril. 2007; 88(2): 438-445.
40. Toshimitsu M, Nagamatsu T, Nagasaka T, Iwasawa-Kawai Y, Komatsu A, Yamashita T, et al. Increased risk of pregnancy-induced hypertension and operative delivery after conception induced by in vitro fertilization/intracytoplasmic sperm injection in women aged 40 years and older. Fertil Steril. 2014; 102(4): 1065-1070.
41. Riely LA, Stark AR. Guidelines for perinatal care. 7th ed. Washington, DC: American Academy of Pediatrics and the American College of Obstetricians and Gynecologists; 2012; 161.
42. Roberts JM, Myatt L, Spong CY, Thom EA, Hauth JC, Leveno KJ, et al. Vitamins C and E to prevent complications of pregnancy-associated hypertension. N Engl J Med. 2010; 362(14): 1282-1291.
43. American College of Obstetricians and Gynecologists; Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' task force on hypertension in pregnancy. Obstet Gynecol. 2013; 122(5): 1122-1131.
44. Coomarasamy A, Honest H, Papaioannou S, Gee H, Khan KS. Aspirin for prevention of preeclampsia in women with historical risk factors: a systematic review. Obstet Gynecol. 2003; 101(6): 1319-1332.
45. Ferrer RL, Sibai BM, Mulrow CD, Chiquette E, Stevens KR, Cornell J. Management of mild chronic hypertension during pregnancy: a review. Obstet Gynecol. 2000; 96(5): 849-860.
46. Regitz-Zagrosek V, Blomstrom Lundqvist C, Borghi C, Cifkova R, Ferreira R, Foidart JM, et al. ESC Guidelines on the management of cardiovascular diseases during pregnancy: the Task Force on the Management of Cardiovascular Diseases during Pregnancy of the European Society of Cardiology (ESC). Eur Heart J. 2011; 32(24): 3147-3197.
47. Lindheimer MD, Taler SJ, Cunningham FG; American Society of Hypertension. ASH position paper: hypertension in pregnancy. J Clin Hypertens. 2009; 11(4): 214-225.
48. Program NHBPE. The seventh report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure. USA; NIH Publication: 2004.
49. Committee on Obstetric Practice The American College of Obstetricians and Gynecologists. Emergent therapy for acute-onset, severe hypertension during pregnancy and the postpartum period. Obstetric Anesthesia Digest. 2015; 35(4): 184-185.
50. Duley L, Meher S, Jones L. Drugs for treatment of very high blood pressure during pregnancy. Cochrane Database Syst Rev. 2013; (7): CD001449.
51. Moroz LA, Simpson LL, Rochelson B. Management of severe hypertension in pregnancy. Semin Perinatol. 2016; 40(2): 112-118.
52. ACOG Committee on Practice Bulletins--Obstetrics. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. Obstet Gynecol. 2002; 99(1): 159-167.
53. Magee LA, Helewa M, Rey E; Hypertension Guideline Committee; Strategic Training Initiative in Research in the Reproductive Health Sciences (STIRRHS) Scholars. Diagnosis, evaluation, and management of the hypertensive disorders of pregnancy. J Obstet Gynaecol Can. 2008; 30(3 Suppl): S1-S2.
54. American College of Obstetricians and Gynecologists. ACOG Practice Bulletin No. 125: Chronic hypertension in pregnancy. Obstet Gynecol. 2012; 119(2 Pt 1): 396-407.
55. Yuce T, Keskin M, Seval MM, Söylemez F. Effect of the timing of delivery on perinatal outcomes at gestational hypertension. Interv Med Appl Sci. 2015; 7(2): 59-62.
56. Cruz MO, Gao W, Hibbard JU. What is the optimal time for delivery in women with gestational hypertension? Am J Obstet Gynecol. 2012; 207(3): 214. e1-6.
57. Goel A, Maski MR, Bajracharya S, Wenger JB, Zhang D, Salahuddin S, et al. Epidemiology and mechanisms of de novo and persistent hypertension in the postpartum period. Circulation. 2015; 132(18): 1726-1733
58. Black MH, Zhou H, Sacks DA, Dublin S, Lawrence JM, Harrison TN, et al. Hypertensive disorders first identified in pregnancy increase risk for incident prehypertension and hypertension in the year after delivery. J Hypertens. 2016; 34(4): 728-735.
59. Sep S, Smits L, Prins M, Peeters L. Prediction tests for recurrent hypertensive disease in pregnancy, a systematic review. Hypertens Pregnancy. 2010; 29(2): 206-230.
60. Excellence NIfHaC. Evidence Update 16 – Hypertension in pregnancy. A summary of selected new evidence relevant to NICE clinical guideline 107 'The management of hypertensive disorders during pregnancy'.Available from: https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0ahUKEwisoqGGnfvRAhVCqxoKHTHvDasQFggZMAA&url=https%3A%2F%2Farms.evidence.nhs.uk%2Fresources%2Fhub%2F712706%2Fattachment&usg=AFQjCNHI3aqPvZ7TkxBFEPC6MZ3fbbZdYw&bvm=bv.146094739,d.d2s. 2012.

Original Article

The Effect of Cognitive Behavioral Therapy on Marital Quality among Women

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Abstract

Background: Marital quality reflects the individual's overall evaluation of marital relationship. The aim of study was examine the effect of cognitive behavioral counseling on marital quality among women.

Materials and Methods: The experimental study was a randomized clinical trial with two groups, on 198 qualified women who referred to selected health care centers in Hamadan, Iran in 2016. The intervention participants attended four 90-minute sessions of cognitive behavioral counseling. Demographic information questionnaire and marital quality scale [Revised Dyadic Adjustment Scale (RDAS)] were completed by the two groups before and after the intervention. To perform the comparisons, t test, Chi-square test and Fisher's test, Logistic Regression and covariance analysis were used. Covariance analysis or change analysis were employed. Statistical analysis was done using SPSS Software, version 21.0. The significance level was set at 5% ($P<0.05$).

Results: According to the results of the present study, the mean age in the control group and the intervention group was 23.58 ± 7.54 and 35.04 ± 7.91 years old, respectively. Covariance analysis was utilized to examine the marital quality scores. In this analysis, after modification of the variables of age, marital quality score of agreement and satisfaction before the intervention, and income status, the total marital quality score experienced a significant change in all dimensions ($P<0.05$) and the mean scores increased remarkably. Moreover, according to the cut-off point of the dimensions, the scores of all dimensions increased remarkably and the proportion of individuals with high marital quality before and after the intervention changed significantly ($P<0.05$).

Conclusion: Due to the role of sexual relations in stabilizing marriage, cognitive behavioral consultation was effective in improving marital quality especially after agreement and can be used in health care centers in order to improve the relationship between couples and reduce divorce rates (Registration number: IRCT201610209014N125).

Keywords: Cognitive Behavioral, Counseling, Marital Relationship

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Introduction

Marital quality has been studied in positive psychology and its role in interpersonal relationships, especially in marital relationships, is remarkable. According to Marx, "quality of marital relations is the result of methods through which married individuals systematically organize this triangle". The quality of marital relations and the level of happiness depend on how wife and husband interact with each other and cope with stressful situations of life (1).

Marital quality reflects the individual's total evaluation of the marital relation (2). The third approach is Marx's theory which is a combined approach developed based on Lewis and Spanier approach and Bowen systematic approach. Marx has a systematic attitude towards individual and the individual's relationship with spouse and others. In his theoretical framework, he stated that a married person has three angles including inner angel, spouse angle, and effective person.

The first angel is the inner angel that includes the individual's personality with his efforts, motivations, and different energies which is formed based on his experience during life. The second angle is the relationship with the spouse. The third angle is any outside concentration point

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except for the spouse. Generally, Marx believes that marital quality is the result of the methods by which married individuals systematically organize themselves within this triangle (2, 3).

Sex education is one of the factors that improves the relationship between couples and enhances marital quality. Lack of enough information about sex and improper attitudes towards this issue in families and couples are among the major problems that today's Iranian society is facing, and leads to collapse of the family. In this regard, marital counseling as a specialized consultation can convey the information necessary to create a favorable sexual life to the couples, so that they can utilize this information to evolve and complete their marriage. In this regard, as midwives are in constant contact with the community and due to their awareness about sexual issues , they play a significant role in making sure about the couples' satisfaction with their marital life, whereby a step towards creating a healthy society, will be taken (4, 5). Statistics indicates that 50% of couples experience sexual dysfunction in some stages of their lives; however, few undergo consultation or treatment (6).

By providing sex education and consultation, sexual problems could be diminished gradually, and unawareness will be replaced with complete awareness. Marital counseling for prevention of marital difficulties is one of the most effective methods of development of individuals and couples health education.

Sexual counseling plays a significant role in family health, as it can decrease sexual violence in the family, prevent sexually transmitted diseases, result in a positive attitude towards sexual relations and sexual pleasure, decrease conflicts within the family, and gain pleasurable sexual experience and sexual satisfaction. Consultation is a process that helps to improve sexual health, interpersonal relationships, affection, intimacy, body image, and gender roles. Sexual counseling is related to a cognitive domain (information and knowledge), affection domain (feelings, values, and attitudes), and behavioral domain (communication skills and decision making) (7).

Cognitive behavioral therapy is today's most popular and widely-used model of psychotherapy, and clinical studies have proven its efficiency in different populations and for treatment of various problems. This approach is characterized by short-term and problem-focused cognitive behavioral intervention strategies that are retrieved from science and cognitive and learning theories (8, 9). In cognitive behavioral approach, an individual learns to fight against his/her negative attitudes toward sexual issues and improve his/her interpersonal relationships by utilizing his/her problem-solving ability. Moreover, this approach helps to promote and maintain good physical and mental feelings between the couples (10).

Cognitive behavioral consultation approach is one of the most common methods of treating sexual dysfunctions (11). The term "cognitive behavioral consultation" is used to refer to an approach in which it is necessary to cope with overt and cognitive components of behavior. Although traditional behavior therapies are still uniquely important, it is believed that intervention affects cognitive aspects of behavior. Most programs that are designed to treat sexual dysfunctions also use behavioral approaches and are based on this premise that cognitive change cause behavioral changes as well (12).

This approach was created by combining behavior therapy approach and cognitive approach either in the form of cognitive therapy or the framework of cognitive psychology and basic cognitive science. In cognitive behavioral therapy, strengths of behavior therapy and cognitive therapy, i.e. objectivism, evaluation, and assessment on one hand and the role of memory in reconstructing and interpreting data, on the other hand, are collected and become one entity. Nowadays, this approach involves relatively different theories and attitudes. Unlike other forms of behavior therapies, cognitive behavioral methods directly deal with thoughts and feelings that are obviously significant in all psychological disorders. Cognitive behavioral therapy fills the gap felt by most merely-behavioral methods and dynamic psychotherapy (13).

One of the main components of this therapy is presenting sexual knowledge and information related to sexual response cycle, anatomy, and sexual techniques (14).

Therefore, due to the importance of sexual relationships and their effect on family and society health, significance of presentation of educational and counseling programs in healthcare centers, and limited studies conducted to confirm the effectiveness of this consultation on marital quality of individuals, the present study was done to examine the effect of cognitive behavioral consultation on marital quality among women referring to healthcare centers in Hamadan, Iran.

Materials and Methods

The experimental study was carried out as a randomized clinical trial including an intervention group (n=99) and a control group (n=99) with a pretest and a posttest on qualified women referring to selected health care centers of Hamadan. The sample size was calculated using formula, $n = \frac{(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta})^2 (S_1^2 + S_2^2)}{(d)^2}$ where S is standard deviation and indices 1 and 2 referring to the intervention and control groups were both equal to 16.1 according to reference (15), d was equal to 10 and Z indicated percentile of normal distribution which was calculated for indices 0.975 and 0.80. Based on this information, the sample size was determined to be 82 in each group, and the final sample size was calculated as 198 considering a 20% loss.

This research plan with codes of ethics was accepted by the Chronic Diseases Research Center, Hamadan University of Medical Sciences, Hamadan, Iran (IR.UMSHA.REC.1394.573) and (IRCT201610209014N125).

Study inclusion criteria

Married women of 15-45 years old (reproductive age), Literacy, Having >6 months of married life, Hamadan residency, No background of remarkable physical and psychological diseases such as psychotic disorders like schizophrenia and severe depression that need special medicine or diet and filling out the questionnaires, age difference of 10 years between the couples.

Study exclusion criteria

Becoming pregnant during the study, Unwillingness to continue the trial, addiction to drugs and/or alcoholic drinks.

Subjects in control and intervention groups were randomly assigned to six health centers. Therefore, the relationship between the two groups was completely omitted. For this purpose, three pairs of health centers (each pair consisted of 2 centers that were similar in terms of social, economic, cultural, and geographical characteristics) were randomly selected (one pair of centers was located in a city region with higher socioeconomic class, one in the middle socioeconomic class, and one in the higher socioeconomic class).

In each pair of centers, one was assigned to the intervention group and one to the control group. In other words, 3 centers were selected for the control group and 3 for the intervention group. The sample size in each center was chosen to be 28 individuals, and due to the probable loss of 20%, 33 individuals were selected in each center, and the total sample size became 33×6=198. It should be noted that the participants of each center were invited through a public invitation of participating in the research (the inclusion criteria were included in the invitation) which could be found on the clinics' bulletin.

The participants were selected using a table of random numbers from women who referred to the clinic. After approval of the Ethics Committee and obtaining informed consent from the participants, all participants were emphatically informed that participation in the study was completely voluntary and they were able to quit at any stage without any restrictions. At the beginning of the study, all of the participants took a pretest.

In the pretest, the participants of both groups filled out the informed consent form, the demographic information questionnaire, and the marital quality scale. The demographic information questionnaire included age, education level, job, spouse age, spouse education level, spouse job, family income level, marriage duration, number of pregnancies, number of children, and addiction background of the couple. Marital quality was measured using the marital quality standard questionnaire. This questionnaire includes 14 questions and aims to evaluate marital quality from different aspects (agreement, satisfaction, and solidarity). It was devised by Busby, Crane, Larson, and Christensen (16).

This 14-question scale is scored through a 6-point Likert scale ranging from 0 to 5 in a way that score 5 represents completely agree and score 0 indicates completely disagree. The tool consists of three subscales of agreement, satisfaction, and solidarity and a high score indicates higher marital quality indicator. The subscale of agreement consists of 6 items as follows: v. I always agree, iv. I almost always agree, iii. I sometimes agree, ii. I often disagree, i. I almost always disagree, and 0. I always disagree. The subscale of satisfaction includes items 7 to 11 as follows: 0. Always, i. Most of the time, ii. Most often, iii. Sometimes, iv. Hardly ever, and v. Never. The subscale of solidarity consists of items 12 to 14. Item 12 was scored using the following scale: v. Every day, iv. Almost every day, iii. Sometimes, ii. Almost sometimes, i. Hardly ever, and 0. Never, and the items 13 and 14 were scored using scales 0. Never, i. Less than once a month, ii. Once or twice a month, iii. Once or twice a week, iv. Once a day, and v. More.

In order to obtain the scores related to each dimension, the total scores of questions relevant to that dimension were added up, and to calculate the total score of the questionnaire, the total scores of all questions were added up. Higher scores indicated higher marital quality and vice versa. The tool used in the present study was a standard questionnaire of which validity and reliability were evaluated in previous studies (16). Cronbach's alpha reliability study of Hollist, Cody and Miller for three subscales of the agreement, satisfaction, and integrity, respectively was reported 79, 80, and 90%. Internal consistency coefficient reliability Cronbach's alpha of the total questionnaire was 92%. The validity coefficient of 39% was obtained (17).

After the primary assessments, the intervention group was provided with consultation while the control group received no intervention. The target consultation was provided in the form of 8 cognitive behavioral counseling sessions of 90 minutes for 8 weeks (18). Each session involved questions and answers, lecturing, group discussion (in groups of maximum 10 individuals), and presentation of teaching slides. In order to provide the consultation, cognitive therapeutic method was used, and each session included cognitive-behavioral group therapy based on eclectic cognitive-behavioral model (19), and the use of valid sexual resources.

Session 1 and 2 was identifying inefficient beliefs and explaining negative thoughts regarding sexual satisfaction and marital quality, and psychological training was examining the cognitive behavioral model and introducing cognitive distortion regarding sexual dissatisfaction and unfavorable marital quality, homework was revising cognitive distortion.

Session 3 and 4 was examining the homework, and psychological training was examining the methods to fight against cognitive distortion, Homework was practicing identification of cognitive distortion using thoughts recording sheets.

Session 5 and 6 was examining the homework, and psychological training was introducing coping and preventing methods of behaviors and thoughts leading to sexual dissatisfaction, Homework was cognitive reconstructing, completing the sheets of recording thoughts, practicing coping and preventing inappropriate behaviors and thoughts.

Session 7 and 8 was examining the homework, and psychological training was discussing and examining the factors, preventing approaches, returning from sexual dissatisfaction, increasing sexual satisfaction, and improving marital quality, Homework was practicing preventing approaches to deal with return.

The sessions were held in a training class, and the intervention group participants were informed about the date of attending the sessions by phone calls. Moreover, one day before the sessions, the individuals were reminded about the sessions in order to prevent sample loss as much as possible. Consultation was provided by a clinical psychologist. Moreover, the trainers agreed to perform a uniform teaching method.

After the 8th session, both groups took the posttest in which marital quality was evaluated again. It should be noted that after the study, a session about sexual issues was held for the control group, and they were provided with booklets. In order to analyze the collected data, independent samples t test and covariance analysis or change analysis were utilized. All of the tests were carried out at a confidence level of 95%.

Results

According to the results of the present study, the average age in the control group was 32.58 ± 7.54 years old and in the intervention group 35.04 ± 7.91 years old; so, the two groups were not homogenous in this regard. However, the two groups were homogenous in terms of husbands' age in the intervention group (37.23 ± 8.01 years old), and in the control group (39.13 ± 7.18 years old), marriage duration in the intervention group (8.95 ± 7.67 years) and control group (8.49 ± 6.99 years), and number of children in the intervention group (1.53 ± 0.96) and control group (1.74 ± 0.86). Moreover, other demographic characteristics such as the level of education in wives and husbands, and drug addiction in wives and husbands, were homogeneous in the two groups (P<0.05) (Table 1).

Covariance analysis was used in order to examine marital quality scores. In this analysis, after modification of the variables of age, marital quality score of agreement and satisfaction before the intervention, as well as income status, marital quality scores were compared between the two groups. Variance analysis and regression coefficients are presented in Table 2. The value of determination coefficient was calculated as 0.85. After the intervention, significant difference was observed between the two groups in all dimensions and total marital quality (P<0.05) and the mean scores of the dimensions increased remarkably (Table 2).

Here, t test was used to compare the two groups before intervention in order to verify if the two groups are different before the intervention, the effect of this difference should be adjusted when comparing them after intervention.

According to the determined cut-off points based on marital quality scale, all dimensions and total marital quality, a limited number of women had a suitable level of marital quality before the intervention. After the intervention, however, marital quality scores increased, and a significant difference was observed between the two groups regarding all dimensions (P<0.05, Table 3).

Table 1: A comparison of demographic characteristics in the two groups

Variable		Control group n (%)	Intervention group n (%)	P value (%)
Education level	Primary	11 (11.1)	8 (8.1)	0.060
	Secondary	16 (16.2)	10 (10.1)	
	Under diploma	28 (28.3)	26 (26.3)	
	Diploma	35 (35.4)	33 (33.3)	
	University	9 (9.1)	22 (22.2)	
Spouse education level	Primary	10 (10.1)	3 (7.1)	0.050
	Secondary	17 (17.2)	14 (14.1)	
	Under diploma	27 (27.3)	19 (19.2)	
	Diploma	37 (37.4)	36 (36.4)	
	University	8 (8.1)	23 (23.2)	
Spouse addiction	Yes	21 (21.2)	26 (26.3)	0.404
	No	78 (78.8)	73 (73.7)	
Addiction	Yes	1 (1)	1 (1)	1
	No	98 (99)	98 (98)	
Income status	<1,000,000	46 (55.4)	40 (40.4)	<0.001
	>1,000,000	53 (11.1)	59 (59.6)	

Table 2: A comparison of the mean scores of different dimensions of marital quality of women before and after the intervention in the two groups

Different dimensions of marital life	Group	Before intervention		After intervention		P value
		Mean ± SD	Min-Max	Mean ± SD	Min-Max	
Agreement	Control	11.18 ± 3.41	3 (19)	11.09 (2.94)	4 (19)	0.603
	Intervention	15.56 ± 4.94	3 (28)	25.11 (3.32)	9 (30)	<0.001 Covariance analysis with adjustment the effect of age, income, agreement score before intervention
	P (Independent t test)	<0.001		<0.001		
Satisfaction	Control	7.60 (1)	2.57 (7.46)	17 (1)	3.04 (7.60)	0.489
	Intervention	9.89 (8)	2.13 (16.53)	18 (1)	4.35 (9.89)	<0.001 Covariance analysis with adjustment the effect of age, income, satisfaction score before intervention
	P (Independent t test)	<0.001		<0.001		
Solidarity	Control	7.64 (2.73)	1 (14)	7.49 (2.96)	1 (14)	0.382
	Intervention	8.1 (2.89)	1 (16)	15.90 (2.54)	9 (20)	<0.001 Covariance analysis with adjustment the effect of age, income
	P (Independent t test)	<0.001		0.199		
Total	Control	26.41 (6.37)	9 (45)	26.05 (5.99)	9 (64)	0.261
	Intervention	33.60 (9.93)	6 (67)	57.54 (5.94)	34 (69)	<0.001 Covariance analysis with adjustment the effect of age, income, total score before intervention
	P (Independent t test)	<0.001		<0.001		

Table 3: Frequency distribution of suitable marital quality based on cut-off point before and after the intervention in both groups

Dimensions of married life	Before intervention n (%)		Comparison before intervention* (P value)	After intervention n (%)		Comparison after intervention** (P value)
	Control group	Intervention group		Control group	Intervention group	
Agreement	0 (0)	14 (100)	<0.001	0 (0)	88 (100)	<0.001
Satisfaction	1 (4.8)	20 (95.2)	<0.001	2 (2.2)	91 (97.8)	<0.001
Solidarity	16 (42.1)	22 (57.9)	0.279	15 (13.4)	97 (86.6)	<0.001
Total	0 (0)	7 (14.3)	0.014	0 (0)	94 (98)	<0.001

***; For comparing agreements, satisfaction and solidarity between the two groups before the intervention, chi-square test was used and to compare total marital quality, Fisher's exact test was used and **; For comparing solidarity between the two groups after the intervention, Chi square test was used of and to compare other cases (because the two groups at baseline were not similar), Wald test resulting from logistic regression with adjustment for the effect of the intervention, was used.**

Discussion

The present study was conducted in order to examine the effect of cognitive behavioral consultation on marital quality among women. The results indicated that cognitive behavioral consultation led to an increase in total marital quality and all its dimensions (solidarity, satisfaction, and agreement). The individuals had a low marital quality before the intervention; however, after the intervention, the scores rose remarkably showing the efficacy of consultation in improving marital relationships.

This means that cognitive behavioral consultation concerning sexual issues, led to an increase in the total score and dimensions of marital quality in the intervention group. Previously, Young and Carlson (20) showed that cognitive behavioral marital consultation can affect the quality of the individuals' marriage, which is in line with the present study.

In the present study, during the sessions, the wives were taught to solve their sexual and marital problems with the help of their husbands. When the problem is regarded as a joint issue, a single individual is not considered as the cause, and the couples become aware of their roles in the emergence of the problem, so, they stop blaming one another, and there will be less argument between them.

Numerous indices are used to show marital quality. Perry (21) proposed satisfaction with marriage, spending time together, management of conflicts, prediction of divorce possibility, and frequency of conflicts between the couples as marital quality indices.

Satisfaction with sexual relationships is an important

factor in marital relationships. Individuals who are highly satisfied with the sexual relationship they have with their spouses, have a remarkably higher quality of life, express higher love and interest to their spouse, and have higher levels of agreement, solidarity, and satisfaction in their marital relationships (22). In this regard, Khajeh et al. (23) and Mangeli (24) indicated the positive effect of sexual counseling on improving marital relationships and satisfaction.

Regarding marital satisfaction and improvement of marital quality in dimensions of solidarity and agreement, the wife's and husband's understanding of one another's behavior is significantly important. Cognitive behavioral therapy tries to fix the incorrect attitude toward spouse and wrong myths about marital relationships and create skills to establish more effective communication and problem solving (25).

In a study, Akbarzadeh (26) indicated that cognitive behavioral education of the couples was effective in family performance and subscales of problem solving, communication, emotional companionship, emotional involvement, and behavior control among divorced applicants. Cognitive foundations of cognitive behavioral therapy of couples highlight the couples' understanding of one another and consider understanding as an inseparable part of change in couples. Finally, the philosophical foundation of this understanding is that change in behavior alone is not enough to correct inefficient interactions and there should be an emphasis on how individuals think about their relationships and their incompatible behavioral patterns (27).

In the study of Khanjani Veshki et al. (28), carried out on 30 women referring to counseling centers in Qom, Iran, researchers utilized a researcher-designed marital quality questionnaire and 6 sessions of sex education. They showed that the intervention could improve marital quality and its dimensions. Due to the role of sexual relations in consolidating marital relationships and its quality, marital consultation leads to an increase in sexual satisfaction, and the women participating in the educational programs reach higher sexual pleasure and express more passion and affection in their marital relationships.

Moreover, Salimi and Fatehizadeh (29) showed that sex education by cognitive behavioral method enhances the married women's knowledge, self-expression, and sexual intimacy, which is in line with the results of the present study.

With regard to the significance of our results, it can be stated that the cognitive behavioral sex consultation used in the present study could improve all dimensions of marital relationships by emphasizing on cognitive dimensions of sex consultation such as pinpointing and challenging the common sexual wrong beliefs through group discussion, determination and improvement of individuals attitude toward sexual activity, presentation of realities and importance of satisfying sexual desires, the role of sexual relation in general relationships and quality dimensions of marital relationships.

In the cultural context of Iranian society, women and men have poor and inaccurate sexual knowledge, and do not have access to reliable sources in this regard. Moreover, they have a lot of incompatible and illogical thoughts, beliefs, attitudes, and understanding of sexual issues which affect the couples' sexual relation. In cognitive behavioral approach, attention is paid to sex education as Iranian couples had not obtained this knowledge by a reliable method (30, 31). According to the mentioned issues, it is determined that in the Iranian culture, paying attention to cognitive factors is highly important to resolve sexual problems, dysfunction, and dissatisfaction, and neglecting them leads to a decrease in treatment success.

Cognitive behavioral family therapy training equip the couples with skills which are necessary for marital life, and can be generalized to other levels of marital and social levels of life. According to the Iranian culture and since an extensive range of treatment techniques and methods is used, cognitive behavioral method can be effectively employed as an extensive method to treat behavioral problems and positively change couples. Using a large population size from various clinics of the city was the strength of the present study, and failure to follow them after the study was one of its limitations.

Due to the effectiveness of the method used in the present study, it is suggested that techniques of this approach could be utilized by family and marriage counselors to increase sexual satisfaction, solve marital conflicts and improve marital quality of the couples' life. Moreover, the applicable drills and skills of this method in the form of educational sessions, workshop, videos, and pamphlets should be employed to prevent marital problems.

Conclusion

Due to the role of sexual relations in consolidating marital life, cognitive behavioral consultation as an effective method for improving marital quality, especially after an agreement, can be employed in health care centers to improve the couples' relationship and reduce divorce rates.

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Author's Contributions

A.S., S.Z.M., M.T., N.B., M.G.; Participated in study design, drafting. A.S., M.G.; Data collection and evaluation. J.F.; Statistical analysis. A.S., S.Z.M., J.F.; Contrib-

uted extensively in interpretation of the data and the conclusion. A.S.; Was responsible for overall supervision. All authors performed editing and approving the final version of the manuscript for submission, also participated in the finalization of the manuscript and approved the final draft.

References

1. Taraban L, Shaw DS, Leve LD, Wilson MN, Dishion TJ, Natsuaki MN, et al. Maternal depression and parenting in early childhood: Contextual influence of marital quality and social support in two samples. Dev Psychol. 2017; 53(3): 436-449.
2. Gong M. Does status inconsistency matter for marital quality? Journal of Family Issues. 2007; 28(12): 1582-1610.
3. Robles TF, Slatcher RB, Trombello JM, McGinn MM. Marital quality and health: a meta-analytic review. Psychol Bull. 2014; 140(1): 140-187.
4. Byrne M, Doherty S, Fridlund BG, Mårtensson J, Steinke EE, Jaarsma T, et al. Sexual counselling for sexual problems in patients with cardiovascular disease. Cochrane Database Syst Rev. 2016; 2: CD010988.
5. Babazadeh R, Najmabadi KM, Masomi Z. Changes in sexual desire and activity during pregnancy among women in Shahroud, Iran. Int J Gynecol Obstet. 2013; 120(1): 82-84.
6. Ghorbani S, Khalaj M. Assessment of education and consolation's effect on premarriage girls' attitude to sex in Qazvin. Alborz University Medical Journal. 2012; 1 (1): 23-30
7. Sabetnejad Z, Assarian F, Omidi A, Najarzadegan MR. Effectiveness of cognitive behavioral therapy and fluoxetine on sexual function of women with obsessive compulsive disorder: A double-blind randomized controlled trial. Electron Physician. 2016; 8(11): 3156-3163.
8. Shayan A, Forouhari S, Ahmadinia H. The effect of body mass index on sexual function. Rese J Pharm Biol Chem Sci. 2015; 6(6): 815-820.
9. Tang NK .Cognitive behavioural therapy in pain and psychological disorders: Towards a hybrid future. Prog Neuropsychopharmacol Biol Psychiatry. 2017. pii: S0278-5846(17)30083-0.
10. Chavooshi B, Saberi M, Tavallaie SA, Sahraei H. Psychotherapy for medically unexplained pain: a randomized clinical trial comparing intensive short-term dynamic psychotherapy and cognitive-behavior therapy. Psychosomatics. 2017. pii: S0033-3182(17)30011-7.
11. Hummel SB, van Lankveld JJ, Oldenburg HS, Hahn DE, Broomans E, Aaronson NK. Internet-based cognitive behavioral therapy for sexual dysfunctions in women treated for breast cancer: design of a multicenter, randomized controlled trial. BMC Cancer. 2015; 15: 321.
12. Hummel SB, van Lankveld JJDM, Oldenburg HSA, Hahn DEE, Kieffer JM, Gerritsma MA, et al. Efficacy of internet-based cognitive behavioral therapy in improving sexual functioning breast cancer survivors: results of a randomized controlled trial. J Clin Oncol. 2017: 35(12): 1328-1340.
13. Hawton K, Salkovskis PM, Kirk J, Clark DM. Cognitive behaviour therapy for psychiatric problems: a practical guide. 1st ed. UK: Oxford University Press; 1989.
14. Rahimi E, Shafiabadi A, Yunesi F. The effect of sexual cognitive behavioral therapy on females' sexual knowledge, sexual attitude, and sexual self-confidence. Armaghane Danesh. 2009; 14(3): 103-111.
15. Rabieepur S, Ebrahimi M, Sadeghi E. Relationship between sexual health and contraception methods in women. J Mazandaran Univ Med Sci. 2015; 25(130): 30-39
16. Hollist CS, Miller RB. Perceptions of attachment style and marital quality in midlife marriage. Family Relations. 2005; 54(1): 46-57.
17. Yousefi N, Etemadi O, Bahrami F, Al-Sadat Fatehezade M, Ahmadi SA, Beshlideh K. Structural relationships between self-differentiation and subjective wellbeing, mental health and marital quality. Fitting Bowen's Theory. IJPBS. 2009; 3 (2): 4-14
18. Breton A, Miller CM, Fisher K. Enhancing the sexual function of women living with chronic pain: a cognitive-behavioural treatment group. Pain Res Manag. 2008; 13(3): 219-224.
19. Dattilio FM. Cognitive-behavioral therapy with couples and families: a comprehensive guide for clinicians. 1st ed. New York: Guilford Press; 2009.
20. Young ME, Carlson RG. Counseling and human development. 2011; 43(7): 1.
21. Perry BJ. The relationship between equity and marital quality among Hispanics, African American and Caucasians. Presented for the Ph.D., Ohio State. Ohio State University. 2004: 135.
22. Movahed M, Azizi T. A study on the relationship between the sexual satisfaction of women in a married life and conflicts between spouses, among married women in Shiraz. Women in Development and Politic (Women Research). 2011; 9(2): 191-217.
23. Khajeh A, Bahrami F, Fatehizadeh M, Abedi M, Sajjadian P. The effect of happiness training based on cognitive behavioral approch on quality of marital life in married males and females. Knowledge and Research in Applied Psychology. 2013; 14(53): 11-21.
24. Mangeli M, Ramezani T, Mangeli S. The effect of educating about common changes in pregnancy period and the way to cope with them on marital satisfaction of pregnant women. Iranian Journal of Medical Education. 2009; 8(2): 305-313.
25. Rajabi GHR, Navrodi SOS. A study of impact of group mindfulness-based cognitive therapy on depression reduction and increase of marital satisfaction in married women. Journal of Guilan University of Medical Sciences. 2012; 20(80): 83-91
26. Akbarzadeh D, Akbarzadeh H, Ganbari S, Mohamadzadeh A. Comparison of type of irrational beliefs, marital conflicts and coping styles in women seeking divorce and women with intact marriages. Journal of Kermanshah University of Medical Sciences. 2014; 18(9): 516-524.
27. Hannson K, Lundbland A. Couple therapy effective of treatment and longterm follow up. J Family Therapy. 2011; 28: 136-152.
28. Khanjani Veshki S, Botlani S, Shahsiah M, Sharifi E. The effect of sex education on marital quality improvement in couples of Qom. Interdisciplinary Journal of Contemporary Research in Business. 2012; 4(7): 134-147.
29. Salimi M, Fatehizadeh M. Investigation of effectiveness of sexual education basd on behavioralcognitive method on sexual intimacy, knowledge and self-expression of married woman in Mobarakeh. Journal of Modern Psychological Researches. 2012; 7(28): 105-122.
30. Bayrami M, Babapour Khirodin J, Hashemi Nosratabad T, Esmali E, Bahadori Khosroshahi J. Prediction of marital satisfaction on the basis of components of emotional intelligence and conflict resolution styles. The Journal of Qazvin University of Medical Sciences. 2013; 17 (3): 20-27.
31. Masoumi SZ, Kazemi F, Tavakolian S, Rahimi A, Oshvandi K, Soltanian A, et al. Effect of Citalopram in combination with omega-3 on depression in post-menopausal women: a triple blind randomized controlled trial. Journal of Clinical and Diagnostic Research. 2016; 10(10): QCO1-QCO5.

Original Article

Evaluating The Impact of Risk Factors on Birth Weight and Gestational Age: A Multilevel Joint Modeling Approach

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Abstract

Background: Abnormalities in birth weight and gestational age cause several adverse maternal and infant outcomes. Our study aims to determine the potential factors that affect birth weight and gestational age, and their association.

Materials and Methods: We conducted this cross-sectional study of 4415 pregnant women in Tehran, Iran, from July 6-21, 2015. Joint multilevel multiple logistic regression was used in the analysis with demographic and obstetrical variables at the first level, and the hospitals at the second level.

Results: We observed the following prevalence rates: preterm (5.5%), term (94%), and postterm (0.5%). Low birth weight (LBW) had a prevalence rate of 4.8%, whereas the prevalence rate for normal weight was 92.4, and 2.8% for macrosomia. Compared to term, older mother's age [odds ratio (OR)=1.04, 95% confidence interval (CI): 1.02-1.07], preeclampsia (OR=4.14, 95% CI: 2.71-6.31), multiple pregnancy (OR=18.04, 95% CI: 9.75-33.38), and use of assisted reproductive technology (ART) (OR=2.47, 95% CI: 1.64-33.73) were associated with preterm birth. Better socioeconomic status (SES) was responsible for decreased odds for postterm birth compared to term birth (OR=0.53, 95% CI: 0.37-0.74). Cases with higher maternal body mass index (BMI) were 1.02 times more likely for macrosomia (95% CI: 1.01-1.04), and male infant sex (OR=1.78, 95% CI: 1.21-2.60). LBW was related to multiparity (OR=0.59, 95% CI: 0.42-0.82), multiple pregnancy (OR=17.35, 95% CI: 9.73-30.94), and preeclampsia (OR=3.36, 95% CI: 2.15-5.24).

Conclusion: Maternal age, SES, preeclampsia, multiple pregnancy, ART, higher maternal BMI, parity, and male infant sex were determined to be predictive variables for birth weight and gestational age after taking into consideration their association by using a joint multilevel multiple logistic regression model

Keywords: Birth Weight, Gestational Age, Multilevel, Statistical Model

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Introduction

World Health Organization definitions on gestational age state that a baby is preterm if born before 37 weeks of gestation, full term if born between 37 through 42 weeks, and late or postterm if born after 42 weeks from the first day of the women's last menstrual period (1, 2). Preterm birth is one of the leading risk factors of infant mortality in which children under 5 years of age are at a higher risk of death (3). Preterm birth is followed by permanent adverse consequences such as increased risk of impaired learning, cerebral palsy, and visual disorders. Chronic disease in adulthood can be an outcome of a preterm birth (4, 5).

Preterm birth may result from risk factors that include multiple pregnancy, infection, advanced maternal age, short interval between pregnancies, low maternal body

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mass index (BMI), poor maternal nutrition, the use of assisted reproductive technology (ART), maternal psychological health, and lifestyle (6). The prevalence of preterm and postterm births range from 5 to 18% across developed and developing countries (5, 7).

Currently, abnormal gestational age is more common due to the increased rate of multiple births, use of ART, and higher numbers of obstetric interventions (5, 8). In addition, postterm births can cause risks for both the mother and the infant such as fetal and neonatal mortality and morbidity, increased maternal morbidity, fetal macrosomia, placental insufficiency, meconium aspiration syndrome, and meconium aspiration (1). Potential reasons for postterm births include nulliparity, maternal age, race, and previous pregnancies with postterm deliveries or anencephaly (9).

Birth weight, or the first weight of a newborn baby, can be categorized as normal (≥2.5 to < 4.0 kg), low (<2.5 kg), and macrosomia (≥4.0 kg) (10). In addition to gestational age, abnormalities in birth weight have negative outcomes for the infant such as higher risk of infant mortality and inappropriate growth velocity for age. Similar to preterm, low birth weight (LBW) is associated with tobacco smoke, drug and alcohol consumption, anemia, bacterial vaginosis, short birth intervals, low BMI, teenage pregnancy, stress, and certain occupational factors (11-13).

Macrosomia is associated with an increased risk of cesarean delivery, prolonged labor, perinatal trauma, maternal diabetes and obesity, excessive weight gain, male infant sex, prolonged gestation, high maternal age, multiparity, and postpartum hemorrhage. Adverse outcomes of macrosomia for the infant include dystocia, birth injury, or death. In addition, these children will be at risk for diabetes and obesity later in life (14, 15). LBW is more prevalent than macrosomia; however, the prevalence of macrosomia in developed countries is between 5% and 20%. In developing countries, macrosomia ranges from 0.5 to 15% (10, 16). The increasing trend of macrosomia in the last two decades may be due to the increasing rate of diabetes and obesity among reproductive age women (14, 16). Birth weight is strongly associated with gestational age so that prevention of preterm births reduces the risk for LBW (16, 17).

Cluster data are widely recorded in medical and clinical areas where the cases are nested in clusters. For instance, students may be clustered in schools. In contrast to the traditional statistical approaches, multilevel models take the correlation among subjects in the same cluster into account (18, 19). Recording and analyzing two or more response variables in the same dataset is widely applied. Jointly analyzing several response variables prevents type I error inflation and increases the statistical power (20).

The detrimental outcomes after abnormalities in birth weight and gestational age are well discussed in the literature. However, regarding the association between these two variables, it is very important to predict the classes of birth weight and gestational age using their potential risk factors. Hence, the current study aims to model birth weight and gestational age jointly for the data from maternity clinic centers in Tehran province by applying a joint multilevel multiple logistic regression model.

Materials and Methods

Participants and study design

We conducted this cross-sectional study on 4415 fertile women who referred to maternity clinic centers in Tehran Province, Iran, from July 6-21, 2015. These centers are supervised by the following universities located in Tehran, Iran: Tehran University of Medical Sciences, Shahid Beheshti University of Medical Sciences, Iran University of Medical Science, and Islamic Azad University School of Medicine.

Ethical consideration

The Ethical Committee of Royan Institute approved our study. Patients received a clear explanation of the study goals as well as assurances for data confidentiality. The participants were assured that their choice to participate in the study would not affect their treatment procedures. Voluntary completion of the questionnaire was considered to be written informed consent.

Questionnaires and variables

The instrument used in this survey was based on a checklist that consisted of the mothers' demographics and information about midwifery and the infant. We completed the checklist by interviewing the mother; medical files in the delivery room were checked by a midwife and well-trained nurse. The checklist contained information about the maternal and paternal age (years), socioeconomic status (SES), mother's BMI (kg/m^2), baby's head circumference (cm), parity (1 and ≥ 2), education of mother (undergraduate and graduate), mother's occupation (housewife or employed), type of pregnancy (wanted, unwanted), history of abortion (yes or no), history of stillbirth (yes or no), preeclampsia (yes or no), multiple pregnancy (yes or no), and ART (yes or no).

Outcome measures

i. Birth weight (g): LBW (<2500 g), normal birth weight (2500-4000 g) and macrosomia (≥4000 g) and ii. Gestational age (weeks) at birth: preterm birth (<37 weeks), term birth (37-42 weeks) and postterm birth (≥42 weeks of gestation).

Statistical analysis

We performed a joint multilevel multiple logistic re-

gression model (18, 20). "Joint" refers to simultaneous modeling of two associated response variables. "Multilevel" refers to the two levels of cases are nested in the hospitals. "Multiple" refers to several predictors in the model. The pregnant women were considered to be the first level whereas hospitals comprised the second level. The correlation between the response variables is induced through random intercepts in each sub-model. The random intercepts are assumed to follow bivariate Bridge distribution with correlation parameters. In contrast to the normal, Bridge distribution provides researchers with the same odds ratio (OR) interpretation both within and between clusters (21). In order to link the systematic component with the response variables, a logit link function is considered. The model is specified as follows:

$$\log\left(\frac{pr(y_{1ij} = Pretrm\ or\ postterm)}{pr(y_{1ij} = term)}\right) = u_{1i} + \omega_c - x_{i'j}\alpha$$

$$\log\left(\frac{pr(y_{2ij} = LBW\ or\ Macrosomia)}{pr(y_{2ij} = Normal)}\right) = u_{2i} + \theta_c - z_{i'j}\beta$$

In the model, y1iJ and y2iJ are the gestational age and birth weight for subject j at hospital I, respectively. Both x and z are predictor variables of the response variables. β and α are the estimated coefficient vectors that correspond to the predictors. The terms u1i and u2i are the random intercepts (hospital specific effects). ω and θ are the thresholds of each response variable category (c). The reference level for gestational age was "term" as well as "normal weight" for birth weight. According to the logit link function, eβ (eα) indicates the OR of LBW (preterm)/ macrosomia (postterm) to normal weight (term) for X=x+1 compared to X=x. A 95% confidence interval (CI) that contained 1 indicated a P>0.05 and a non-significant effect size. Simple univariate multilevel nominal logistic regression models were separately applied for predictors. Those variables with P<0.20 were used in the joint multilevel multiple logistic regression model.

The data analysis was carried out recruiting the PROC NLMIXED in SAS software version 9.2 (SAS Institute, Inc.). A P<0.05 was considered significant. Two-sided tests were run for the statistical hypothesis.

Results

We observed the following prevalence rates for preterm (5.5%), term (94%), and postterm (0.5%). LBW had a prevalence rate of 4.8%. The prevalence rate for normal weight was 92.4 and 2.8% for macrosomia. The independent chi-square test exposed a strong association between gestational age and weight at birth (Pearson chi-square=940.308, df=4, P<0.001).

The distribution of cases' characteristics in three groups of gestational age and weight at birth are illustrated (Tables 1, 2). Patients had a mean ± SD age of 29.18 ± 5.35 years.

The joint multilevel multiple logistic regression model analysis adjusted the association between gestational age and birth weight as well as the interaction among several predictors (Table 3). Based on several simple univariate multilevel logistic regression models, we entered mother's age and BMI, SES, mother's education, preeclampsia, ART, multiple pregnancy, parity, history of stillbirth, and infant sex into the joint model. The joint multiple multilevel logistic regression model was fitted to the data (Table 3). A comparison of preterm to term in the joint model showed that mother's age, preeclampsia, multiple pregnancy, and ART were significant predictors. Postterm to term comparison showed that mother's age and SES were significant predictors. The OR of preterm to term among older mothers was strongly more likely than younger mothers (OR=1.04, 95% CI: 1.02-1.07) while postterm was not affected by mother's age (OR=0.92, 95% CI: 0.85-1.01). Mothers with a better SES were less prone to have postterm births compared to term births (OR=0.53, 95% CI: 0.37-0.74). Mothers with preeclampsia were 4.14 (95% CI: 2.71-6.31) times more likely to experience preterm than term births. Patients with multiple pregnancy were 18.04 (95% CI: 9.75-33.38) times more prone to undergo preterm delivery compared to term delivery. Mothers in the ART group were 2.47 (95% CI: 1.64-33.73) times more prone to experience a preterm rather than term delivery.

We assessed infant weight at birth, mother's age, mother's BMI, mother's education, parity, history of stillbirth, multiple pregnancy, preeclampsia, and infant sex as the candidate affective predictors by the univariate models. According to 95% CI, parity, multiple pregnancy, and preeclampsia had a statistical association with LBW. Macrosomia showed a significant association with mother's BMI and infant sex. Mothers with more than two pregnancies were less likely to deliver a child with LBW (OR=0.59, 95% CI: 0.42-0.82). The patients with multiple pregnancy were 17.35 (95% CI: 9.73-30.94) times more prone for LBW than normal weight. Children from mothers with preeclampsia were 3.36 (95% CI: 2.15-5.24) times more likely to experience LBW compared to normal mothers. The OR of macrosomia to normal weight for a mother with a higher BMI was 1.02 (95% CI: 1.01-1.04). Macrosomia was more common among male infants compared to females (OR= 1.78, 95% CI: 1.21-2.60).

Table 1: Distribution of patients' characteristics in the preterm, term and postterm groups

Variable	Preterm n=244	Term n=4149	Postterm n=22	P value
Mother's age (Y)	30.51 ± 5.95	29.11 ± 5.29	26.86 ± 6.36	<0.001
Father's age (Y)	34.64 ± 6.29	33.43 ± 5.73	33.05 ± 7.11	0.006
SES	0.16 ± 2.11	0.03 ± 2.02	-1.93 ± 1.57	<0.001
Mother's BMI (kg/m^2)	25.01 ± 4.12	24.99 ± 5.60	24.10 ± 5.31	0.752
Baby's head circumference (cm)	32.78 ± 2.81	34.98 ± 4.93	35.36 ± 1.18	<0.001
Parity				0.816
1	122 (5.6)	2026 (93.8)	12 (0.6)	
≥2	122 (5.4)	2123 (94.1)	10 (0.4)	
Mother's education				0.007
Undergraduate	149 (5)	2800 (94.3)	20 (0.7)	
Graduate	95 (6.6)	1349 (93.3)	2 (0.1)	
Father's education				0.253
Undergraduate	156 (5.2)	2827 (94.2)	17 (0.6)	
Graduate	88 (6.2)	1322 (93.4)	5 (0.4)	
Mother's occupation				0.324
Housewife	209 (5.4)	3645 (94.1)	21 (0.5)	
Employed	35 (6.5)	504 (93.3)	1 (0.2)	
Type of pregnancy				0.882
Wanted	194 (5.4)	3351 (94)	18 (0.5)	
Unwanted	50 (5.9)	798 (93.7)	4 (0.5)	
History of abortion				0.251
No	190 (5.3)	3353 (94.1)	20 (0.6)	
Yes	54 (6.3)	796 (93.4)	2 (0.2)	
History of stillbirth				0.354
No	237 (5.5)	4079 (94)	22 (0.5)	
Yes	7 (9.1)	70 (90.9)	0 (0)	
Preeclampsia				<0.001
No	198 (4.7)	3961 (94.8)	21 (0.5)	
Yes	46 (19.6)	188 (80)	1 (0.4)	
ART				<0.001
No	197 (4.8)	3867 (94.7)	19 (0.5)	
Yes	47 (14.2)	282 (84.9)	3 (0.9)	
Infant sex				0.113
Female	108 (5)	2054 (94.4)	14 (0.6)	
Male	136 (6.1)	2095 (93.6)	8 (0.4)	
Multiple pregnancy				<0.001
No	210 (4.8)	4121 (94.7)	22 (0.5)	
Yes	34 (54.8)	28 (45.2)	0 (0)	
Birth weight				<0.001
LBW	111 (52.1)	102 (47.9)	0 (0)	
Normal	131 (3.2)	3928 (96.3)	19 (0.5)	
Macrosomia	2 (1.6)	119 (96)	3 (2.4)	

Values are given as mean ± SD or number (%).
SES; Socioeconomic status, BMI; Body mass index, ART; Assisted reproductive technology, and LBW; Low birth weight.

Table 2: Distribution of patients' characteristics in the LBW, normal, and macrosomia groups

P value	Macrosomia n=124	Normal n=4078	LBW n=213	Variable
0.247	29.90 ± 4.73	29.15 ± 5.35	29.40 ± 5.61	Mother's age (Y)
0.013	34.96 ± 5.61	33.44 ± 5.74	33.75 ± 6.41	Father's age (Y)
0.625	0.12 ± 1.93	0.02 ± 2.03	0.13 ± 2.01	SES
0.009	26.49 ± 3.81	24.94 ± 4.92	24.98 ± 12.71	Mother's BMI (kg/m^2)
<0.001	36.59 ± 1.43	34.96 ± 4.96	32.01 ± 2.39	Baby's head circumference (cm)
				Parity
0.016	54 (2.5)	1983 (91.8)	123 (5.7)	1
	70 (3.1)	2095 (92.9)	90 (4)	≥2
0.176				Mother's education
	82 (2.8)	2756 (92.8)	13 (4.4)	Undergraduate
	42 (2.9)	1322 (91.4)	82 (5.7)	Graduate
0.155				Father's education
	94 (3.1)	2764 (92.1)	142 (4.7)	Undergraduate
	30 (2.1)	1314 (92.9)	71 (5)	Graduate
0.487				Mother's occupation
	111 (2.9)	3582 (92.4)	182 (4.7)	Housewife
	13 (2.4)	496 (91.9)	31 (5.7)	Employed
0.449				Type of pregnancy
	100 (2.8)	3284 (92.2)	179 (5)	Wanted
	24 (2.8)	794 (93.2)	34 (4)	Unwanted
0.957				History of abortion
	101 (2.8)	3238 (92.3)	173 (4.9)	No
	23 (2.7)	789 (92.6)	40 (4.7)	Yes
0.061				History of stillbirth
	119 (2.7)	4012 (92.5)	207 (4.8)	No
	5 (6.5)	66 (85.7)	6 (7.8)	Yes
<0.001				Preeclampsia
	115 (2.8)	3887 (93)	178 (4.3)	No
	9 (3.8)	191 (81.3)	35 (14.9)	Yes
0.281				ART
	115 (2.8)	3777 (92.5)	191 (4.7)	No
	9 (2.7)	301 (90.7)	22 (6.6)	Yes
0.009				Infant sex
	45 (2.1)	2019 (92.8)	112 (5.1)	Female
	79 (3.5)	2059 (92)	101 (4.5)	Male
<0.001				Multiple pregnancy
	123 (2.8)	4045 (92.9)	185 (4.2)	No
	1 (1.6)	33 (53.2)	28 (45.2)	Yes

Values are given as mean ± SD or number (%).
SES; Socioeconomic status, BMI; Body mass index, ART; Assisted reproductive technology, and LBW; Low birth weight.

Table 3: The results of joint multilevel multiple logistic regression model determining gestational age and birth weight categories

Postterm to term OR (95% CI)	Preterm to term OR (95% CI)	Predictor
0.92 (0.85-1.01)	1.04 (1.02-1.07)	Mother's age (Y)
0.53 (0.37-0.74)	0.97 (0.89-1.07)	SES
0.82 (0.17-3.91)	1.29 (0.90-1.86)	Mother's education
		Preeclampsia
109 (0.14-8.39)	4.14 (2.71-6.31)	Yes
	Reference category	No
		ART
109 (0.14-8.39)	2.47 (1.64-33.73)	Yes
	Reference category	No
		Multiple pregnancy
0.61 (0.001-4.68)	18.04 (9.75-33.38)	Yes
	Reference category	No
Macrosomia to normal OR (95% CI)	**LBW to normal OR (95% CI)**	
1.01 (0.98-1.05)	1.01 (0.98-1.04)	Mother's age (Y)
1.02 (1.01-1.04)	1.01 (0.97-1.03)	Mother's BMI (kg/m^2)
1.10 (0.72-1.69)		Mother's education
	1.15 (0.82-1.61)	Graduate
	Reference category	Underggraduate
		History of stillbirth
2.47 (0.94-6.52)	2.17 (0.89-5.28)	Yes
	Reference category	No
		Multiple pregnancy
0.72 (0.08-6.84)	17.35 (9.73-30.94)	Yes
	Reference category	No
		Preeclampsia
1.4 (0.68-2.87)	3.36 (2.15-5.24)	Yes
	Reference category	No
		Infant sex
1.78 (1.21-2.60)	0.87 (0.65-1.17)	Male
	Reference category	Female

SES; Socioeconomic status, BMI; Body mass index, ART; Assisted reproductive technology, OR; Odds ratio, and CI; Confidence interval.

Discussion

Numerous studies reported a direct, positive correlation between weight at birth and gestational age (1, 13, 22, 23). We conducted the current study to determine the risk factors for the adverse categories of these two pregnancy outcomes regarding their association where the OR of preterm or postterm to term as well as LBW or macrosomia to normal birth weight have been demonstrated. Our study has shown that postterm birth did not have any association with mother's age. However, older mothers are more prone to have preterm deliveries. This may be due to certain conditions at older ages such as elevated blood pressure.

A systematic review Flenady et al. (24) has sought to determine whether older maternal age could be a risk factor for preterm birth. They reported that most studies indicated that preterm birth was significantly affected by older maternal age. The current study demonstrated that better SES reduced the chance of preterm or postterm delivery. This might be due to the fact that families with relatively better SES have greater access to facilities. Whitehead assessed the relationship between SES and preterm delivery

and contractions. SES appeared to be associated with the basic factors of spontaneous preterm delivery, but not directly with preterm delivery (25).

Kistka et al. (26) assessed the risk for postterm delivery. They showed that low SES scores had an association with increased risk for recurrent postterm birth. Joseph et al. (27) assessed the relationship between spontaneous preterm and socioeconomic position; they observed significantly higher preterm births among low income families.

We showed that the presence of preeclampsia increased the odds for preterm and LBW. However, our study showed that the odds for postterm delivery and macrosomia were higher among those with preeclampsia. These ratios were not significant. Davies et al. (28) investigated the association between preeclampsia and preterm in a large population of primiparous women. Their study confirmed that preeclampsia contributed to preterm delivery and reduction in preterm rates could be achieved by controlling preeclampsia. The same results were reported by Goldenberg et al. (23). In contrast to postterm births, preterm births had a significant association with the use of ART.

Dunietz et al. (29) assessed the association of preterm delivery and ART among primiparous women. They demonstrated that the use of ART increased the risk for preterm birth, even in the cases with male factor infertility. Our results revealed that multiple pregnancy significantly predicted preterm birth and LBW, but did not affect postterm and macrosomia. It has been shown that the majority of twins are born preterm. The relationship between preterm and multiple pregnancy was well discussed by Liem et al. (30). The leading cause of preterm births was over-distension of the uterus (31).

Based on the results from our study, macrosomia was affected by mother's BMI. Rockhill et al. (32) reported the same findings when they assessed the effects of prepregnancy BMI on fetal macrosomia. Jolly et al. (33) studied affected variables of macrosomia as well as its clinical outcomes using a large data on pregnancies. They demonstrated a higher rate of macrosomia among mothers with higher BMI. The current study also showed that male infant sex was more common in macrosomia. This result was consistent with the study from Ju et al. (34) who assessed fetal macrosomia and pregnancy outcomes. Although the current study had less numbers of birth results at more normal weight compared to macrosomia, this finding was not significant. However, increased numbers of births were associated with decreased odds for LBW. The same result was found by Nazari et al. They compared maternal characteristics in LBW and normal birth weight infants. They found that primiparous mothers were more at risk for LBW infants in comparison with multiparous mothers (12).

The strength of this study was the association between two ordinal outcomes of pregnancy and determining their potential risk factors by using an advanced statistical joint modeling approach. Ignoring the strong association between the two response variables, weight at birth and gestational age, reduced the statistical power to find their significant risk factors. In contrast to univariate models and traditional approaches, jointly modeling several response variables increases the statistical power of data analysis. In a study by Santos et al. (35), multivariate and univariate GARCH models were fitted to forecast portfolio value-at-risk. They determined that more valid results were provided by the multivariate approaches.

Kassahun et al. used a joint model for hierarchical continuous and zero-inflated overdispersed count data to assess the diarrhoeal disease burden. To do so, the combined infant body weight and number of days of diarrhoeal illness using a longitudinal design (36). Moreover, a multilevel structure of a dataset has been shown to cause some variances in which multilevel modeling approaches must be applied (37).

These types of data are widely used in medical and clinical areas; ignoring its' natural variance causes misleading estimations (19). Nkansah-Amankra et al. (38) have evaluated the effects of maternal stress on LBW and preterm birth outcomes using multilevel logistic analysis. The multilevel analysis simultaneously modeled individual and neighborhood contexts to determine the odds of LBW and preterm delivery. In a similar study, Ota et al. (39) assessed the risk factors of preeclampsia and its adverse outcomes in low- and middle-income countries. They used multilevel regression models to determine the associations between preeclampsia and its risk factors.

Conclusion

The study results showed an association between preterm and postterm births to maternal age and SES. In contrast to postterm births, preeclampsia, multiple pregnancy, and ART affected preterm births. Macrosomia was caused by higher maternal BMI. Macrosomia was more common among male infants. We observed an association between LBW and parity, preeclampsia, and multiple pregnancy. We have determined that the joint multilevel multiple logistic regression model is a proper statistical tool for these types of data.

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Author's Contributions

P.A.; Participated in study design, drafting, statistical analysis and interpretation, editing and revising of the manuscript. A.M.; Contributed to the statistical analysis,

and interpretation of data and was responsible for overall supervision. F.Z.; Helped with statistical consultant, editing and revising the manuscript. H.M.; Contributed to statistical consultant, and conception and statistical interpretation of the results. S.M.; Helped with data collection and evaluation, drafting and statistical analysis. R.O.S.; Contributed to the study design and concepts, supervising the data collection, and clinical interpretation and discussion of the results. All authors read and approved the final manuscript.

References

1. Galal M, Symonds I, Murray H, Petraglia F, Smith R. Postterm pregnancy. Facts Views Vis Obgyn. 2012; 4(3): 175-187.
2. WHO: recommended definitions, terminology and format for statistical tables related to the perinatal period and use of a new certificate for cause of perinatal deaths. Modifications recommended by FIGO as amended October 14, 1976. Acta Obstet Gynecol Scand. 1977; 56(3): 247-253.
3. Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, et al. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet. 2015; 385(9966): 430-440.
4. Mwaniki MK, Atieno M, Lawn JE, Newton CR. Long-term neurodevelopmental outcomes after intrauterine and neonatal insults: a systematic review. Lancet. 2012; 379(9814): 445-452.
5. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012; 379(9832): 2162-2172.
6. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008; 371(9606): 75-84.
7. Roos N, Sahlin L, Ekman-Ordeberg G, Kieler H, Stephansson O. Maternal risk factors for postterm pregnancy and cesarean delivery following labor induction. Acta obstet gynecol Scand. 2010; 89(8): 1003-1010.
8. Zeitlin J, Szamotulska K, Drewniak N, Mohangoo AD, Chalmers J, Sakkeus L, et al. Preterm birth time trends in Europe: a study of 19 countries. BJOG. 2013; 120(11): 1356-1365.
9. Caughey AB, Stotland NE, Washington AE, Escobar GJ. Who is at risk for prolonged and postterm pregnancy? Am J Obstet Gynecol. 2009; 200(6): 683. e1-5.
10. Abubakari A, Kynast-Wolf G, Jahn A. Prevalence of abnormal birth weight and related factors in Northern region, Ghana. BMC Pregnancy Childbirth. 2015; 15: 335.
11. McDonald SD, Han Z, Mulla S, Murphy KE, Beyene J, Ohlsson A; Knowledge Synthesis Group. Preterm birth and low birth weight among in vitro fertilization singletons: a systematic review and meta-analyses. Eur J Obstet Gynecol Reprod Biol. 2009; 146(2): 138-148.
12. Nazari M, Sharifah Zainiyah SY, Lye MS, Zalilah MS, Heidarzadeh M. Comparison of maternal characteristics in low birth weight and normal birth weight infants. East Mediterr Health J. 2013; 19(9): 775-781.
13. Nelson SM, Lawlor DA. Predicting live birth, preterm delivery, and low birth weight in infants born from in vitro fertilisation: a prospective study of 144,018 treatment cycles. PLoS Med. 2011; 8(1): e1000386.
14. Henriksen T. The macrosomic fetus: a challenge in current obstetrics. Acta Obstet Gynecol Scand. 2008; 87(2): 134-145.
15. Harder T, Rodekamp E, Schellong K, Dudenhausen JW, Plagemann A. Birth weight and subsequent risk of type 2 diabetes: a meta-analysis. Am J Epidemiol. 2007; 165(8): 849-857.
16. Koyanagi A, Zhang J, Dagvadorj A, Hirayama F, Shibuya K, Souza JP, et al. Macrosomia in 23 developing countries: an analysis of a multicountry, facility-based, cross-sectional survey. Lancet. 2013; 381(9865): 476-483.
17. Yu DM, Zhai FY, Zhao LY, Liu AD, Yu WT, Jia FM, et al. Incidence of fetal macrosomia and influencing factors in China in 2006. Chin J Prevent Med. 2008; 16: 11-13.
18. Goldstein H. Multilevel statistical models. 4th ed. UK: John Wiley and Sons; 2011.
19. Twisk JW. Applied multilevel analysis: a practical guide for medical researchers. 1st ed. Cambridge: Cambridge University Press; 2006.
20. Twisk JW. Applied longitudinal data analysis for epidemiology: a practical guide. 2nd ed. Cambridge: Cambridge University Press; 2013.
21. Wang Z, Louis TA. Matching conditional and marginal shapes in binary random intercept models using a bridge distribution function. Biometrika. 2003; 90(4): 765-775.
22. Amini P, Maroufizadeh S, Hamidi O, Samani RO, Sepidarkish M. Factors associated with macrosomia among singleton live-birth: A comparison between logistic regression, random forest and artificial neural network methods. Epidemiol Biostat Public Health. 2016; 13(4).
23. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008; 371(9606): 75-84.
24. Flenady V, Koopmans L, Middleton P, Frøen JF, Smith GC, Gibbons K, et al. Major risk factors for stillbirth in high-income countries: a systematic review and meta-analysis. Lancet. 2011; 377(9774): 1331-1340.
25. Whitehead NS. The relationship of socioeconomic status to preterm contractions and preterm delivery. Matern Child Health J. 2012; 16(8): 1645-1656.
26. Kistka ZAF, Palomar L, Boslaugh SE, DeBaun MR, DeFranco EA, Muglia LJ. Risk for postterm delivery after previous postterm delivery. Am J Obstet Gynecol. 2007; 196(3): 241. e1-6.
27. Joseph K, Fahey J, Shankardass K, Allen VM, O'Campo P, Dodds L, et al. Effects of socioeconomic position and clinical risk factors on spontaneous and iatrogenic preterm birth. BMC Pregnancy and Childbirth. 2014; 14(1): 117.
28. Davies EL, Bell JS, Bhattacharya S. Preeclampsia and preterm delivery: a population-based case-control study. Hypertens Pregnancy. 2016; 35(4): 510-519.
29. Dunietz GL, Holzman C, McKane P, Li C, Boulet SL, Todem D, et al. Assisted reproductive technology and the risk of preterm birth among primiparas. Fertil Steril. 2015; 103(4): 974-979. e1.
30. Liem S, Schuit E, Hegeman M, Bais J, de Boer K, Bloemenkamp K, et al. Cervical pessaries for prevention of preterm birth in women with a multiple pregnancy (ProTWIN): a multicentre, open-label randomised controlled trial. Lancet. 2013; 382(9901): 1341-1349.
31. Mercer BM, Goldenberg RL, Meis PJ, Moawad AH, Shellhaas C, Das A, et al. The preterm prediction study: prediction of preterm premature rupture of membranes through clinical findings and ancillary testing. Am J Obstet Gynecol. 2000; 183(3): 738-745.
32. Rockhill K, Dorfman H, Srinath M, Hogue C. The effects of prepregnancy body mass index and gestational weight gain on fetal macrosomia among American Indian/Alaska Native Women. Matern Child Health J. 2015; 19(11): 2480-2491.
33. Jolly MC, Sebire NJ, Harris JP, Regan L, Robinson S. Risk factors for macrosomia and its clinical consequences: a study of 350,311 pregnancies. Europ J Obstet Gynecol Reprod Biol. 2003; 111(1): 9-14.
34. Ju H, Chadha Y, Donovan T, O'ROURKE P. Fetal macrosomia and pregnancy outcomes. Australia New Zealand J Obstet Gynaecol. 2009; 49(5): 504-509.
35. Santos AA, Nogales FJ, Ruiz E. Comparing univariate and multivariate models to forecast portfolio value-at-risk. Journal of Financial Econometrics. 2013; 11(2): 400-441.
36. Kassahun W, Neyens T, Molenberghs G, Faes C, Verbeke G. A joint model for hierarchical continuous and zero-inflated overdispersed count data. J Stat Comput Simul. 2015; 85(3): 552-571.
37. Mahmoodi M, Moghimbeigi A, Mohammad K, Faradmal J. Semiparametric models for multilevel overdispersed count data with extra zeros. Stat Methods Med Res. 2016. pii: 0962280216657376. (A head print).
38. Nkansah-Amankra S, Luchok KJ, Hussey JR, Watkins K, Liu X. Effects of maternal stress on low birth weight and preterm birth outcomes across neighborhoods of South Carolina, 2000-2003. Matern Child Health J. 2010; 14(2): 215-226.
39. Ota E, Ganchimeg T, Mori R, Souza JP. Risk factors of pre-eclampsia/eclampsia and its adverse outcomes in low-and middle-income countries: a WHO secondary analysis. PLoS One. 2014; 9(3): e91198.

Original Article

Attitude of Law and Medical Students to Oocyte Donation

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Abstract

Background: Among the young generation, medical and law students' attitude towards third party reproduction is very important because they will be directly involved in restricting or developing the programs that will support it in the future. The aim of this survey was to investigate attitude of law and medical students to oocyte donation and key aspects of this kind of third party.

Materials and Methods: In analytical cross-sectional study, 345 medical and law students were randomly selected using stratified sampling. Data was collected using attitude toward donation- oocyte (ATOD-O) questionnaire. Responses were on a 5-point Likert scale. Data were analyzed according to established statistical approach by Heeren and D'Agostino.

Results: The majority of the participants agreed with oocyte donation being the last choice for infertility treatment. There was a significant difference between medical students and law students regarding the acceptance of oocyte donation (3.23 vs. 3.53, P=0.025). In addition, female participants were more tolerant on receiving donated oocytes from their sisters than male participants (3.01 vs. 2.58, P=0.002) and finally, a higher number of the participants had a positive attitude towards anonymity of the donor and the recipient to one another (3.93 vs. 3.86, P=0.580). The vast majority of female students believed that the oocyte recipient naturally likes that child (P<0.0001).

Conclusion: In the current study, a great majority of law and medical students support oocyte donation as an alternative way of starting a family. There is an interest among female students in donating oocytes anonymously. The majority believed that the oocyte recipient family will like the donor oocyte child naturally.

Keywords: Attitude, Disclosure, Infertility, Oocyte Donation

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Introduction

Since 1980s, oocyte donation (OD) has become an increasingly more accepted method of assisted reproduction, leading to a high number of OD children being born every year worldwide (1-3). Therefore, OD continues to grow in popularity and is considered as an established method to aid infertile couples in achieving their reproductive goals (4, 5). There are a few reports on OD families that have indicated no negative effects on the mother-child relationship, quality of parenting or emotional health of the children despite the absence of a genetic connection between the mother and the child (6, 7).

As the use of OD increases, so do the concerns about its psychological, social and ethical impacts on the children created in this way. Parents of OD children face important challenges during the treatment, bearing and the development of their child. These challenges include making decisions on selecting known or anonymous donors, and whether to tell others and/or the child about his or her oocyte origin (8). Not only the recipient couple, but the donor also faces emotional and social challenges regarding egg-sharing (9).

However, in recent years, particularly in developed countries, there has been a trend of couples delaying parenthood well into their fourth decade (7, 10). Subsequently, it is expected that the growing use of assisted reproductive treatments, especially third party reproduction, takes place in these countries. Among countries that are governed by Islamic law, Iran is the only country, in which third party reproduction is not illegal and any such donation as oocyte, sperm, gamete, or embryo donation, and surrogacy are currently practiced. Iran is also equipped with tourism trade (7) to meet the needs of third party treatment by couples from other countries. In addition to Iran, third party reproduction is allowed in two other countries, Azerbaijan and Turkish Republic of Northern Cyprus.

Compared to Iran, much more is known about a couple's attitude regarding OD in the rest of the world (7, 8), but there needs to be more sufficient data on this matter in Iran. This study is aimed to investigate the attitude of law and medical students, who are directly involved in restricting or developing such reproduction programs, towards OD, and to measure the amount of their agreement on some key aspects of OD.

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Materials and Methods

A questionnaire-based study was carried out between October and December of 2014. Participants were randomly selected from medical and law students who attended in the largest university, which has two schools of law and medical together, in Tehran (capital of Iran). We used stratified sampling, in which 4th year and above medical students and 3rd and 4th year law students were stratified by the number of years of education in each field. Then, simple random sampling was done in each stratum.

The instrument

An analytical cross-sectional study was constructed on the basis of an earlier qualitative research on infertile couples who had referred to Royan Institute, the largest referral fertility clinic in Iran. Based on the existing literature, a pool of domains and statements was designed.

The final version of the tool for measuring attitudes towards OD, entitled attitude toward donation-oocyte (ATOD-O), contained 52 questions in 12 domains, including the importance of having children (2 statements), decision making and acceptance of oocyte donation (7 statements), playing the role of oocyte donor (5 statements), characteristics of the donor (8 statements), characteristics of the recipient (8 statements), being an anonymous child toward the donor (4 statements), disclosure of the use of this treatment method with others (3 statements), legal issues (4 statements), tendency to the use of different methods of OD (2 statements), the parents-child relationship (4 statements), and ownership of the child (2 statements).

For each statement, the responses were on a 5-point Likert scale, strongly disagree, disagree, somewhat, agree, and strongly agree (scoring 1 to 5, respectively). More detailed information on measuring the validity and reliability of ATOD-O questionnaire qualitatively has been published (11). To our knowledge, this tool is the first valid and comprehensive questionnaire that measures different psychological aspects of OD, with a potential for further investigations.

Training before the Study

It was necessary to provide information on the OD process for the participants because attitude measurement is useless without awareness about the subject. Before distribution of the questionnaires, training was conducted for about 15 to 20 minutes by a trained expert. Initially, a brief history of OD in Iran as well as in the rest of the world, was given to the students.

The process was then explained and the characteristics of potential candidate donors and recipients were described: which couples are candidates for OD (e.g., women with old age, early menopause, and who have no high quality oocytes), general characteristics of recipients and donors (donor: age from 21upto 34 years, preferably having a child/children, similar physical specifications to a recipient according to skin, eye, and hair color and the body, lack of genetic diseases even in her family history, syphilis, gonorrhea, hepatitis, and AIDS; recipient: up to 35 years, general physical examination, routine laboratory tests before pregnancy, determining the blood type and Rh, human immunodeficiency virus (HIV), hepatitis, pelvic exams, ultrasound evaluation of the uterine cavity to measure the size of the uterus, and a history of hysteroscopy, laparoscopy and spermiogram examination).

Ethical approval to conduct the study was obtained by the Ethics Committee of Royan Institute. The aims of the study were clearly explained for all participants prior to the investigation. Voluntarily filling the questionnaire was considered as consent. Participants were also made assure about their confidentiality and anonymity for attending this investigation.

Data analysis

Statistical analyses were carried out using SPSS version 22.0 (SPSS Inc, Chicago, IL, USA). Questionnaires with missing values were not considered in the analyses. Continuous variables were expressed as mean ± SD and categorical variables as number (percentage). In this paper, responses (as 4-point Likert scale, ranging from 1 to 4) to women's and men's attitude and medical and law students' attitude questions were compared using independent samples t test. Heeren and D'Agostino, in 1987, demonstrated that the t test is robust even once the outcome variable is assessed as ordinal scaled data. More details were explained elsewhere (12). $P<0.05$ was considered statistically significant.

Results

General characteristics of participants

In this study, 345 medical and law students participated. Of them, 57.7% (199 participants) were women and 42.3% (146 participants) men. About 344 participants (99.7%) were urban and 93.9% (234 participants) single. The mean age was 21.66 ± 2.07 years. Approximately 226 students (65.5%) were in medical school and 34.5% (119 participants) in law school. General characteristics of the participants are shown in Table 1.

Table 1: General characteristics of the participants

Men n=146	Women n=199	Demographic variables
		Marital Status
16 (8)	183 (92)	Single
5 (3.4)	141 (96.6)	Married
		Field
76 (38.2)	123 (61.8)	Medicine
43 (29.5)	103 (70.5)	Law

Values in parentheses are percentages.

Decision making on oocyte donation

Male and female respondents were in favor of OD as the last choice of infertility treatment, while there was a significant difference between medical and law students (3.23 ± 1.18 vs. 3.53 ± 1.15, respectively, P=0.025). The difference between male and female students with regards to the recipient relationship to the donor was not significant (2.68

± 1.17 vs. 2.67 ± 1.06, P=0.924). The difference between law and medical students with regards to supportive of relatives donating and receiving oocytes was not significant (2.58 ± 1.09 vs. 2.72 ± 1.11, P =0.275). Women were more supportive of receiving donated oocytes from their sisters compared to men. Men, on the other hand, received a question about their wife's sister rather than their own sister (3.01 vs. 2.58, P=0.002), but in both fields there was no significant mean difference regarding this statement (2.98 ± 1.29 vs. 2.75 ± 1.30, P=0.118, Table 2).

Anonymity and disclosure

The difference between male and female participants with regards to the statement "anonymity of the donor and the recipient to one another" was not significant (3.93 ± 1.20 vs. 3.86 ± 1.17, P=0.580). Similarly, a significant mean difference was not found between the students of the two fields. However, most of the women and the men in both fields believed that disclosure of some of the donor's characteristics, such as age, ethnicity and religion, to the recipient couple was necessary. However, a significant difference was not observed between the groups that were compared (P=0.165 for gender groups and P=0.620 for education groups).

Medical and law students had similar attitudes towards an OD child's anonymity to the donor before 18 years (2.67 ± 1.24 vs. 2.55 ± 1.38, P=0.421), and there was no significant difference between men and women regarding the same statement (2.67 ± 1.30 vs. 2.57 ± 1.28, P=0.487). The mean scores obtained by the students in both genders and both fields indicated that the participants had relatively negative attitudes towards the statement "The child can meet the genetic or biologic mother after 18 years" (P=0.527 for gender groups and P=0.802 for education groups (Table 3).

The importance of child-parent relationship

Female participants than male respondents believed that an oocyte recipient (the mother) naturally likes that OD child (4.21 ± 0.88 vs. 3.82 ± 1.07, P<0.0001). They also believed that the husband of the oocyte recipient (the father), naturally likes the child (4.17 ± 0.91 vs. 3.79 ± 1.01, P<0.0001). Compared to the men, the women had a more positive attitude towards the two statements regarding "the child will naturally like the mother (oocyte recipient) if oocyte donation is disclosed" and "the child will naturally like the father (the husband of an oocyte recipient) if oocyte donation is disclosed" (3.95 ± 1.07 vs. 3.59 ± 1.02, P=0.002 and 4.08 ± 0.96 vs3.66 ± 1.01, P<0.0001, respectively, Table 4).

Table 2: Attitudes towards decision making on oocyte donation

P value	Law Mean ± SD	Medicine Mean ± SD	P value	Men Mean ± SD	Women Mean ± SD	Statement
0.025*	3.53 ± 1.15	3.23 ± 1.18	0.849	3.36 ± 1.14	3.33 ± 1.20	I am ready to use oocyte donation treatment if there are no other treatments for infertility
0.388	4.33 ± 0.87	4.23 ± 0.99	0.364	4.21 ± 0.99	4.31 ± 0.92	Psychological conditions of my spouse are important for oocyte donation
0.275	2.58 ± 1.09	2.72 ± 1.11	0.924	2.68 ± 1.17	2.67 ± 1.06	I support a decision on oocyte donation by my relatives or friends
0.118	2.98 ± 1.29	2.75 ± 1.30	0.002*	2.58 ± 1.20	3.01 ± 1.35	Donated oocyte from my sister is acceptable for me

*; P<0.05 was considered significant statistically.

Table 3: Attitudes towards disclosure or secrecy of oocyte donation

Statement	Women Mean ± SD	Men Mean ± SD	P value	Medicine Mean ± SD	Law Mean ± SD	P value
The donor and the recipient should be anonymous to each other	3.93 ± 1.20	3.86 ± 1.17	0.580	3.82 ± 1.19	4.05 ± 1.16	0.097
Donor's characteristics (such as age, ethnicity, and religion) can be given to the recipient	4.19 ± 1.02	4.04 ± 1.01	0.165	4.11 ± 1.05	4.17 ± 0.98	0.620
Recipient's characteristics (such as age, ethnicity, and religion) can be given to the donor	3.96 ± 1.16	3.88 ± 1.06	0.532	3.89 ± 1.11	4.00 ± 1.11	0.383
The child should be aware of his/her own genetic origin after 18 years	2.67 ± 1.30	2.57 ± 1.28	0.487	2.67 ± 1.24	2.55 ± 1.38	0.421
The child can meet the genetic or biologic mother after 18 years	2.10 ± 1.15	2.18 ± 1.51	0.527	2.15 ± 1.09	2.11 ± 1.25	0.802

Table 4: Attitudes towards the parent-child relationship

Statement	Women Mean ± SD	Men Mean ± SD	P value	Medicine Mean ± SD	Law Mean ± SD	P value
Oocyte recipient (the mother), naturally likes the child	4.21 ± 0.88	3.82 ± 1.07	0.000*	4.02 ± 0.94	4.08 ± 1.06	0.607
The husband of oocyte recipient(the father), naturally likes the child	4.17 ± 0.91	3.79 ± 1.01	0.000*	3.95 ± 0.95	4.12 ± 1.00	0.105
The child naturally will like the mother (oocyte recipient) if oocyte donation is disclosed	3.95 ± 1.07	3.59 ± 1.02	0.002*	3.76 ± 1.03	3.86 ± 1.12	0.408
The child naturally will like the father (the husband of oocyte recipient) if oocyte donation is disclosed	4.08 ± 0.96	3.66 ± 1.01	0.000*	3.88 ± 0.98	3.95 ± 1.04	0.498

*; P<0.05 was considered significant statistically.

Discussion

The present findings revealed that law and medical students who participated in this study support OD as an alternative way of childbearing and starting a family. A study on attitudes of Christians and Muslims to an oocyte donation program in Iran also revealed that 74% of Christians and 59% of Muslims supported the OD for infertile couples (13).

Another survey on public opinion regarding OD in Sweden suggested that the majority of the participants believed that OD is a good way to help childless couples. However, in contrast to the findings of the present study, the Swedish women were more supportive of friends to become oocyte donors and recipients compared to the Swedish men (14).

In the present study, the law students were significantly more in favor of this idea compared to the medical students. Interestingly, in conservative or religious societies, such as in Islamic countries, legal rights are usually consistent with the religious commands or recommendations, which are obtained from religious authorities (11). However this type of cell donation with the purpose of reproduction is not permitted to be practiced in any of the Islamic countries (15) except for in Iran-an Islamic country, which is a theocracy and is directed by the Shia laws (7). Unlike Shiites, third party reproduction is forbidden by Sunni religious scholars. However, in all Islamic countries married couples can use and benefit from assisted reproductive treatment (16).

The finding that the responders thought that the oocyte donor and the receiving couple should be anonymous (unknown) to one another was a line with earlier studies (13, 17). Although anonymity would be preferred in OD, there has been interest among donors and recipients to receive information about each other (3, 18, 19). Similar to the results of a study by Soderstrom-Anttila et al. (20), we found that some personal information, such as age, ethnicity, and religion of the oocyte donor, is an important factor in decision making by the recepient couple.

Women also had more tendency to receiving donated oocyte from their sisters than men. Yee et al. (21), suggest that known donation will have potential challenges and problems due to an emotional relationship between the donor and the recipient. It is important to keep in mind that the feelings of both responsibility and guilt to recipient's family appear by the donor with a family tie, especially when donor and recipient were sisters (22). The current legislation in Iran allows the donor to be anonymous for an OD procedure.

Importantly, disclosure or non-disclosure of the genetic origin to the children is a challenging issue in OD families (23). We observed that both medical and law students had a positive attitude towards child's anonymity to the donor before the age of 18 years. The present study confirmed the previous findings, but previous findings do not confirm a future study (6, 13, 24). At this point, a little is known about the parents' decisions on whether to disclose their child's origin in OD families, but there is a general agreement in favor of telling the OD children about their origins (25).

In a questionnaire-based study, Laruelle et al. (22) extracted anonymity and secrecy options of recipient couples and donors from semi-structured counseling sessions for all those who wanted to undergo oocyte donation. The participants' motivations towards the secrecy of OD to the child were the fear of rejection by the family or their social circle and/or by the child, the fear of stigmatization by the family or their social circle, the fear of weakening the mother-child relationship, the fear of a negative psychological impact upon the child and the idea that this is intimate and does not concern the child.

Motivations towards disclosure included matters such as to give honesty in the relationship with the child, to prevent accidental disclosure by others, to give the child potential access to his/her origins and the opportunity to meet the donor (specially the known donation group), and to avoid potential disadvantageous effects of secrecy on the parent-child relationship (23).

In a study on increasing openness in oocyte donation families regarding disclosure over 15 years, it was concluded that the professionals have more and more actively encouraged the parents to inform their presumptive children of their conceptions (3). Nonetheless, the nature of counseling provided by the medical team and psychologists is the most important factor affecting disclosure decisions (3, 26).

Surprisingly, in this study, women valued parenthood more than men and they believed that the OD child will naturally like her/his family member (the mother and the father or recipient couple), even if oocyte donation is disclosed. Since the participants of the current study were from two schools in the country, a more extensive study will on both law and medical students will help in achieving more comprehensive results.

Conclusion

This was the first report on the attitudes of medical and law students towards OD in a Muslim country. The present findings indicated that a great majority of law and medical students support OD as an alternative way of starting a family. There is an interest amongst female students in donating oocytes anonymously. The majority of the participants believed in the importance of the relationship between parents and their child. They were concerned about the oocyte recipient family loving their OD child and vice versa naturally. We truly believe that these studies may potentially influence the law and medical students in a positive manner.

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Author's Contributions

S.V.; Performed preparing, writing, editing and approving this paper for submission, and also participated extensively in interpretation of the data and the conclusion, the finalization of the manuscript, and approved the final draft. R.O.S.; Participated in conception and study design and was responsible for overall supervision. E.K.; Performed data collection. M.M.; Contributed to statistical analysis of data. All authors read and approved the final manuscript.

References

1. Lutjen P, Trounson A, Leeton J, Findlay J, Wood C, Renou P. The establishment and maintenance of pregnancy using in vitro fertilization and embryo donation in a patient with primary ovarian failure. Nature. 1984; 307(5947): 174-175.
2. Trounson A, Leeton J, Besanko M, Wood C, Conti A. Pregnancy established in an infertile patient after transfer of a donated embryo fertilised in vitro. Br Med J (Clin Res Ed). 1983; 286(6368): 835-838.
3. Soderstrom-Anttila V, Salevaara M, Suikkari AM. Increasing openness in oocyte donation families regarding disclosure over 15 years. Hum Reprod. 2010; 25(10): 2535-2542.
4. Boutelle AL. Donor motivations, associated risks and ethical considerations of oocyte donation. Nurs Womens Health. 2014; 18(2): 112- 121.
5. Remohi J, Gallardo E, Guanes PP, Simon C, Pellicer A. Donor-recipient synchronization and the use of gonadotrophin-releasing hormone agonists to avoid the premature luteinizing hormone surge in oocyte donation. Hum Reprod. 1995; 10 Suppl 2: 84-90.
6. Golombok S, Murray C, Brinsden P, Abdalla H. Social versus biological parenting: family functioning and the socioemotional development of children conceived by egg or sperm donation. J Child Psychol Psychiatry. 1999; 40(4): 519-527.
7. Isikoglu M, Senol Y, Berkkanoglu M, Ozgur K, Donmez L, Stones-Abbasi A. Public opinion regarding oocyte donation in Turkey: first data from a secular population among the Islamic world. Hum Reprod. 2006; 21(1): 318-323.
8. Klock SC, Greenfeld DA. Parents' knowledge about the donors and their attitudes toward disclosure in oocyte donation. Hum Reprod. 2004; 19(7): 1575-1579.
9. Gürtin ZB, Ahuja KK, Golombok S. Emotional and relational aspects of egg-sharing: egg-share donors' and recipients' feelings about each other, each others' treatment outcome and any resulting children. Hum Reprod. 2012; 27(6): 1690-1701.
10. Wang YA, Farquhar C, Sullivan EA. Donor age is a major determinant of success of oocyte donation/recipient programme. Hum Reprod. 2012; 27(1): 118-125.
11. Omani Samani R, Mounesan L, Ezabadi Z, Vesali S. Development of A questionnaire to measure attitude toward oocyte donation. Int J Fertil Steril. 2015; 9(3): 387-392.
12. Heeren T, D'Agostino R. Robustness of the two independent samples t-test when applied to ordinal scaled data. Stat Med. 1987; 6(1): 79-90.
13. Khalili M, Isikoglu M, Ghasemi M, Attitudes of christians and muslims to an oocyte donation program in Iran. Eubios J Asian Int Bioethics. 2006; 16: 66-70.
14. Svanberg AS, Lampic C, Bergh T, Lundkvist O. Public opinion regarding oocyte donation in Sweden. Hum Reprod. 2003; 18(5): 1107-1114.
15. Schenker JG. Jewish and moslem aspects of in vitro fertilization and embryo transfer. Ann N Y Acad Sci. 1985; 442: 601-607.
16. Gooshki ES, Allahbedashti N. The process of justifying assisted reproductive technologies in Iran. Indian J Med Ethics. 2015; 12(2): 57-96.
17. Halvaei I, Khalili MA, Ghasemi-Esmailabad S, Nabi A, Shamsi F. Zoroastrians support oocyte and embryo donation program for infertile couples. J Reprod Infertil. 2014; 15(4): 222-228.
18. Kalfoglou AL, Geller G. A follow-up study with oocyte donors exploring their experiences, knowledge, and attitudes about the use of their oocytes and the outcome of the donation. Fertil Steril. 2000; 74(4): 660-667.
19. Blyth E, Kramer W, Schneider J. Perspectives, experiences, and choices of parents of children conceived following oocyte donation. Reprod Biomed Online. 2013; 26(2): 179-188.
20. Soderstrom-Anttila V, Sajaniemi N, Tiitinen A, Hovatta O. Health and development of children born after oocyte donation compared with that of those born after in-vitro fertilization, and parents' attitudes regarding secrecy. Hum Reprod. 1998; 13(7): 2009-2015.
21. Yee S, Hitkari JA, Greenblatt EM. A follow-up study of women who donated oocytes to known recipient couples for altruistic reasons. Hum Reprod. 2007; 22(7): 2040-2050.
22. Laruelle C, Place I, Demeestere I, Englert Y, Delbaere A. Anonymity and secrecy options of recipient couples and donors, and ethnic origin influence in three types of oocyte donation. Hum Reprod. 2011; 26(2): 382-390.
23. Greenfeld DA, Klock SC. Disclosure decisions among known and anonymous oocyte donation recipients. Fertil Steril. 2004; 81(6): 1565-1571.
24. Golombok S, Brewaeys A, Giavazzi MT, Guerra D, MacCallum F, Rust J. The European study of assisted reproduction families: the transition to adolescence. Hum Reprod. 2002; 17(3): 830-840.
25. Gottlieb C, Lalos O, Lindblad F. Disclosure of donor insemination to the child: the impact of Swedish legislation on couples' attitudes. Hum Reprod. 2000; 15(9): 2052-2056.
26. Guillou J, Sejourne N, Chabrol H. Pregnancy following anonymous oocyte donation: Experience and feelings. Gynecol Obstet Fertil. 2009; 37(5): 410-414.

Original Article

Relationship between Quality of Life, Relationship Beliefs and Attribution Style in Infertile Couples

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Abstract

Background: Many infertile couples experience psychological distress and suffer from impaired quality of life. Generally, when couples are dealing with uncontrolled events such as infertility, it is important to manage it well and to use the suitable coping style; so this can represent an example of attribution style. The purpose of this study is to investigate the quality of life, relationship beliefs and attribution style in infertile couples.

Materials and Methods: This cross-sectional study consisted of 50 infertile couples, who were at least 18 years of age and could read and write in Persian. Participants provided demographic and general characteristics and completed the quality of life (SF-12), relationship belief inventory (RBI) and attribution style (ASQ) forms. Data was analyzed by the paired t test, Pearson correlation tests and multiple linear regression analysis, using SPSS version 22 statistical software.

Results: Overall, 50 infertile couples participated in our study. The males had a significantly higher score for quality of life compared to the females (P=0.019). In RBI subscales except "Disagreement is Destructive" all others significantly higher in wives than husbands. All subscales of RBI had a negative correlation with the quality of life. The quality of life had a significant correlation with positive internal (r=0.213, P=0.033). The adjusted regression model showed that the quality of life for males was higher than in females (β=-3.098, P=0.024).

Conclusion: The current data indicate that in infertile couples, the husbands have a higher quality of life in comparison to their wives. Also, all subscales of relationship beliefs have a negative correlation with the quality of life, but in attribution style, just internal attribution style for positive events is associated with the quality of life. In general, there is a correlation between relationship beliefs and the quality of life in infertile couples.

Keywords: Assisted Reproduction Technique, Attribution Style, Infertility, Quality of Life, Relationship Beliefs

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Introduction

It is known well Having children has always been considered as a major sociocultural value in many societies (1). Prevalence of infertility is currently estimated at 9% worldwide (2), and affects approximately 2.5% of the Iranian population (3). Infertility could lead to serious emotional problems and psychological stress (4-7). It potentially has negative impact on marital happiness, sexual satisfaction and the general quality of life (8-11). Generally, the quality of life is affected by a person's physical health, psychological condition, social constraints and personal belief (12, 13), the latter being a key determinant in the quality of the relationship between spouses (14).

Rational relationship is the most common problem explained by dissatisfied couples. Many mental health issues occur if spouses not to speak about or meet their needs (15). A number of studies have shown that there is a negative association between irrational believes and marital satisfaction (16-18). When infertile couples are dealing with this uncontrolled event, it is very important how to evaluate it in order to use a suitable coping style; so this can represent an example of attribution style. Peterson and Seligman (19) argue the causal styles in three main attribution dimensions as (internality versus externality), (globality versus specificity) and (stability versus instability).

Attribution style theory provides a framework for understanding the causal statements that individuals explain their own behavior as well as the behavior of others (20). Based on this theory, when individuals tend to interpret negative events to internality (self), globality and stability, they are more prone to experience mental problems (21). Some studies suggest that individuals with depression tend to use internal attributions for negative events (20, 22).

Therefore, it is import to know how relationship beliefs and attribution styles can impact on one's quality of life. To our knowledge, this represents the first study to examine this relationship in infertile couples. The aim of this

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study is to investigate the connection between the quality of life based on relationship beliefs and attribution styles in infertile couples.

Materials and Methods

A cross-sectional study was conducted on infertile couples who referred to the Royan Institute, a specialty infertility clinic in Tehran, the capital of Iran, between January and February 2014. Correlation sample size formula with a 0.4 correlation between variables from same study and was used to find an optimal sample size. The sample size with a confidence interval of 95%, power of 80%, and with a significance level of 0.05, was estimated to be 50 couples (50 males and 50 females). The sampling method was available sampling. The inclusion criteria were being 18 years or older, having a history of infertility, and being able to read and write in Persian.

The study was approved by the Ethics Committee of Royan Institute. Aims of the study and the confidentiality of the data were clearly explained for all participants. Eligible individuals were also assured that acceptance or refusal to participate in the research had no influence on their treatment procedures. Voluntarily filling the questionnaire was considered as consent.

Instruments

Demographic

The demographic questionnaire include age (years), educational levels (under diploma,under diploma and academic), type of infertility (male factor, female factor, unknown, both), duration of infertility (year), duration of marriage (year).

12-Item Short-Form Survey (SF-12)

The quality of life (the short form health survey) SF-12 used in our study is a Persian version of the SF-36 instrument. The SF-12 was developed in 1996 by Ware et al. (23) in English as part of the International Quality of Life Project Assessment, a shorter alternative to the SF-36, and was translated subsequently into Persian and other languages. In the Persian version of SF-12 validated in 2007 by Montazeri et al. (24), Cronbach's alpha coefficient (to test reliability) was 0.85, and intraclass correlation coefficient (ICC) was 0.90. Cronbach's alpha coefficient in this study was 0.77. The SF-12 questionnaire was used as a common tool for evaluating the quality of life of infertile couples (25). The SF-12 is comprised of eight subscales: physical functioning, physical role, bodily pain, general health, vitality, social functioning, emotional role, and mental health. These were summarized into two scales: a physical component score (PCS) and a mental component score (MCS), in accordance with the guidelines for the SF-12 instrument. Both scores ranged between 12 and 48, with a higher score indicating better health. These SF-12-based summaries have been shown to reproduce accurately both PCS and MCS, which have been derived from the full SF-36 (26).

Relationship Belief Inventory

Relationship Belief Inventory (RBI) questionnaire was published in 1981 by Eidelson and Epstein (26). It is a 40-item self-report instrument and contains disagreement is destructive (D), partners cannot change (C), and mindreading is expected (M), sexual perfectionism (S) and sexes are different (MF). By summing questions for each subscales, the score of each subscale was calculated. In the Persian version of RBI, which was validate by Mazaheri and Pooretemad in 2001 (27), Cronbach's alpha coefficient was 0.75 and ICC was 0.78. Higher scores indicate a worse relationship belief. Cronbach's alpha coefficient of total score in this study was 0.80 and Cronbach's alpha coefficients were 0.60, 0.75, 0.62, 0.67, 0.71 for D, M, C, S and MF respectively.

Attribution Style Questionnaire

The Attribution Style Questionnaire (ASQ) was built in 1982 by Peterson et al. (28). This questionnaire contains hypothetical situations split equally into positive AS for (a) good affiliative and (b) good achievement-related situations, and negative AS for (a) bad affiliative and (b) bad achievement-related situations. Respondents rated each causal attribution on three 7-point scales: i. Internality-externality, ii. Stability-instability, and iii. Globality-specificity. Internality, stability, and globality are usually simply summed to derive composite positive attribution style and composite negative attribution style. Cronbach's alpha coefficient of total score in this study was 0.72 and cronbach's alpha coefficients were 0.62, 0.71 and 0.76 for internality, stability, and globality respectively.

Statistical analysis

Statistical analysis were performed using the Statistical Package for the Social Sciences; SPSS V.22.0 for Windows (IBM SPSS V.22.0.0). Continuous variables were expressed as mean ± SD and categorical variables as frequencies (%). Normality of the variables was checked with Kolmogorov-Smirnov Test. Pearson correlation coefficient was used to examine the relationship between study variables, and paired t test was used to evaluate the difference between male and female data (wives and husbands). Finally, multiple linear regression analysis was performed by controlling confounders. $P<0.05$ was considered statistically significant.

Results

Participants characteristics

In this study, 50 infertile couples consisting of 50 (50%) men and 50 (50%) women participated. The mean age was 33.30 ± 5.13 for males and 28.94 ± 5.26 for females ($P<0.001$). Amongst the participants 35 individuals (35%) had an academic education. According to the cause of infertility, 49 couples (49%) involved in male factor infertility, 8 (8%) female factor, 7 (7%) both and 36 (36%) unknown. The mean dura-

tion of infertility was 4.7 ± 3.84 years and the mean duration of marriage was 6.8 ± 3.89 years in the participants (Table 1).

Table 1: Demographic characteristics of the infertile couples

	Male Mean ± SD or n (%)	Female Mean ± SD or n (%)	P value
Age (Y)	33.30 ± 5.13	28.94 ± 5.26	<0.001
Education			0.017
Under diploma/ Diploma	33 (66)	33 (66)	
Academic	17 (34)	13 (34)	
Cause of infertility			
Male factor	49 (49)		
Female factor	8 (8)		
Both	7 (7)		
Unknown	36 (36)		
Duration infertility	4.7 ± 3.84		
Duration of marriage	6.8 ± 3.89		

Univariate analysis

The mean score for the quality of life was significantly different between wives and husbands and it was higher in husbands than wives (P=0.019, Table 2).

Between the factors of RBI questionnaire, Mindreading is Expected (M), Partners Cannot Change (C), Sexual Perfectionism (S) and Sexes Are Different (MF) were significantly different between wives and husbands and they were all higher in wives than in their husbands (P=0.008, 0.001, 0.035 and 0.010 respectively). The husbands had a relatively higher score of Disagreement is Destructive (D) compared to their wives, but the difference was not significant (P=0.152). Overall, the wives expressed a higher mean total RBI compared to their husbands and the overall difference was significant (P=0.002). In the attribution style questionnaire, the scores for negative internality and cynical attributions were not significantly different among males and females have a higher mean score in husbands compared to their wives but not significant. Also, there was no significant difference between males and females with regard to casual attribution categories.

Table 2: SF-12, RBI and ASQ subscales between couples

	Male Mean ± SD	Female Mean ± SD	P value*
SF-12	38.02 ± 5.12	34.64 ± 5.45	0.019*
RBI subscales			
Disagreement is destructive	19.90 ± 4.19	18.74 ± 4.60	0.152
Mindreading is expected	21.90 ± 3.89	24.16 ± 5.28	0.008
Partners cannot change	18.44 ± 6.24	21.90 ± 6.09	0.001
Sexual perfectionism	22.68 ± 7.79	25.48 ± 6.61	0.035
Sexes are different	17.48 ± 7.55	20.44 ± 5.24	0.010
Total	100.40 ± 21.94	110.72 ± 20.31	0.002
ASQ subscales			
Negative internal	4.18 ± 1.15	3.79 ± 1.06	0.448
Positive internal	4.75 ± 1.17	5.08 ± 1.01	0.702
Negative stability	3.81 ± 0.81	3.89 ± 0.82	0.179
Positive stability	4.78 ± 0.99	4.87 ± 1.04	0.408
Negative public	3.82 ± 1.26	4.04 ± 0.89	0.200
Positive public	4.58 ± 1.34	4.79 ± 1.07	0.573
Cynical attribution	11.81 ± 1.92	11.72 ± 1.72	0.244
Optimistically attribution	14.12 ± 2.98	14.75 ± 2.60	0.263

RBI; Relationship Belief Inventory, ASQ: Attribution Style Questionnaire, SF-12: 12-Item Short Form Survey, and *; Paired t test.

Table 3: The relationship of SF-12 with RBI and ASQ subscales

	1	2	3	4	5	6	7	8	9
1. SF-12	1	0.380*	0.210*	0.119	0.101	0.206*	0.386**		
2. Disagreement is destructive	0.380**	1	0.319**	-0.012	0.191	0.202*	0.663**		
3. Mindreading is expected	0.210**	0.319**	1	-0.151	0.082	0.097	0.490**		
4. Partners cannot change	0.119	-0.012	-0.151	1	0.195	0.257*	0.456**		
5. Sexual perfectionism	0.101	0.191	0.082	0.195	1	-0.027	0.487**		
6. Sexes are different	0.206*	0.202*	0.097	0.257*	-0.027	1	0.600**		
7. Total	0.386**	0.663**	0.490**	0.456**	0.487*	0.600**	1		
1. SF-12	1	0.061	0.213*	-0.166	0.110	-0.029	0.118	-0.054	0.175
2. Negative internal	0.061	1	0.100	0.144	-0.127	-0.031	-0.135	0.661	-0.065
3. Positive internal	0.213*	0.100	1	-0.226	0.561**	0.265*	0.644**	0.120	0.876**
4. Negative stability	-0.166	0.144	-0.226*	1	-0.017	0.010	-0.099	0.540	-0.138
5. Positive stability	0.110	-0.127	0.561**	-0.017	1	0.125	0.466**	-0.009	0.786**
6. Negative public	-0.029	-0.031	0.265*	0.010	0.128	1	0.526	0.586**	0.378*
7. Positive public	0.118	-0.135	0.644**	-0.099	0.466**	0.526**	1	0.189	0.855**
8. Cynical attribution	-0.054	0.661**	0.120	0.540**	-0.009	0.586**	0.189	1	0.126
9. Optimistically attribution	0.175	-0.065	0.876**	-0.138	0.786**	0.378**	0.855**	0.126	1

RBI; Relationship Belief Inventory, ASQ; Attribution Style Questionnaire, SF-12; 12-Item Short Form Survey, r; Pearson correlation coefficient, *; P<0.05, and **; P<0.001

Correlations among study variables

Additionally, bivariate correlations were conducted among subscales of two questionnaires (RBI and ASQ) with the score of quality of life (SF-12), as shown in Table 3. In subscales of the RBI questionnaire, D, M, C, S, MF and a total of RBI were positively correlated with the total score of quality of life, but only D, M, S and the total score of RBI had a significant correlation. In addition, positive internality was directly and significantly correlated with total score of SF-12 (P=0.033).

Multiple linear regression analysis

For the quality of life, the enter method was used as a covariate selection method, in step 1, demographics and subscales of RBI ad ASQ entered to model, sex and "Disagreement is Destructive" were significantly related to quality of life (β=-3.098, P=0.024 and 0.254, P=0.023 respectively). When gender, education, duration of marriage, duration of infertility, subscales of RBI and subscales of ASQ were in the quality of life model, the model adjusted R^2 was equal to 0.134. On the other hand, variance inflation factor (VIF) and tolerance of variables showed the model does not have collinearly. In step 2, sex and "Disagreement is Destructive" entered to model "Disagreement is Destructive" was positively correlated with the quality of life (β=0.319, P=0.001) and gender was negatively correlated with the quality of life. (β=-2.499, P=0.001) When sex and "Disagreement is Destructive" were in model, there was an improvement in the model (adjusted R^2=0.176, P<0.001, Table 4).

Table 4: The results of hierarchical multiple linear regressions, including factors related to the quality of life

Quality of life				
B	**SE**	**Beta**	**P**	
				Step 1:
0.024	-0.282	7.986	-3.098	Sex (female vs. male)
0.307	1.225	1.225	1.260	Education (educated vs. under diploma/diploma)
0.739	-0.071	0.303	-0.101	Duration of marriage
0.633	0.101	0.305	0.146	Duration of infertility
0.385	-0.135	0.152	-0.133	Age
0.023	0.257	0.110	0.254	Disagreement is destructive
0.349	0.102	0.129	0.121	Mindreading is expected
0.714	0.041	0.130	0.048	Partners cannot change
0.680	0.044	0.133	0.055	Sexual perfectionism
0.631	0.054	0.113	0.055	Sexes are different
0.914	0.012	0.538	0.058	Negative internal
0.819	-0.070	1.532	-0.351	Positive internal
0.869	0.018	0.751	0.125	Negative stability
0.450	-0.147	1.049	-0.797	Positive stability
0.523	-0.079	0.627	-0.402	Negative public
0.242	0.118	0.458	0.539	Positive public
0.593	-0.054	0.307	-0.165	Cynical attribution
0.747	0.790	0.378	0.347	Optimistically attribution
Model characteristics		Adjusted R^2=0.134, F=1.958, P=0.026		
Step 2:				
-2.499	1.036	-0.227	0.018	Sex (female vs. male)
0.319	0.093	0.323	0.001	Disagreement is Destructive
		Adjusted R^2=0.176, F=11.561, P<0.001		

B; Unstandardized coefficient, SE; Standard error, Beta; Standardized coefficient, and R2; Coefficient of determination.

Discussion

Our results revealed that husbands have a higher quality of life in comparison to their wives and generally males have a higher quality of life than females. In the Iranian culture, the paternalistic beliefs for fertility and the lack of social and economic supports for many women are some factors that may amplify the psychological problems of infertile women. Based on cultural conditions, such women are more likely to be under mental and emotional pressures (29). The results presented here are in line with previous studies that had shown that males reported a higher quality of life than females (25, 30-32).

Our study about factors related to relationship beliefs showed that the wives expressed a higher mean score in relationship beliefs than their husbands, meanings that wives had unreasonable relationship beliefs compared to their husbands. Also, our results indicated that relationship beliefs negatively correlate with the quality of life except for "Disagreement is Destructive" subscale, which is positively correlated with the quality of life. Many couples believe that if they oppose to their spouses, it is destructive and makes them more compatible with marital life, and as a result, their quality of life goes up. Irrational beliefs were positively related to different types of distress, such as general distress, anxiety, depression and anger (33).

A previous study reported that irrational beliefs relate to health-related quality of life (34, 35). The result of this study is nearly in line with another previous study in marital satisfaction, which reported a correlation between marital dissatisfaction and unreasonable relationship beliefs (15, 36). The results of this study also revealed that only one n internal attribution style for positive events was associated with the quality of life and the remaining dimensions of attribution style (ASQ total score, global and stable causal attributions of negative and positive events) did not display a significant relationship with the quality of life. Goli et. al. (37) have examined the roles of locus of control and attributional style in coping strategies and the quality of life between patients with cancer. The results from their study approved a positive correlation among optimistic attributional style and the quality of life.

A study reported that attribution style was significantly associated with psychopathology scores as the patients that tend to internalize, showed greater overall psychopathology (20). Also, a number of studies have shown that individuals with depression tend to excessively use inter-

nal attributions for negative events (38, 39). Therefore, attribution styles are useful for social cognitive predictors and psychosis improvement. Since the relationship beliefs have an essential role in satisfaction or dissatisfaction of life, we can change the attribution styles to improve psychosis disorders. It means that changing these beliefs can help couples to have longer and happier marital lives.

Finally, there is a need to educate infertile couples, so that they have realistic expectations, successful relationships and good quality of life. These could be provided with the help of the couple's therapists, family life educators, or psychologists. The findings of this study enable us to design and analyze future studies, which will have larger sample sizes from multiple infertility centers, with improvements.

Conclusion

The results of the current study indicate that in infertile couples, men have a higher quality of life in comparison to their wives. Also, all subscales of relationship beliefs have a negative correlation with the quality of life. However, in attribution style only internal attribution style for positive events was associated with the quality of life. In general, there is a correlation between relationship beliefs and the quality of life in infertile couples.

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Author's Contributions

B.N.; Contributed to conception and design and write introduction also revised drafted the manuscript. M.M.; Contributed to statistical analysis, interpretation of data and revised drafted the manuscript. P.A.; Contributed to interpretation of data and write the discussion. Z.S.; Participated in data collection and interpretation of the data. S.M; R.O.S.; Were responsible for overall supervision. All authors read and approved the final manuscript.

References

1. van Balen F, Bos HM. The social and cultural consequences of being childless in poor-resource areas. Facts Views Vis Obgyn. 2009; 1(2): 106-121.
2. Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Hum Reprod. 2007; 22(6): 1506-1512.
3. Vahidi S, Ardalan A, Mohammad K. The epidemiology of primary infertility in the Islamic Republic of Iran in 2004-5. J Reprod Infertil. 2006; 7(3): 243-251.
4. Verhaak CM, Smeenk JM, Evers AW, Kremer JA, Kraaimaat FW, Braat DD. Women's emotional adjustment to IVF: a systematic review of 25 years of research. Hum Reprod Update. 2007; 13(1): 27-36.
5. Drosdzol A, Skrzypulec V. Quality of life and sexual functioning of Polish infertile couples. Eur J Contracept Reprod Health Care. 2008; 13(3): 271-281.
6. Maroufizadeh S, Karimi E, Vesali S, Samani RO. Anxiety and depression after failure of assisted reproductive treatment among patients experiencing infertility. Int J Gynecol Obstet. 2015; 130(3): 253-256.
7. Omani Samani R, Maroufizadeh S, Navid B, Amini P. Locus of control, anxiety, and depression in infertile patients. Psychol Health Med. 2017; 22(1): 44-50.
8. Benyamini Y, Gozlan M, Kokia E. Variability in the difficulties experienced by women undergoing infertility treatments. Fertil Steril. 2005; 83(2): 275-283.
9. Maroufizadeh S, Ghaheri A, Omani Samani R. Factors associated with poor quality of life among Iranian infertile women undergoing IVF. Psychol Health Med. 2016: 22(2): 145-151.
10. Maroufizadeh S, Ghaheri A, Samani RO, Ezabadi Z. Psychometric properties of the satisfaction with life scale (SWLS) in Iranian infertile women. Int J Reprod Biomed. 2016; 14(1): 57-62.
11. Ghaheri A, Shojaei Shahrokhabadi M, Zayeri F, Maroufizadeh S, Karimi M. Relationship among life satisfaction, anxiety and fertility quality of life in women. Koomesh. 2016; 17(4): 957-965.
12. Wang M, Schalock RL, Verdugo MA, Jenaro C. Examining the factor structure and hierarchical nature of the quality of life construct. Am J Intellect Dev Disabil. 2010; 115(3): 218-233.
13. Andrews FM, Abbey A, Halman LJ. Is fertility-problem stress different? The dynamics of stress in fertile and infertile couples. Fertil Steril. 1992; 57(6): 1247-1253.
14. Chambers C, Markson L, Diamond JJ, Lasch L, Berger M. Health beliefs and compliance with inhaled corticosteroids by asthmatic patients in primary care practices. Respir Med. 1999; 93(2): 88-94.
15. Khodabakhshi AK, Adibrad N, Sedgh Poor BS. The comparison of relationship beliefs and couples burnout in women who apply for divorce and women who want to continue their marital life. Iran J Psychiatry. 2010; 5(1): 35-39.
16. Addis J, Bernard ME. Marital adjustment and irrational beliefs. J Ration Emot Cogn Behav Ther. 2002; 20(1): 3-13.
17. Hamamci Z. Dysfunctional relationship beliefs in marital satisfaction and adjustment. Social Behavior and Personality: an international Journal. 2005; 33(4): 313-328.
18. Möller AT, Rabe HM, Nortje C. Dysfunctional beliefs and marital conflict in distressed and non-distressed married individuals. J Ration Emot Cogn Behav Ther. 2001; 19(4): 259-270.
19. Peterson C, Seligman ME. Causal explanations as a risk factor for depression: theory and evidence. Psychol Rev. 1984; 91(3): 347-374.
20. Mizrahi R, Addington J, Remington G, Kapur S. Attribution style as a factor in psychosis and symptom resolution. Schizophr Res. 2008; 104(1): 220-227.
21. Peterson SP, Schiller N, Stricker RB. Failure of two-dimensional echocardiography to detect aspergillus endocarditis. Chest J. 1984; 85(2): 291-294.
22. Leposavić I, Leposavić L. Attribution style of patients with depression. Srp Arh Celok Lek. 2009; 137(9-10): 529-533.
23. Ware J Jr, Kosinski M, Keller SD. A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. Med Care. 1996; 34(3): 220-233.
24. Montazeri A, Vahdaninia M, Mousavi SJ, Omidvari S. The Iranian version of 12-item Short Form Health Survey (SF-12): factor structure, internal consistency and construct validity. BMC Public Health. 2009; 9: 341.
25. Mousavi SA, Masoumi SZ, Keramat A, Pooralajal J, Shobeiri F. Assessment of questionnaires measuring quality of life in infertile couples: a systematic review. J Reprod Infertil. 2013; 14(3): 110-119.
26. Eidelson RJ, Epstein N. Cognition and relationship maladjustment development of a measure of dysfunctional relationship beliefs. J Consult Clin Psychol. 1982; 50(5): 715-720.
27. Mazaheri M, Pooretemad H. Relationship beliefs scale. Tehran: Unprinted report from Family Research Center: Shahid Beheshti University; 2003.
28. Peterson C, Semmel A, von Baeyer C, Abramson LY, Metalsky GI, Seligman MEP. The attributional style questionnaire. Cogn Ther Res. 1982; 6(3): 287-299.
29. Fahami F, Quchani SH, Ehsanpour S, Zargham A. Women's lived experiences of female infertility. Iran J Obstet Gynecol Inferti. 2010; 13(4): 45-58.
30. Rashidi B, Montazeri A, Ramezanzadeh F, Shariat M, Abedinia N, Ashrafi M. Health-related quality of life in infertile couples receiving IVF or ICSI treatment. BMC Health Serv Res. 2008; 8: 186.
31. Ragni G, Mosconi P, Baldini MP, Somigliana E, Vegetti W, Caliari I, et al. Health-related quality of life and need for IVF in 1000 Italian infertile couples. Hum Reprod. 2005; 20(5): 1286-1291.
32. El Kissi Y, Amamou B, Hidar S, Ayoubi Idrissi K, Khairi H, Ali BB. Quality of life of infertile Tunisian couples and differences according to gender. Int J Gynecol Obstet. 2014; 125(2): 134-137.
33. Vîslă A, Flückiger C, Grosse Holtforth M, David D. Irrational beliefs

and psychological distress: a meta-analysis. Psychother Psychosom. 2016; 85(1): 8-15.
34. Stanculete MF, Matu S, Pojoga C, Dumitrascu DL. Coping strategies and irrational beliefs as mediators of the health-related quality of life impairments in irritable bowel syndrome. J Gastrointestin Liver Dis. 2015; 24(2): 159-164.
35. Bahremand M, Saeidi M, Komasi S. Non-coronary patients with severe chest pain show more irrational beliefs compared to patients with mild pain. Korean J Fam Med. 2015; 36(4): 180-185.
36. Sullivan BF, Schwebel AI. Relationship beliefs and expectations of satisfaction in marital relationships: Implications for family practitioners. The Family Journal. 1995; 3(4): 298-305.
37. Goli F, Scheidt CE, Gholamrezaei A, Farzanegan M. The role of locus of control and attributional style in coping strategies and quality of life among Iranian breast cancer and colorectal cancer patients: a pilot study. Int J Body Mind Culture. 2014; 1(1): 65-72.
38. Joiner TE. Negative attributional style, hopelessness depression and endogenous depression. Behav Res Ther. 2001; 39(2): 139-149.
39. Moore MT, Fresco DM. Depressive realism and attributional style: Implications for individuals at risk for depression. Behav Ther. 2007; 38(2): 144-154.

Original Article

Evaluation of Factors Associated with Sexual Function in Infertile Women

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Abstract

Background: Infertility is a major and problem influencing different aspects of couples life, especially those of women. Sexual dysfunction is the silent partner of infertility. This study aimed to identify the above-mentioned factors to make necessary decisions and perform efficient interventions to improve the sexual health of infertile women. This study investigated the factors influencing sexual dysfunction in infertile women in Mashhad, Iran.

Materials and Methods: This cross-sectional study was conducted on 85 infertile women visiting governmental Infertility Clinic and Research Center in Mashhad, Iran. The convenience sampling method was used in this study. The research tools included a demographic and infertility information form, a sexual self-efficacy questionnaire based on Schwarzer's General Self-Efficacy Scale, Female Sexual Function Index (FSFI), and Evaluation and Nurturing Relationship Issues, Communication, and Happiness (ENRICH) Marital Satisfaction Scale. The descriptive statistical tests and logistic regression method were used to analyze data.

Results: The mean age of women was 31.18 ± 5.56 years old. The majority of participants (36.7%) had higher educations, and 60% of them were housewives. Most of their husbands (49.4%) were self-employed. The mean period of infertility awareness was 6.02 ± 4.47 years, and the mean period of infertility treatment was 4.11 ± 4.46 years. The following variables influenced the sexual function of infertile women: sexual self-efficacy, sexual satisfaction, marital satisfaction, the educational level of both wife and husband, income, satisfaction with spouse appearance, and the high costs of infertility treatment.

Conclusion: The findings indicated that some factors such as sexual self-efficacy, marital satisfaction, sexual satisfaction, education, and cost of infertility treatment are associated with sexual function in infertile women.

Keywords: Female, Infertility, Self-Efficacy, Sexual Behavior

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Introduction

Infertility is be defined as being unable to become pregnant after having regular sexual contacts for one year without using contraceptive methods (1, 2). Infertility is a health issue in today's world. According to the statistics published by the World Health Organization on January 16, 2013, in developed countries one out of every four couples is suffering from infertility (3). In general, male factor, female factor, joint factor and unknown factor account for 30-40%, 40-50%, 10-20% and 5-10% of infertility cases, respectively (4). Infertility is observed in 10-15% of American couples (5).

Several studies have been conducted on infertile couples in Iran. In an epidemiological study in 2004, the prevalence of infertility was reported 24.9% in Iranian women of 19 to 49 years old (6). According to the statistics presented by Iran's Ministry of Health and Medical Education in 2009, the prevalence of infertility was estimated to be 20.2% in Iran (7). Sexual dysfunctions comprise a heterogeneous group of disorders that are typically characterized by a clinically significant disturbance in a person's ability to respond sexually or to experience sexual pleasure (8). Sexual problems are highly prevalent in women. In the United States, approximately 40% of women have sexual concerns and 12% report distressing sexual problems. Female sexual dysfunction takes different forms, including lack of sexual desire, impaired arousal, inability to achieve orgasm, pain with sexual activity, or a combination of these issues (9).

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The most important causes of sexual dysfunction in women include lack of desire, excitement disorders, lack of orgasm, dyspareunia and vaginism. The prevalence rates of sexual dysfunction are 40 and 19.6% in the US and Sweden respectively (2). Since an active and effective sexual contact can increase the chance of fertility, it is believed that sexual dysfunction has a more marked impact on infertile women rather than fertile women (10). Sexual dysfunction is associated with infertility. It is caused by the reduction or lack of sexual activity. Sexual dysfunction is usually created or intensified during the diagnosis and treatment of infertility. In fact, sexual dysfunction is the silent partner of infertility. It prevents effectiveness treatment of infertility (11).

Sexual behavior disorders can be a prior cause of infertility. Generally, infertile couples seek treatment for infertility instead of searching for deeper problem in their sexual relationships (12). It was reported that many infertile women suffer from one of the different types of sexual dysfunction (13). Many factors can influence sexual dysfunction. The physical factors include the ages of husband and wife, BMI, physical activities, etc. The mental-emotional factors include feelings for a sexual partner, sexual self-efficacy, self-confidence, the mental image of body, the feeling of sexual attraction, etc. Finally, the social factors include the educational levels of a women and her husband, occupation, duration of marriage, the quality of spousal relationships, socioeconomic status, substances abuse, etc. (14). Many couples believe that conception is the only result of a sexual relationships; otherwise, this relationship is fruitless (15).

Since fertility is regarded as a value in Iranian culture and notions, infertility questions the individual and social competencies of women, i.e. the feelings of maternity and spousal values); therefore, a women's entire marital and sexual relationships with her husband will be affected. Given the importance of identifying the factors influencing sexual dysfunction in infertile women, the current study employed a modern approach using statistical models to investigate the subject. This study aimed to identify the above-mentioned factors to make necessary decisions and perform efficient interventions to improve the sexual health of infertile women. This study investigated the factors influencing sexual dysfunction in infertile women in Mashhad, Iran.

Materials and Methods

In this cross-sectional study, type I error (Z_{α}=1.96) and the statistical power (Z_{β}=1.28) were taken into account to select 85 infertile women as the population sample. The following formula was used for sampling:

$$n \geq 2\frac{(z_{\alpha}+z_{\beta})^2\sigma^2}{(\mu_1-\mu_2)^2}$$

The convenience sampling method was employed to select the participants from the women visiting Montasarieh Infertility Clinic and Research Center in Mashhad, Iran. The Ethics Committee of Shahid Beheshti University of Medical Sciences approved this study (approval No. 2881/116). All participants received information about the purpose of this study and gave their verbal informed consent to participate.

The participants were aged between 20 and 45 years old. Participants, did not suffer from any physical, mental, or medical problems. They were not addicted to any substances or alcohol. The information collection tools included an informational form with demographic and infertility information and Female Sexual Function Index (FSFI) and at demographic variables included age, mental and physical status, surgery history, medication and drugs, having a child or foster child. Infertility variables were duration of infertility and treatment, cause of infertility, kind of treatment, hope for cure, cost of treatment, and information about sexual relationship sexual. FSFI is one of the gold standard for female sexual dysfunction (FSD) assessment and in this study, the nominal and content validities of FSFI were re-evaluated by obstetricians and gynecologists, psychologists, midwives, and health education experts.

The reliability of this index was determined 0.9 through a retest. The researcher obtained the information from participants individually. The standard FSFI includes 19 items regarding sexual desire, sexual excitement, dyspareunia, and the inability to reach orgasm. Each item has five choices. According to the scoring system, a score below 28 indicates a poor sexual function, whereas a score between 28 and 36 shows a desirable sexual function (9). Sexual self-efficacy questionnaire developed based on Schwarzer's General Self-Efficacy Scale includes 10 items. Each item has three choices. Following completion of the questionnaire, a score of -0-10- was considered low, -10-20- moderate and -20-30- high efficacy (16).

ENRICH Marital Satisfaction Scale includes 47 items regarding idealistic distortion, marital satisfaction, personality issues, communication, conflict resolution, etc & with 5-Likert scale. According to the scoring system, a score below 30 indicates very poor satisfaction, -30-40- poor satisfaction, -40-60- moderate satisfaction, -60-70- high satisfaction and above 70 very high satisfaction (17). Then the descriptive-analytical tests were used to analyze the results. Finally, the logistic regression method was employed to investigate the relation between influencing and predicting factors and sexual function in infertile women, because sexual function possesses qualitative variables with two state (undesirable=0 when sexual function less than 28 according FSFI and desirable=1 when sexual function is between 36-28).

Results

Infertile women were investigated with respect to demographic characteristics (Table 1). The mean age of infertile women was 31.18 ± 5.56 years old, and 32% of them were aged between 26 and 30 years old. The cause of infertility was the female factor in 28 participants (32.9%), the male factor in 24 participants (28.3%), joint factor in 15 participants (17.6%), and unknown factor in 15 participants (17.6%). Moreover, three of them (3.5%) visited the clinic for the first time. The average duration of diagnosed infertility was 6.02 ± 4.47 years, and the average duration of infertility treatment was 4.11 ± 4.41. Furthermore, 54.1% of infertile women reported

high levels of marital satisfaction, and 60% of them (51 participants) reported medium self-efficacy in their sexual relationships. 71.8% of infertile women show poor sexual performances (Table 2).

Table 1: Distribution of some demographic variables among infertile women

Variable	n (%)
Education	
Primary education	23 (34.0)
High school	25 (29.4)
Higher education	31 (36.7)
Husband education	
Primary education	31 (36.55)
High school	26 (30.6)
Higher education	28 (32.9)
Job	
Housewife	60 (70.6)
Practitioner	25 (29.4)
Income	
Less than 1,700,000,0 Rials	22 (25.9)
Equal to 1,700,000,0 Rials	62 (72.9)
More than 1,700,000,0 Rials	1 (1.2)

Table 2: "Number and percent" of sexual function among infertile women

Variable	n (%) or mean + SD
Sexual function desirable	24 (28.2)
Sexual function undesirable	61 (71.8)
Total score of sexual function	25.93 ± 4.32

Table 3 indicated some infertility information. Based on the logistic regression model, the following variables influenced social function: sexual self-efficacy, sexual satisfaction, marital satisfaction, couple satisfaction with spouse appearance, and the high costs of infertility treatment (Table 4).

Table 3: Distribution of some infertility information

Variable	n (%)
Using of ART	
Yes	67 (78.8)
No	18 (21.2)
ART	
IUI	25 (30.6)
IVF	21 (24.7)
Ovulation and intercourse	20 (23.5)
None	18 (21.2)
Hope for a cure	
Yes	57 (67)
Somewhat	26 (30.6)
No	2 (2.4)
The cost problem	
Yes	58 (68.2)
Somewhat	21 (24.7)
No	6 (7.1)

ART; Assisted reproductive technology, IUI; Intrauterine inseminations, and IVF; *In vitro* fertilization.

Table 4: Regression logistic of related factors of sexual function

Related factor	OR (CI)	P value
low	13.77 (1.74-10.89)	0.01
Average	4.16 (2.35-7.33)	0.00
High	1	-
No	3.78 (2.19-6.51)	0.00
Yes	1	-
Sexual satisfaction		
No	5.52 (2.66-11.43)	0.00
Yes	1	-
Primary education	3.42 (1.70-6.94)	0.00
High school	1.89 (1.04-3.41)	0.00
After high school	1	-
Primary education	2.95 (1.40-6.20)	0.01
High school	3.12 (1.73-5.61)	0.00
After high school	1	-
Less than 1,700,000,0 Rials	10.31 (3.03-34.99)	0.00
Equal to 1,700,000,0 Rials	4.20 (1.56-11.31)	0.00
More than 1,700.000.0 Rials	-	-
No	5.14 (1.50-17.64)	0.00
Yes	1	-
No	4.67 (1.75-12.44)	0.00
Yes	1	
Yes	2.66 (0.39-17.91)	0.31
Somewhat	8.54 (1.38-52.73)	0.01
No	1	

OR; Odds ratio and CI; Confidence interval

Discussion

This research indicated that 71.8% of infertile women were suffering from sexual dysfunction. Regardless of the duration and causes, infertility causes mental health and sexual problems and infertile women suffer from these conditions more than fertile women (16). Previous studies indicate that sexual self-efficacy is related to sexual function, as individuals with a poor sexual self-efficacy and individuals with average sexual self-efficacy face problems in sexual function fourteen times and five times more than individuals with high sexual self-efficacy, respectively.

In 2013, Champion and et al. (17) conducted a study entitled sexual self-efficacy and marital satisfaction on 194 university students. They showed that sexual satisfaction referred to an individual's pleasure from the type of sexual relationships. Also, the concept of marital-sexual satisfaction depends on an individual's perception of self-efficacy whether as sexual activity satisfaction or emotional satisfaction. According to another study, self-efficacy and self-confidence should increase in sexual issues to have better and healthier sexual functions (18). Previous investigations indicated that marital satisfaction was significantly related to sexual function. In other words, sexual satisfaction can result in fewer complaints made by women with sexual

disorders (19). In fact, a sexual partner's behavior, sexual adequacy, and martial life status influence sexual function.

Women having happy and exciting relationships with their husbands experience sexual disorders less often. They feel more self-confident and think that their husbands like their bodies; therefore, they feel that they are more sexually attractive to their husbands. On the other hand, women having negative attitudes towards their bodies are nervous in private and romantic relationships with their husbands. Such women are not sure about having sexual activities (20-22). The results of this study indicate that satisfaction with spouse appearance improves sexual function by five times in both men and women. Likewise, Kalra et al. (23) stated that the shape of body is one of the factors influencing the emergence of sexual dysfunction. Moreover, this study indicated a relation between educational attainment and sexual function. As individuals with high school diplomas or lower educations face sexual dysfunction problems three times more than the individuals with higher educations.

Fajewonyomi et al. (24) indicated that women with higher educations would face sexual dysfunction less often. In fact, higher educational attainments increase the chance that individuals can speak about their sexual problems or their spouses. Training and high educational attainments are necessary to have desirable and normal sexual activities (25).

Income is another effective factor (22). To confirm this statement, the results of this study showed that low-income individuals face sexual problems four times more than high-income individuals. Moreover, Audu (26) indicated that income would influence sexual function. Also, Cayan et al. (27) suggested low income as a risk factor for the emergence of sexual dysfunctions. Difficulty in providing the costs of infertility treatment increases the chance of sexual dysfunction by nine times. Similarly, Mollaiy nezhad et al. (28) stated that sexual dysfunction was significantly related to the duration of infertility treatment, treatment costs, the number of unsuccessful pregnancies, and the hope for successful treatment. Likewise, Noorani et al. (29) and Karamidehkordi and Latifnejad Roudsari (30) showed that infertile women whose husbands helped them during treatment and covered the costs, experienced better marital and sexual satisfaction. One of our research limitations was inclusion of women with primary infertility. In addition, the infertile women were selected from only one infertility clinic in Mashhad, we used self-report scales and clinician rated psychological parameters.

Conclusion

The finding indicated that some factors such as sexual self-efficacy, marital satisfaction, sexual satisfaction, education, cost of infertility treatment are associated with sexual function in infertile women.

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Author's Contributions

S.A., G.O., H.A.M.; Contributed to conception and design. S.A.; Contributed to all experimental work, data collection and data statistical analysis, and interpretation of data. G.O.; Was responsible for overall supervision. S.A.; Drafted the manuscript, which was revised by her. All authors read and approved the final manuscript.

References

1. Fitz MA, Speroff L. Clinical gynecologic endocrinology and infertility. 7th ed. Lippincott Philadelphia: Lippincott Williams and Wilkins; 2011; 1112-1186.
2. Novak E, Berek SJ. Berek & Novak's gynecology. 15th ed. London: Lippincott Williams and Wilkins; 2012; 1028-1040.
3. Chandra A, Copen CE, Stephen EH. Infertility and impaired fecundity in the United States, 1982-2010: data from the National Survey of Family Growth. Natl Health Stat Report. 2013; (67): 1-18.
4. Nourani S, Jonaidy E, Shakeri MT, Mokhber N. Sexual Satisfaction in fertile and infertile women attending state clinics in Mashhad. J Reprod Infertil. 2010; 10(4): 269-277.
5. Millheiser LS, Helmer AE, Quintro RB, Westphal LM, Milki AA, Lathi RB. Is infertility a risk factor for female sexual dysfunction? A case-control study. Fertil Steril. 2010; 94(6): 2022-2025.
6. Goshtasbi A, Vahdaninia M, Rahimi forooshani A. Prevalence and potential risk factors of female sexual difficulties: an urban Iranian population-based study. J Sex Med. 2009; 6(11): 2988-2996.
7. Jones D, Bagga R, Nehra R, Deepika, Sethi S, Walia K, et al. Reducing sexual risk behavior among high-risk couples in Northern India. Int J Behav Med. 2013; 20(3): 344-354.
8. Sadock BJ, Kaplan HI, Sadock VA. Kaplan and Sadock comprehensive textbook of psychiatry. 11th ed. Philadelphia: Lippincott Williams and Wilkins; 2009; 287-294.
9. West SL, Vinikoor LC, Zolnoun D. A systematic review of the literature on female sexual dysfunction prevalence and predictors. Annu Rev Sex Res. 2004; 15: 40-172.
10. Gibson DM, Myers JE. Gender and infertility: a relational approach to counseling women. J Couns Dev. 2000; 78(4): 400-410.
11. Cuijpers P, Sijbrandij M, Koole SL, Andersson G, Beekman AT, Reynolds CF 3rd. The efficacy of psychotherapy and pharmacotherapy in treating depressive and anxiety disorders: a meta-analysis of direct comparisons. World Psychiatry. 2013; 12(2): 137-148.
12. Read J. Sexual problems associated with infertility, pregnancy, and ageing. BMJ. 2004; 329(7465): 559-561.
13. Hock RR. Human sexuality. 3rd ed. UK: Mandocino College; 2010; 696.
14. Khademi A, Alleyasin A, Amini M, Ghaemi M. Evaluation of sexual dysfunction prevalence in infertile couples. J Sex Med. 2007; 5(6): 1402-1410.
15. Nazarpour S, Simbar M, Tehrani FR. Factors affecting sexual function during menopause: a review of the literature. Taiwan J Obstet Gynecol. 2016; 55(4): 480-487.
16. Cousineau TM, Green TC, Corsini EA, Barnard T, Seibring AR, Domar AD. Development and validation of the infertility self-efficacy scale. Fertil Steril. 2006; 85(6): 1684-1696.
17. Champion VL, Ziner KW, Monahan PO, Stump TE, Cella D, Smith LG, et al. Development and psychometric testing of a breast cancer survivor self-efficacy scale. Oncol Nurs Forum. 2013; 40(6): E403-E410.
18. Kudo Y. Effectiveness of a condom use educational program developed on the basis of the information-motivation-behavioral skills model. Jpn J Nurs Sci. 2013; 10(1): 24-40.
19. Rostosky SS, Dekhtyar O, Cupp PK, Anderman EM. Sexual self-concept and sexual self-efficacy in adolescents: a possible clue to promoting sexual health? J Sex Res. 2008; 45(4): 277-286.
20. Safarinejad MR. Infertility among couples in a population-based study in Iran: prevalence and associated risk factors. Int J Androl. 2008; 31(3): 303-314.
21. Jamali S, Mosalanejad L. Sexual dysfnction in Iranian pregnant

women. Iran J Reprod Med. 2013; 11(6): 479-486.
22. Meltzer AL, Mcnulty JK. Body image and marital satisfaction: Evidence for the mediating role of sexual frequency and sexual satisfaction. J Fam Psych. 2010; 24(2): 156.
23. Kalra G, Tandon A, Sathyanarayana Rao TS. Sexual disorders in Asians: a review. Asian J Psychiatr. 2014; 7(1): 80-82.
24. Fajewonyomi BA, Orji EO, Adeyemo AO. Sexual dysfunction among female patients of reproductive age in a hospital setting in Nigeria. J Health Popul Nutr. 2007; 25(1): 101-106.
25. Ramezani N, Dolatian M, Shams J, Alavi H. The relationship between self-esteem and sexual dysfunction and satisfaction in women. AMUJ. 2012; 14(6): 57-65.
26. Audu BM. Sexual dysfunction among infertile Nigerian women. J Obstet Gynaecol. 2002; 22(6): 655-657.
27. Cayan S, Akbay E, Bozlu M, Canpolat B, Acar D, Ulusoy E. The prevalence of female sexual dysfunction and potential risk factors that may impair sexual function in Turkish women. Urol Int. 2004; 72(1): 52-57.
28. Mollaiy nezhad M, Jaafarpour M, Jahanfar Sh, Jamshidi R. Infertility related stress and marital life in Iranian infertile women who referred to Isfahan infertility treatment clinics. J Reprod Infertil. 2001; 2(1): 26-39.
29. Noorani Sh, Jonaidy E, Shakeri MT, Mokhber N. Comparing the marital satisfaction in infertile and fertile women referred to the public clinic in Mashhad. J Obstet Gynecol Infertil. 2009; 15(7): 24-31.
30. Karamidehkordi A, Latifnejad Roudsari R. Body image and its relationship with sexual function and marital adjustment in infertile women. Iran J Nurs Midwifery Res. 2014; 19(7 Suppl 1): S51-S58.

Original Article

The Persian Version of Fertility Adjustment Scale: Psychometric Properties

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Abstract

Background: Infertility is a common clinical problem. Psychological adjustment to infertility refers to changing the viewpoint and attitude of an infertile person toward infertility problems, treatments and possible outcomes. The present study aims to prepare a valid and reliable scale for assessing the psychological adjustment to infertility, by determining the cultural adaptation, validity and reliability of the Persian version of the Fertility Adjustment Scale.

Materials and Methods: This is a cross-sectional study performed to localize and validate the Fertility Adjustment Scale, in which 40 infertile women and 40 healthy subjects (fertile or having children) were detected by a gynecologist and the subjects who completed the Fertility Adjustment Scale (FAS) questionnaire were recruited. This study had four steps: in the first step, the literature was reviewed, in the second step, the scale was translated, in the third step, the content and construct validity indicators were calculated, and in the fourth step, reliability of the scale was validated.

Results: The mean (± SE and range) of fertility adjustment total scores in the infertile group and the control group were 43.2 (1.2 and 27-57) and 42.3 (1.5 and 18-57), respectively (P=0.623). The content validity was good according to Content Validity Index score (0.7-0.8). A two-component structure was extracted from factor analysis which approximately justifies 52.0% of the cumulative variations. A Cronbach's alpha value of 0.68 showed moderate reliability.

Conclusion: The results of this study revealed that the infertility adjustment scale is a useful tool for the analysis of psychological reactions towards infertility problems and evaluation of the consequences of treating this social-clinical problem.

Keywords: Adjustment, Fertility, Infertility, Iran, Psychometrics

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Introduction

As a natural stage of life and one of the most important aims of each marital bond, fertility, reproduction and breeding are considered the basis of human survival (1, 2). Fertility is a physiological process in living creatures, which also involves social and mental dimensions of human life (2). Infertility is defined as the inability to get pregnant after one year or more of regular unprotected sexual intercourse during the menstrual fertility cycles, without using contraceptive methods (1, 3, 4).

Infertility is a common clinical problem which, as estimated by the World Health Organization (WHO), has affected about 60 to 80 million couples all over the world and its prevalence is estimated to be around 10 to 15%, worldwide. Meanwhile, this problem is more prevalent in developing countries (1, 5-7). A meta-analysis on the prevalence of infertility in Iran showed that its prevalence was about 13.2% in Iran, the lowest rate being 2.8% in 2001, and the highest being 24.9% in 2010 (8). Also, a study in 2015 found a prevalence of 17.3% for primary infertility in Iranian couples (9).

Having the features of a traumatic event including the length of time, complicated condition, unpredictability and uncontrollability, infertility creates a full-scale crisis in the lives of infertile couples, and has been described as a global health problem with physical, mental and social dimensions (6, 10).

The person who is not able to have children or experience the natural reproduction process is called "infertile" and this may trigger psychological problems especially in the Iranian culture where parents and relatives have a key role in the couple's life; under this condition, infertility can be considered as one of the worst experiences of life (6, 7, 10). Infertile people experience depression, grief, fear, inefficiency, lack of control, and high levels of anxiety and guilt, and they are concerned about their body and sexual function disorders all of which are the symptoms of lack of fertility problems adjustment (6, 11, 12).

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Most Iranian infertile couples face a type of stigma and consider themselves as a misfit in the community due to the inability to have their own children (13, 14). Sultan and Tahir (7) studied the psychological consequences of infertility in 400 couples (200 fertile and 200 infertile couples), samples were randomly selected from different cities in Pakistan. The results showed that fertile couples have lower levels of depression, aggression and anxiety, but higher levels of self-esteem and marital satisfaction than infertile couples. Since most infertile couples, especially those under-treatment, are reported to have psychological problems, the couples' psychological problems should also be considered in medical treatments and interventions (13, 15).

Adjustment to infertility refers to changing the viewpoint and attitude of an infertile person towards infertility problems, treatments and possible outcomes. Fertility Adjustment Scale (FAS) was introduced by Glover et al. (14) to evaluate the psychological adjustment to fertility. The results showed satisfactory reliability and validity of the scale. In addition, Arslan and Okumuş (16) localized a Turkish version of the fertility adjustment scale. The study was carried out on 240 women with infertility who referred to the infertility center of a hospital in Turkey.

In this scale, adjustment is defined as an appropriate management of behavioral, mental and emotional responses to infertility (11, 14). Adjustment does not mean that the couple do not have the desire to have children anymore or to accept their current situation, rather it reflects the extent to which the couple are able to cognitively, emotionally and behaviorally process the possibilities of having or not having children and how to get prepared for both situations (14).

After evaluation of the validity and reliability of this scale, it has been used for investigating the psychological consequences of infertility treatment in infertile couples (11, 13, 15). To the best of our knowledge, despite the importance of adjustment to fertility, no study has been conducted in this regard in Iran. Hence, the present study aims to prepare a valid and reliable scale for assessing the psychological adjustment to infertility, by determining the psychometric properties of the Persian version of FAS.

Materials and Methods

This is a cross-sectional study conducted on 40 infertile and 40 fertile women (with or without children) in Birjand, east of Iran from November 2016 to January 2017. Fertility/infertility was diagnosed by a gynecologist, and the participants were selected from the available subjects who completed the FAS.

Given that there has been no similar study in Iran, and the desirable conditions for conducting the pilot study were not known, hence the sample size was estimated with a sensitivity of 0.85 and a specificity of 0.70. Also, as there was no infertility center in the city of Birjand, the patients and healthy subjects (women with a history of having children) were selected from those referring to obstetricians' clinics. The inclusion criteria involved being infertile, completion of the informed consent for participation, being within the age range of 18-45 years and having the ability to read and write. According to previous studies (17, 18), there were four steps. In the first step, the literature was reviewed, in the second, the tool was translated, in the third step, content and construct validity indicators were calculated and in the fourth step, the reliability of the tool was evaluated.

Review of the literature

In the present research the terms, adjustment, fertility and infertility were selected as the search keywords. Documents were obtained from scientific databases such as PubMed, Science Direct, Medline, Embase, Scopus and Google Scholar as well as Persian electronic resources namely, SID, Irandoc, Iran-Medex and Magiran. One of the main aims of this step was to examine the possibility of existence of a Persian version of this scale, but no such version was found.

Translation of Fertility Adjustment Scale

Translation and back-translation method was used in this step (19). For this purpose, the questionnaire was first translated into Persian (Farsi) independently by an English-language expert and a nursing and midwifery expert fluent in English. Afterwards, the translated versions were reviewed by two nursing and midwifery experts, and then the Persian version was translated into English by an English-language expert and a nursing and midwifery expert fluent in English. Finally, all experts reviewed and approved the compliance of the Persian version with the original one.

Calculation of content and construct validity

In order to evaluate the face and content validity of the tool, the translated draft was handed to 2 obstetricians, 3 nursing experts and 4 gynecologists working in the Faculty of Medicine and Faculty of Nursing and Midwifery. In order to determine the content validity of the questions of the above mentioned questionnaire, the experts group was asked to judge the suitability of every question based on a 3-point Likert scale. Then, using the face and content validity indicators, the beneficial questions were selected. At this point, the content validity ratio (CVR) was calculated for each statement by the following equation (20).

$$CVR = \frac{n_e - \frac{N}{2}}{\frac{N}{2}}$$

Where, n_e is the number of the experts who considered the question as necessary and N is the total number of the

experts. The obtained value for each question was then compared with the Lawshe Table criterion for 9 experts which equaled to 0.77 (21). Some of the statements and phrases were modified, and in order to have a better evaluation of the final version regarding the difficulty level, 10 questionnaires were given to infertile women, and no particular problem was observed. The Exploratory Factor Analysis (EFA) method was used in order to assess the construct validity. Given that the questionnaire was translated for the first time in Iran and the original designer did not use exploratory factor analysis to present basic model, we used EFA and believed that next studies on this questionnaire in Iran need to perform CFA based on our findings.

EFA was utilized for evaluating the presence of possible subscales and the construct validity, using the principal component analysis and varimax orthogonal rotation. In this procedure, before performing the exploratory factor analysis, the Kaiser-Meyer-Olkin (KMO) index was evaluated and Bartlett test was done. The EFA can be performed if the KMO index is >0.5 and the Bartlett test P value is <0.05. In addition, the explained variance indicators (>0.6), eigenvalues (>1) and rotated factor loadings (>0.4) were used for selecting the components (22).

Reliability of the tool

Reliability of the questionnaire in terms of internal consistency was assessed using Cronbach's alpha coefficient. This index ranges from 0 to 1, and values close to 1 indicate better reliability. Cronbach's alpha of more than >0.7 reflects a good internal reliability (23).

Fertility adjustment questionnaire details

Fertility adjustment questionnaire was developed in 1999 by Glover et al. (14). It contains 12 questions to which the participants respond and is evaluated using a 6-point Likert scale (strongly agree=6 and strongly disagree=1). Statements with positive aspects (2, 4, 6, 9, 10, 12) were inversely coded. The minimum score is 12 and the maximum score is 72, and the fertility adjustment total score is the sum of scores. A high score means low adjustment level. The internal consistency of the scale has been confirmed with a Cronbach's alpha coefficient value of 0.85. The split half reliability of the scale was approved with a correlation coefficient of 0.68 and Guttman coefficient of 0.8. Reliability of the scale was also approved by test-retest method with a correlation coefficient of 0.88. At the same time, validity of the scale was confirmed by evaluation of the correlation between the scale scores and the scores of the hospital anxiety (r=0.43) and depression (r=0.49) scale.

Demographic data

The demographic information checklist included the following variables: age of patients, job of patients, length of marriage, length of infertility, length of treatment, age of mates and job of mates. These variables were compared between the two fertile and infertile groups, using Chi-square and Mann-Whitney tests.

Ethical considerations

The Ethics Committee of Birjand University of Medical Sciences approved the present study (approval No. IR.BUMS.REC.1395.210). Afterwards, written informed consent was obtained from each patient, showing that the participants were recruited voluntarily and with full knowledge and could quit the study at any time and this would not have an impact on their treatment process. Moreover, they were informed that the information will be reported in a general manner, without revealing the patients' personal information. Also, the written authorization was obtained from Glover et al. (14) who invented the questionnaires for the first time.

Statistical analysis

The collected data were entered into the SPSS software, version 18 (SPSS, Inc., Chicago, IL, USA). The mean, standard deviation, percentage and indicators of reliability and validity were assessed. Moreover, normality of the demographic variables was examined, using Kolmogorov-Smirnov test, and non-parametric tests were used for comparing the two groups.

Results

Demographic information

A total of 80 patients divided into two groups of 40 fertile or infertile subjects who completed the FAS questionnaire. The mean (± SD) of demographic and clinical variables is reported in Table 1.

Table 1: Demographic and clinical variables

Variable	Infertile n=40 Mean (SD)	Fertile n=40 Mean (SD)	P value
0.487	27.6 (1.0)	28.6 (1.0)	Age of patients (Y)
0.592	31.4 (0.9)	32.1 (0.8)	Age of mates (Y)
0.903	5.3 (0.8)	5.5 (0.6)	Length of marriage (Y)
--	--	3.2 (0.4)	Length of infertility (Y)
--	--	18.7 (3.3)	Lenght of treatment (months)
	n (%)	n (%)	Job of patients
0.020	25 (62.5)	34 (85.0)	Housewife
	6 (15.0)	5 (12.5)	Employee
	9 (22.5)	1 (2.5)	University student
			Job of mates
0.116	17 (42.5)	9 (22.5)	Employee
	21 (52.5)	24 (60.0)	Self-employed
	2 (5.0)	7 (17.5)	Worker

Table 2: Explanatory factor analysis

Item number and descriptor	Factor loading	Eigenvalue	Percentage of variance	Cumulative (%)
Factor 1				
5. I have made plans for a possible future life without a child*	0.72	2.80	23.55	23.55
6. I will always feel unfulfilled if I am unable to have my own child	0.64			
7. I think I could adjust to a future life without a child*	0.66			
8. I am sure that I can continue my normal life activities*	0.76			
10. I think life could be rewarding either with or without children*	0.69			
Factor 2				
1. I will continue with investigations/treatment until I succeed in having a child	0.73	1.3	16.62	40.17
3. I cannot plan for the future until I know for certain whether or not I can have a child	0.40			
4. I want a child of my own more than anything else in life	0.72			
Factor 3				
2. There are both advantages and disadvantages to having a child*	0.82	1.1	11.80	52.06
9. I cannot imagine a future without a child	0.51			

Content and construct validity

Content validity was examined using CVR and the obtained values for the questions were in the range of 0.7 to 0.8. Construct validity was assessed using EFA. The results showed that the KMO index was about 0.68, Bartlett's Chi-Square test result was 126.0, and the P value was less than 0.001. The results indicated the sufficiency of samples to perform this procedure. A total of 3 eigenvalues was more than 1, which justifies approximately 52.0% of the cumulative variations. Since the orthogonal varimax rotation method was utilized, factors with non-shared components were identified. All factor loadings were greater than 0.3. The first factor involved questions 5, 6, 7, 8 and 10; the second factor involved questions 1, 3 and 4; and the third factor involved questions 2 and 9, as explained in Table 2. The correlation coefficient between the first factor and the second and third factors were 0.12 and -0.02, respectively, and the correlation coefficient between the second and the third factors was -0.01. Spearman's correlation coefficient showed no significant relationship (Table 2).

Reliability

Reliability of the tool was evaluated using two methods. First, the correlation between the statements and the total score was evaluated and the statements with low insignificant correlations were excluded. In the second method, a Cronbach's alpha coefficient value was utilized to show the internal consistency of the tool. According to the first method, statement 7 had low correlation (0.14) with the fertility adjustment total score which was not statistically significant. Besides, Cronbach's alpha coefficient value for the questionnaire was 0.62, which was promoted to 0.65 after eliminating statement 7. Following the elimination of statement 4, Cronbach's alpha value increased to 0.68, which is close to the 0.7 criteria.

Fertility Adjustment Total Score

The mean (± SE and range) of fertility adjustment total scores in the infertile group and the control group were 43.2 (1.2 and 27-57) and 42.3 (1.5 and 18-57), respectively (P=0.623). Also, the mean (SD) of each of the statements for the infertile group is reported in Table 3. In the infertile women group, no significant correlation was observed between the fertility adjustment total score and the age, age of the mate, length of marriage, length of infertility (year), and length of treatment (month). Furthermore, no relationship was observed between the fertility adjustment total score and the job of patients and job of mates (Table 4).

Table 3: Mean ± SE item scores and item-to-total correlations for the Total score of Fertility Adjustment Scale

Item	Mean (SE)	Item to total correlation
1. I will continue with investigations/ treatment until I succeed in having a child	5.7 (0.1)	0.40
2. There are both advantages and disadvantages to having a child*	2.6 (0.2)	0.26
3. I cannot plan for the future until I know for certain whether or not I can have a child	3.8 (0.3)	0.43
4. I want a child of my own more than anything else in life	5.3 (0.2)	0.44
5. I have made plans for a possible future life without a child*	4.8 (0.2)	0.43
6. I will always feel unfulfilled if I am unable to have my own child	4.7 (0.2)	0.76
7. I think I could adjust to a future life without a child*	4.9 (0.2)	0.52
8. I make sure that I carry on with my normal life activities*	3.8 (0.3)	0.70
9. I cannot imagine a future without a child	4.4 (0.3)	0.61
10. I think life could be rewarding either with or without children*	2.8 (0.3)	0.63

*; Reverse-scored

Table 4: Correlation of demographic and clinical variables with Total score of Fertility Adjustment Scale

Item	Correlation coefficient	P value
Age of patient	-0.11	0.325
Job of patient	-0.06	0.548
Age of husband	-0.12	0.275
Job of husband	0.008	0.941
Marriage time	-0.03	0.782
Infertility time	0.12	0.447
Treatment time	0.10	0.520

Discussion

Infertility creates a full-scale crisis in infertile couples lives, and it has been described as a global health problem with physical, mental and social dimensions. Having a tool for evaluation of the maladjustment of spouses with respect to pregnancy is of great importance. This useful tool can be used to measure the maladjustment of infertile women or men (14). The results of the present study showed that family adjustment tool along with the remaining questions can be a trustworthy scale for measurement of infertility in in Iranian population. For attaining adjustment, a change in the patients' behavior, emotion and recognition of their position should be made. For some patients, passing of time, studying and treatment accelerate the adjustment, while for some other patients hoping to have a baby prolongs the patients' adjustment process.

Evaluation of the correlation of each item with the total adjustment score using Cronbach's alpha coefficient showed that by eliminating the two questions of "I cannot talk to my husband about the possibility of not having a baby" and "I feel like I am losing my life month by month", the reliability of the tool reached the acceptable level of 0.68 and finally we had a 10-question 3-component reliable scale for measuring the adjustment to infertility. Arsalan and OKUMUŞ (16) indicated two components for Turkish version of FAS with 10 questions by factor analysis method with Cronbach's alpha values of 0.80 and 0.71. In addition, Glover et al. (14) as the original designer, presented a 12-question FAS with a Cronbach's alpha value of 0.85 without performing EFA method.

Due to the time constraints and lack of sufficient human resources, it was not possible to collect data from all patients. Almost all participants in this study completed the questionnaire. The subjects were recruited from patients referring to specialists' offices; hence, it can be said that our subjects formed a representative sample.

Considering the problems faced by infertile couples and the Iranian culture unique features on the issue, nowadays, the consequences of infertility have attracted special attentions, and tools like FAS can be useful for evaluating the psychological problems caused by infertility. Based on the obtained CVR for different items and the conducted exploratory factor analysis, this tool can be considered a valid scale for screening individuals referring to clinics for receiving psychological counseling on infertility problems. As a clinical tool, it can be the starting point for the couples' psychotherapy sessions, which somewhat specifies the way they look at the infertility issue.

In this pilot study, the infertility adjustment tool was given to 80 patients in two groups of 40 fertile and 40 infertile women. The results showed that in the infertile women group, no significant correlation was observed between the fertility adjustment total score and the patient's age, age of the mate, length of marriage, length of infertility (year), and length of treatment (month). Furthermore, no relationship was observed between the fertility adjustment total score and the job of patients and job of mates. Lack of relationship between this score and the mentioned demographic variables indicated that this scale is indeed a useful tool for measuring the psychological adjustment. Results were similar to those reported by Arsalan and OKUMUŞ (16).

Further clinical studies on larger population are needed to standardize the information obtained by using this tool in different medical centers. It is suggested that this questionnaire should be used in infertility treatment centers as the first step to provide the couple's with a better understanding of each other's point of view, and also an initial screening for the physician and health care team to understand psychological status of patients.

Conclusion

The results of this study revealed that Persian infertility adjustment scale is an appropriate tool for the analysis of psychological reactions towards infertility problems and the consequences of treating this social-clinical problem.

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Author's Contributions

M.T., S.A.V., M.H.; Contributed to conception and design. A.T., N.B.; Contributed to data collection. M.H., S.A.V.; Contributed to statistical analysis, and interpretation of data and were responsible for overall supervision. M.H.; Drafted the manuscript, which was revised by M.T. and S.A.V. All authors read and approved the final manuscript.

References

1. Bahrami N, Sattarzadeh N, Ranjbar Koochaksariie F, Ghojazadeh M. Comparing depression and sexual satisfaction in fertile and infertile couples. J Reprod Infertil. 2007; 8(30): 52-59.
2. Goli M, Ahmadi SM, Goli Sh. Irrational perception of parenthood in infertile women. Journal of Research in Behavioural Sciences.

2012; 10(3): 213-220.
3. Hosseini J, Emadedin M, Mokhtarpour H, Sorani M. Prevalence of primary and secondary infertility in four selected provinces in Iran, 2010-2011. Iranian Journal of Obstetrics, Gynecology and Infertility. 2012; 15(29): 38.
4. Ying LY, Wu LH, Loke AY. Gender differences in experiences with and adjustments to infertility: a literature review. Int J Nurs Stud. 2015; 52(10): 1640-1052.
5. Kamel RM. Management of the infertile couple: an evidence-based protocol. Reprod Biol Endocrinol. 2010; 8: 21
6. Molid HA, Varaghchi J. Psychological meta-analysis of the infertility researches in Iran. Iranian Journal of Obstetrics, Gynecology and Infertility. 2012; 15(13): 26-33.
7. Sultan S, Tahir A. Psychological consequences of infertility. Hellenic Journal of Psychology. 2011; 8: 229-47.
8. Direkvand Moghadam A, Delpisheh A, Sayehmiri K. The trend of infertility in Iran, an original review and meta-analysis. Nursing Practice Today. 2014; 1: 46-52.
9. Kazemijaliseh H, Ramezani Tehrani F, Behboudi-Gandevani S, Hosseinpanah F, Khalili D, Azizi F. The prevalence and causes of primary infertility in iran: a population-based study. Glob J Health Sci. 2015; 7(6): 226-232.
10. Tamannaifar M. A comparative study of mental health, marital adjustment and coping responses among fertile-infertile women. Clinical Psychology and Personality (Daneshvar Raftar). 2011; 3(4): 51-60.
11. Mahajan NN, Turnbull DA, Davies MJ, Jindal UN, Briggs NE, Taplin JE. Adjustment to infertility: the role of intrapersonal and interpersonal resources/vulnerabilities. Hum Reprod. 2009; 24(4): 906-912.
12. Ozkan B, Orhan E, Aktas N, Coskuner ER. Depression and sexual dysfunction in Turkish men diagnosed with infertility. Urology. 2015; 85(6): 1389-1393.
13. Ferreira M, Antunes L, Duarte J, Chaves C. Influence of infertility and fertility adjustment on marital satisfaction. Procedia-Social and Behavioral Sciences. 2015; 171: 96-103.
14. Glover L, Hunter M, Richards JM, Katz M, Abel PD. Development of the fertility adjustment scale. Fertil Steril. 1999; 72(4): 623-628.
15. Durning PE, Williams RS. Factors influencing expectations and fertility-related adjustment among women receiving infertility treatment. Fertil Steril. 2004; 82(2): S101.
16. Arslan İ, OKUMUŞ H. Psychometric properties of the Turkish version of the fertility adjustment scale. Turkiye Klinikleri J Nurs Sci. 2016; 8(3): 224-231.
17. McDowell I. Measuring health: a guide to rating scales and questionnaires. 3rd ed. NY: Oxford University Press; 2006.
18. Streiner DL, Norman GR, Cairney J. Health measurement scales: a practical guide to their development and use. 5th ed. USA: Oxford University Press; 2014.
19. Hilton A, Skrutkowski M. Translating instruments into other languages: development and testing processes. Cancer Nurs. 2002; 25(1): 1-7.
20. Polit DF, Beck CT, Owen SV. Is the CVI an acceptable indicator of content validity? Appraisal and recommendations. Res Nurs Health. 2007; 30(4): 459-467.
21. Lawshe CH. A quantitative approach to content validity. Personnel Psychology. 1975; 28(4): 563-575.
22. Watson R, Thompson DR. Use of factor analysis in Journal of Advanced Nursing: literature review. J Adv Nurs. 2006; 55(3): 330-341.
23. Polit DF, Beck CT. Essentials of nursing research: appraising evidence for nursing practice. 9th ed. USA: Lippincott Williams & Wilkins; 2010.

Original Article

Relationship between Health Literacy and Sexual Function and Sexual Satisfaction in Infertile Couples Referred to The Royan Institute

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Abstract

Background: Health science and technology today is a rapidly growing field. Health is a multifaceted concept influenced by several factors, and health literacy is essential to deal properly with the current situation. In this study, the association between health literacy and sexual function and sexual satisfaction were investigated in 2016.

Materials and Methods: This descriptive and correlational study was conducted on 193 couples in the Royan Institute, Tehran. Data collection instruments were three standard questionnaires which included the Test of Functional Health Literacy, the Female Sexual Function Index (FSFI) and the International Index of Erectile Function, and the Iranian version of the Sexual Satisfaction Scale. The data were analyzed using SPSS-v23 software at a significance level of 0.05.

Results: Marginal health literacy, 49.7% among men and 44.1% among women, was more common than adequate or inadequate health literacy. Erectile function for the majority of men was appropriate (53.3%), compared to 16.6% who had perfect function and 30.1% for whom function was less than appropriate. The majority of women (57.0%) had sexual dysfunction. One hundred and three (53.3%) men had appropriate sexual function and 57% of women had normal sexual function. The greater proportion of men (50.8%) and women (46.1%) had good, rather than very good or less than good, sexual satisfaction. The results of chi-square tests indicated that greater health literacy was associated with higher levels of sexual function and sexual satisfaction among men and women. However, application of the Cramer's V test indicates that the strength of these associations is moderate to weak.

Conclusion: Health literacy was marginal among most couples and its adverse impacts on sexual function and sexual satisfaction were confirmed. Accordingly, it is recommended that plans be developed to promote health literacy among infertile couples.

Keywords: Health Literacy, Infertility, Sexual Dysfunction, Sexual Satisfaction

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Introduction

Health literacy represents the capacity of a person to access, interpret, and apply health information as well as make decisions to use existing health services (1). This concept was used for the first time in the field of health education in 1974. In the first two decades after the development of this concept, little attention was paid to it. However, once it started to be used formally in health promotion texts from 1997, attention to the concept has increased (2). Health literacy is one of the components that determines an individual's health-related behaviors and likelihood of following treatment recommendations. Effective decisions about health are also affected by health literacy, and general health and the adoption of preventive behaviors depend on it (3, 4).

Several studies have been conducted to determine the effects of health literacy on different health outcomes. In this regard, we can refer to the impact of health literacy on quality of life, medication and adherence to clinical recommendations, taking advantage of contraceptive and emergency services, reducing the risk of mortality, utilization of services and knowledge related to asthma, the use of mammography and effective use of health information sources (5-11). This in turn can affect the quantity and quality of an individual's sexual relations and sexual satisfaction.

Good sexual function between couples includes regular willingness to participate in sexual acts, sexual arousal, and reaching orgasm and increase their marital satis-

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faction (12). Sexual satisfaction is dependent on mental state as well as attitudes to sexual relations and the level of pleasure derived (13). Sexual function and satisfaction also have physical and psychological dimensions. Sexual functioning and the quality of that functioning are affected not only by an individual's physical condition, but also by their mental state. Indeed a person's mental state may be the main component in determining sexual satisfaction (14). In this regard, results of a study conducted on 176 women with cervical cancer and a history of pelvic radiation therapy showed one third of them had sexual dysfunction due to their unfavorable physical condition and consequently psychological problems (15). Therefore, we can conceptually explain the relationship between health literacy and sexual satisfaction and function. Health literacy affects the behavior of individuals, how they make use of information and services, and their physical and mental condition (16). These variables affect people's sexual function and satisfaction.

Studies of health literacy among pregnant women in Tehran showed literacy to be adequate in 45.4%. The proportion of people with marginal and inadequate health literacy was respectively 24.6 and 30% (3). In addition, results of a study which conducted on 525 adults in Isfahan showed that 46.5 percent of people had adequate health literacy (17). The results of a study of 130 infertile women referred to the Montaserieh Infertility Research Centre in Mashhad showed that 54.6 had inadequate or poor sexual function (18). Investigation of sexual function among women referred to health centers in north Tehran showed the prevalence of sexual dysfunction to be 64% and the level of sexual satisfaction only 20% (19). The results of a study conducted in Turkey showed sexual dissatisfaction in 102 infertile couples to be 37.3 and 33.3% for women and men respectively (20).

Health literacy is one of the factors that affect health behaviors and several aspects of health. These include sexual function and sexual satisfaction, which in turn affect quality of life in terms marital satisfaction. Therefore, in this study, the relationship between health literacy and sexual function and sexual satisfaction was investigated in infertile couples referred to the Royan Institute in 2016.

Materials and Methods

In this descriptive study conducted in 2016 health literacy, sexual function and sexual satisfaction and correlations between them were examined. The research field was Royan Institute in Tehran and the research population included all of the couples (with primary and secondary infertility) referred to the Institute during the study. The Cochran formula was used to calculate the sample size ($z=1.96$, $\alpha=0.05$, $p=0.5$, $q=0.5$, and $d=0.05$). A sample of 386 people was selected to give the ability to detect differences with a significance level of 0.05 and a power of 80%.

The sample of 193 women and 193 men was selected using availability sampling by researchers who visited the Institute on specific days. Participants of the study included couples who lived together and were both willing to participate in the study. They also had either a diagnosis of primary infertility (no pregnancy for 12 months despite marital relations without contraception and no history of previous pregnancy) or a diagnosis of secondary infertility (no pregnancy despite a history of previous pregnancy) (21). In order to obtain the required data, four valid and reliable questionnaires were administered.

These included a demographic characteristics questionnaire, the Test of Functional Health Literacy in Adults (TOFHLA), a standard sexual functioning questionnaire (separate versions for women and men) and the Iranian version of the sexual satisfaction questionnaire (separate versions for women and men). The first questionnaire collected information on age, education, housing, household income, age at marriage, occupation, disease and habit (cigarette, alcohol, and drug) history of each individual and their relatives, marriage duration, weight, height, number of children, pregnancy history, abortion experience, treatment seeking, diagnosis, infertility cause, and contraception method. The TOFHLA questionnaire of Parker et al. (22) has two components, one for reading and one for numeracy, together comprised of 67 questions. Overall score for this tool was 100, with a score of 0-59 representing inadequate health literacy, 60-74 marginal health literacy, and 75-100 adequate health literacy.

Face validity, content validity and reliability of this tool (translated version) were studied and verified in the study conducted by Javadzade et al. (23) (reading component-Cronbach's alpha=0.88, numeracy component Cronbach's alpha=0.79). The 19-item Female Sexual Function Index (FSFI) of Rosen et al. (24) was used to assess sexual function in women. It covers six functional areas including desire, arousal, vaginal moisture, orgasm, pain, and sexual satisfaction. The numerical range for these fields is defined from 1 to 5. Total score less than 19 indicates the presence of sexual dysfunction. Karamidehkordi and Roudsari (18) confirmed the validity of the Persian version of FSFI, it was also confirmed its (Cronbach's alpha=0.79).

To measure sexual function in men, the International Index of Erectile Function (IIEF) was used. The questionnaire, developed by Rosen et al, includes five dimensions of erectile function, ejaculatory function, sexual stimulation, satisfaction during intercourse, and overall satisfaction in 15 questions. Each items are scored from 0 to 5, or 1 to 5, in which higher scores indicate better sexual performance (25). Content validity of the instrument (Persian version) was confirmed in a study conducted by Fakhri et al. (26) and reliability was confirmed by Cronbach's alpha. A self-assessment tool, developed in a study by Nasiri Nejad et al. (27), was used to measure sexual

satisfaction. The total score for this tool was 108 for men and 144 for women. Low, moderate, good, and very good satisfaction levels were categorized by scores that differ by increments of 27 in men and 36 in women. Face validity, content validity and reliability of this tool (Cronbach's alpha 0.89for the male questionnaire and 0.859 for the female questionnaire) were confirmed. Data were collected by interview after explaining the purpose of the research to the participants and obtaining informed consent.

Ethical approval

The study was designed in accordance with ethical rules approved by the Ethical Committee of the Islamic Azad University of Tehran Medical branch (IR.IAU.TMU.REC.1394.23).

Statistical analysis

The data collected in this study were analyzed using SPSS 23.0 statistical software (SPSS, Inc., Chicago, IL, USA). Descriptive statistics including percent's and frequencies are reported. Chi-square tests were used to analyze the associations between sexual function or sexual satisfaction and heath literacy at significant level of 0.05. Although the chi-squared test can indicate whether or not there is a relationship between 2 qualitative variables, it cannot indicate the strength of the association. The Cramer's V test was thus used to determine the strength of the associations and, consequently, their importance.

Results

Demographic variables are shown in Table 1. Besides that, about 64% of the participants weighed 60 kg or above, and the height of the majority (48.7%) was in the range of 150-170 cm. Although 86% of subjects had no children, only 10.6% had a history of non-pregnancy among family members and close relatives. Additionally, 85.5% of participants had no history of previous pregnancy and the percentage that had experienced an abortion was estimated 3.1%. For 63.2% of the participants this was their first referral to a health center for follow up of their infertility status, and only 40.4% of them had a diagnosis of the cause of their infertility. Twenty eight percent of the 40.4% infertility was due to the woman, and 59.1% of the participants used medicinal methods of contraception.

Descriptive results related to health literacy, sexual function, and sexual satisfaction variables are shown in Table 2. The results reported in Table 2 show that a marginal level of health literacy (44.1% in women and 49.7% in men) was more common that an adequate or inadequate level. In both sexes the percentages of participants with an inadequate level of health literacy was lower than those with an adequate level. In addition, 57.0% of women had normal sexual function and 69.9% of men had appropriate or perfect sexual function. The percentage of participants with good or very good sexual satisfaction was 57.0% in women and 67.4% in men.

Table 1: demographic variables for infertile couples referred to the Royan Institute in 2016

Variable	Subgroup	Frequency (%)	Variable	Subgroup	Frequency (%)
Age (Y)	<20	9 (2.4)	Marriage age (Y)	<20	41 (10.6)
	20-30	143 (37.0)		20-30	265 (68.7)
	31-40	173 (44.8)		31-40	61 (15.8)
	>40	61 (15.8)		>40	19 (4.9)
	Total	386 (100)		Total	386 (100)
Education	Illiterate	5 (1.3)	Occupation	Housewife	27 (7.0)
	Primary	56 (14.5)		Worker	88 (22.8)
	≤Diploma	163 (42.3)		Employee	99 (25.7)
	Bachelor	133 (34.4)		Self-Employed	160 (41.4)
	>Bachelor	29 (7.5)		Unemployed	12 (3.1)
	Total	386 (100)		Total	386 (100)
Housing	Owner- Occupied	108 (28.0)	History: individual (I), relatives (R)	Special diseases (I&R)	17 (12.0)
	Rented	218 (56.5)		Chronic diseases (I)	38 (27.0)
	Corporate Home	42 (10.9)		Genetic diseases (I&R)	13 (9.2)
	Relative's	18 (4.6)		Cigarette, alcohol, and drug (I)	73 (51.8)
	Total	386 (100)		Total	141 (100)
Household income (Million Rial)	<1	6 (1.5)	Marriage duration (Y)	<1	8 (2)
	1-2	154 (39.9)		1-5	152 (39.4)
	2-3	174 (45.1)		6-10	174 (45.1)
	>3	52 (13.5)		>10	52 (13.5)
	Total	386 (100)		Total	386 (100)

Table 2: Health literacy, sexual function, and sexual satisfaction in infertile couples referred to the Royan Institute in 2016

Variable	Sample	Level of variable	Frequency (%)
Health literacy	Woman	Inadequate	46 (23.8)
		Marginal	85 (44.1)
		Adequate	62 (32.1)
		Total	193 (100)
	Man	Inadequate	42 (21.8)
		Marginal	96 (49.7)
		Adequate	55 (28.5)
		Total	193 (100)
Sexual function	Woman	Dysfunction	83 (43.1)
		Normal	110 (57.1)
		Total	193 (100)
	Man	Inappropriate	3 (1.6)
		Medium	11 (5.7)
		Medium to Good	44 (22.8)
		Appropriate	103 (53.3)
		Perfect	32 (16.6)
		Total	193 (100)
Sexual satisfaction	Woman	Low	27 (14.0)
		Medium	56 (29.0)
		Good	89 (46.1)
		Very Good	21 (10.9)
		Total	193 (100)
	Man	Low	17 (8.8)
		Medium	46 (23.8)
		Good	98 (50.8)
		Very Good	32 (16.6)
		Total	193 (100)

The relationships between health literacy, sexual function, and sexual satisfaction were examined using the chi-square test and are presented in Table 3. The results show a significant relationship ($P<0.05$) between the level of health literacy, sexual function, and sexual satisfaction in infertile women and men referred to the Royan Institute. Cramer's V indicated that the strength of the association between health literacy and sexual function in women is 0.33 and 0.18 for men. The Cramer's V results indicate that these relationships can be considered as moderate to weak.

Discussion

Marginal health literacy, 49.7% among men and 44.1% among women, was more common than adequate or inadequate health literacy. Ghanbari et al. (3) in their study of fertile women concluded that 45.4% had an adequate literacy level and the percentage with a marginal literacy level was only 24.6%. Results of a study of 525 adults in Isfahan city showed that 46.5% had adequate health literacy and the proportion with marginal and inadequate literacy was 38.0 and 15.5%, respectively (17). Another study conducted by Protheroe et al. (28) in an English city showed that 28.5% of adult papulation had inadequate health literacy levels. However, the results of a study conducted among American adults showed that half the participants do not have basic health literacy (29). In another study conducted among African-American adults, it was shown that 65% of participants had low and inadequate health literacy levels (30). Due to the dependency of health literacy on socio-economic and cultural conditions, the difference between our results whit other studies which conducted in different regions is natural.

In relation to sexual functioning, our study showed 43 percent of the women had sexual dysfunction and 53.3% of men had appropriate sexual function. Results of other studies in Iran are not in line whit our study. In the North of Tehran, the prevalence of sexual dysfunction among women was 64% (19). Another study conducted on 405 women in South Tehran showed that sexual function in the largest proportion was moderate (31). The study of Karamidehkordi and Roudsari (18) on 130 infertile women in Mashhad showed that 45.4% of infertile women had normal sexual function.

Cai et al. (32) conducted a study of 105 infertile women in China which showed the mean sexual function score in infertile women was 25.2 (out of 28). In Turkey, sexual dysfunction in women affected by male infertility was 51.9%, while it was 54.8% in the group affected by female infertility (33). The results of a study conducted on 236 men in South Korea (sex partners of infertile couples) showed that only 49.2% did not suffer sexual dysfunction. The remaining 50.8% had different levels of sexual dysfunction, a rate that is high in comparison with the present study (34). In addition, a study conducted in Turkey on 56 men showed 85.9% of participants had mild to moderate sexual dysfunction (35).

The third variable examined in this study was sexual satisfaction, and, based on the findings, 57% of women and 67.4% of men had good or very good sexual satisfaction. Ramazani et al. (19) concluded that 20% of participants had sexual satisfaction with their spouse. In south

Table 3: Results of Chi-square and Cramer's V tests to examine the relationship between health literacy, sexual function, and sexual satisfaction in infertile couples referred to the Royan Institute in 2016

Variable	Sample	Sexual function			Sexual satisfaction		
		df	P value	Cramer's V	df	P value	Cramer's V
Health literacy	Woman	2	0.005	0.33	6	0.007	0.17
	Man	8	0.017	0.18	6	0.038	0.16

df; Degree of freedom.

of Tehran, a significant percentage of women (58.2%) had moderate sexual satisfaction level (31). A study conducted in Egypt on infertile couples (due to male infertility) showed that 89% of men and 80% of women had sexual satisfaction with their spouses (36). The difference in sexual satisfaction found between the study conducted in Egypt and the present study can be due to racial, ethnic, and cultural differences that affect the level of the expectations of the people. A study conducted in Turkey on 102 infertile couples showed that the share of sexual dissatisfaction for men and women was respectively 37.3 and 33.3% (20). In comparing the results of studies in the area of sexual satisfaction, characteristics of the research subjects should be considered. The findings from 64 papers showed that infertility is one of the factors influencing sexual satisfaction (37).

A significant relationship between health literacy and sexual function and between health literacy and sexual satisfaction was observed in this study. Health literacy affects attitude, mental condition, behaviors related to health, and thus, physical health status. In addition, health literacy can affect the use of information and sexual function in couples (38), and, thereby have a positive impact on quality of life (39). A study of 290 women by Mogadam Banaem et al. (40) showed that the rate of sexual satisfaction is related to health literacy level. In this regard, it can be stated that health literacy can affect mental states and attitude of the couples to sexual and marital relations, and thus, they can affect their sexual satisfaction.

Although the research has reached its aims, there were some unavoidable limitations. First, this study was cross-sectional and conducted in one center. Second, research variables are dependent on the socio-cultural environment. Therefore, to generalize the results for large groups in different settings, it is recommended that studies be performed in other places and with a larger sample. Also, it can be recommended to conduct a meta-analysis of existing literature to further understanding in this field.

Conclusion

The results of this study showed that marginal health literacy was more common than adequate or inadequate health literacy in both sexes. Additionally, our study confirmed the relationship between health literacy and sexual function and sexual satisfaction. Accordingly, it is recommended that applied and practical plans be developed in order to improve health literacy at the level of the community, especially among infertile couples.

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Author's Contributions

M.S., Z.M., H.F., M.S.; Contributed to the design and implementation of the research. Data collection and analyzing were done by Z.M. and H.F. M.S., M.S; Wrote the first draft of manuscript. All authors discussed the results and contributed to the final manuscript.

References

1. LeBreton, M. Implementation of a validated health literacy tool with teach-back education in a super utilizer patient population. Widener University: Widener University; 2015; 1-402.
2. Abel T, Hofmann K, Ackermann S, Bucher S, Sakarya S. Health literacy among young adults: a short survey tool for public health and health promotion research. Health Promot Int. 2015; 30(3): 725-735.
3. Ghanbari S, Majlessi F, Ghaffari M., Mahmoodi Majdabadi M. Evaluation of health literacy of pregnant women in urban health centers of Shahid Beheshti Medical University. Daneshvarmed. 2012; 19(97): 1-12.
4. Peerson A, Saunders M. Health literacy revisited: what do we mean and why does it matter? Health Promot Int. 2009; 24(3): 285-296.
5. Berkman ND, Sheridan SL, Donahue KE, Halpern DJ, Crotty K. Low health literacy and health outcomes: an updated systematic review. Ann Intern Med. 2011; 155(2): 97-107.
6. Sheridan SL, Halpern DJ, Viera AJ, Berkman ND, Donahue KE, Crotty K. Interventions for individuals with low health literacy: a systematic review. J Health Commun. 2011; 16 Suppl 3: 30-54.
7. Schumacher JR, Hall AG, Davis TC, Arnold CL, Bennett RD, Wolf MS, et al. Potentially preventable use of emergency services: the role of low health literacy. Med Care. 2013; 51(8): 654-658.
8. Wolf MS, Feinglass J, Thompson J, Baker DW. In search of 'low health literacy': Threshold vs. gradient effect of literacy on health status and mortality. Soc Sci Med. 2010; 70(9): 1335-1341.
9. Rosas-Salazar C, Apter AJ, Canino G, Celedón JC. Health literacy and asthma. J Allergy Clin Immunol. 2012; 129(4): 935-942.
10. Komenaka IK, Nodora JN, Hsu CH, Martinez ME, Gandhi SG, Bouton ME, et al. Association of health literacy with adherence to screening mammography guidelines. Obstet Gynecol. 2015; 125(4): 852-859.
11. Jordan JE, Buchbinder R, Briggs AM, Elsworth GR, Busija L, Batterham R, et al. The health literacy management scale (HeLMS): a measure of an individual's capacity to seek, understand and use health information within the healthcare setting. Patient Educ Couns. 2013; 91(2): 228-235.
12. Finkelstein JS, Lee H, Burnett-Bowie SA, Pallais JC, Yu EW, Borges LF, et al. Gonadal steroids and body composition, strength, and sexual function in men. N Engl J Med. 2013; 369(11): 1011-1022.
13. Sánchez-Fuentesa MM, Santos-Iglesiasb P, Carlos Sierraa J. A systematic review of sexual satisfaction. Int J Clin Health Psychol. 2014; 14(1): 67-75.
14. Bois K, Bergeron S, Rosen NO, McDuff P, Grégoire C. Sexual and relationship intimacy among women with provoked vestibulodynia and their partners: Associations with sexual satisfaction, sexual function, and pain self-efficacy. J Sex Med. 2013; 10(8): 2024-2035.
15. Hasanzadeh Mofrad M, Karami Dehkordi A, Mozaffar Tizabi N, Amirian M. Survey of sexual dysfunction in women with cervical cancer and a history of pelvic radiation therapy in 2009 to 2013 in Ghaem and Omid hospitals, Mashhad. The Iranian Journal of Obstetrics, Gynecology and Infertility. 2015; 18(144): 9-18.
16. McMichael C, Gifford S. It is good to know now… before it's too late: promoting sexual health literacy amongst resettled young people with refugee backgrounds. Sexuality & Culture. 2009; 13(4): 218-236.
17. Javadzade H, SharifiradGh, Reisi M, Tavassoli E, Rajati F. Health literacy among adults of Isfahan, Iran. J Health Syst Res. 2013; 9(5): 540-549.
18. Karamidehkordi A, Roudsari RL. Body image and its relationship

with sexual function and marital adjustment in infertile women. Iran J Nurs Midwifery Res. 2014; 19(7 Suppl 1): S51-58.
19. Ramezani N, Dolatian M, Shams J, Alavi H. The relationship between self-esteem and sexual dysfunction and satisfaction in women. Arak Medical University Journal. 2011; 14(659): 57-65.
20. Yangin H, Kukulu K, Gulşen S, Aktaş M, Sever B. A survey on the correlation between sexual satisfaction and depressive symptoms during infertility. Health Care Women Int. 2016; 37(10): 1082-1095.
21. Davari Tanha F, Mohseni M, Ghajarzadeh M. Sexual function in women with primary and secondary infertility in comparison with controls. Int J Impot Res. 2014; 26(4): 132-134.
22. Parker RM, Baker DW, Williams MV, Nurss JR. The test of functional health literacy in adults: a new instrument for measuring patients' literacy skills. J Gen Intern Med. 1995; 10(10): 537-541.
23. Javadzade SH, Sharifirad G, Radjati F, Mostafavi F, Reisi M, Hasanzade A. Relationship between health literacy, health status, and healthy behaviors among older adults in Isfahan, Iran. J Educ Health Promot. 2012; 1: 31.
24. Rosen R, Brown C, Heiman J, Leiblum S, Meston C, Shabsigh R, et al. The female sexual function index (FSFI): a multidimensional self-report instrument for the assessment of female sexual function. J Sex Marital Ther. 2000; 26(2): 191-208.
25. Rosen RC, Wei JT, Althof SE, Seftel AD, Miner M, Perelman MA, et al. Association of sexual dysfunction with lower urinary tract symptoms of BPH and BPH medical therapies: results from the BPH Registry. Urology. 2009; 73(3): 562-566.
26. Fakhri A, Morshedi H, Soleymanian A, Hosaini M. Psychometric properties of Iranian version of male sexual function index. Jundishapur Scientific Medical Journal. 2014; 12(6): 655-663.
27. Nasiri Nejad F, Nazari AM, Bahrainian A. Investigating the effect of training positive thinking on intimacy, satisfaction and sexual performance in housewives of 5th region in Tehran. Bull Env Pharmacol Life Sci. 2014; 3(4): 26-32.
28. Protheroe J, Whittle R, Bartlam B, Estacio EV, Clark L, Kurth J. Health literacy, associated lifestyle and demographic factors in adult population of an English city: a cross-sectional survey. Health Expect. 2017; 20(1): 112-119.
29. Dennison Himmelfarb CR, Hughes S. Are you assessing the communication" vital sign"?: Improving communication with our low-health-literacy patients. J Cardiovasc Nurs. 2011; 26(3): 177-179.
30. Manganello JA, Sojka CJ. An exploratory study of health literacy and African American adolescents. Comprehensive Child and Adolescent Nursing. 2016; 39(3): 221-239.
31. Tavakkol Z, Mirmovlayee ST, Momenimovahed Z, Mansouri A. The relationship between sexual function and sexual satisfaction among women referred to health centers in South of Tehran. Scientific Journal of Nursing and Midwife School of Hamedan. 2011; 19(2): 50-54.
32. Cai L, Liu J, Lu S, Yin J. Female sexual dysfunction and timed intercourse: a prospective study of 105 infertile women. Advances in Reproductive Sciences. 2015; 3(4): 92-96.
33. Kucur Suna K, Ilay G, Aysenur A, Kerem Han G, Eda Ulku U, Pasa U, et al. Effects of infertility etiology and depression on female sexual function. J Sex Marital Ther. 2016; 42(1): 27-35.
34. Song SH, Kim DS, Yoon TK, Hong JY, Shim SH. Sexual function and stress level of male partners of infertile couples during the fertile period. BJU Int. 2016; 117(1): 173-176.
35. Ozkan B, Orhan E, Aktas N, Coskuner ER. Depression and sexual dysfunction in Turkish men diagnosed with infertility. Urology. 2015; 85(6): 1389-1393.
36. Gadalla A, Nasreldin M, Mostafa T, Ahmed A, Eid A. Psychosexual satisfaction of infertile couples due to male factor infertility. Human Andrology. 2011; 1(2): 51-56.
37. Samadaee-Gelehkolaee K, McCarthy BW, Khalilian A, Hamzehgardeshi Z, Peyvandi S, Elyasi F, et al. Factors associated with marital satisfaction in infertile couple: a comprehensive literature review. Glob J Health Sci. 2015; 8(5): 96-109.
38. Matsuyama RK, Wilson-Genderson M, Kuhn L, Moghanaki D, Vachhani H, Paasche-Orlow M. Education level, not health literacy, associated with information needs for patients with cancer. Patient Educ Couns. 2011; 85(3): e229-e236.
39. González-Chica DA, Mnisi Z, Avery J, Duszynski K, Doust J, Tideman P, et al. Effect of health literacy on quality of life amongst patients with ischaemic heart disease in Australian General Practice. PLoS One. 2016; 11(3): e0151079.
40. Moghaddam Banaem L, Gashtasbi A, Tamrtash M. The Relationship between marital satisfaction and sexual satisfaction with health literacy among women with breast cancer in fertility age. Presented for the M.Sc., Tehran. Tarbiat Modares University. 2015.

Original Article

Difference between Primary and Secondary Infertility in Morocco: Frequencies and Associated Factors

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Abstract

Background: The main objective of this survey was to determine the difference between primary and secondary infertility in Morocco and the associated factors among women, who are referred to public and private health centers in Morocco.

Materials and Methods: In this cross-sectional study, 619 infertile women referring to public and private health centers in Marrakech-Safi region, were selected by simple random sampling method. This study was conducted between 1 October 2013 and 31 December 2015. Socio-economic status, demographic characteristics, couple's age, nutritional status and other data related to both male and female reproductive organs were collected by a questionnaire. Logistic regression was used to identify the associated factors to infertility. Statistical significance was set at 0.05.

Results: The rates of primary and secondary infertility were 67.37, and 32.63%, respectively. Multivariate analysis identified a model with three significant predictive factors of secondary infertility: duration of marriage [odds ratio (OR)=12.263: 2.289-65.685], socio-economic status (OR=3.83: 1.011-14.70) and the ages of women (OR=1.268: 1.038-1.549).

Conclusion: The causes of primary and secondary infertility were not always a woman's problem, but both man and woman contribute to infertility. Multiple regression analysis showed that women's age, duration of marriage, and socio-economic status are predictive variables that decrease the chance of fertility among women with secondary infertility.

Keywords: Infertility, Morocco, Women

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Introduction

Infertility is a public health problem during the reproductive age, affecting about 10-15% of couples attempting to achieve pregnancy in worldwide (1). Infertility is defined by the failure to achieve a natural pregnancy after 12 months or more of regular unprotected sexual intercourse (2). For many couples, the inability to bear children is a shocking tragedy leading to serious physical, social, psychological and sexual dysfunction in their lives (3).

According to World Health Organization (WHO), the term primary infertility is used when a woman has never conceived and secondary infertility is the incapability to conceive in a couple who have had at least one successful conception in the past (4). Infertility can be attributed to anomalies associated with either male or female reproductive systems or with both partners. Several factors can disturb the process of fertility at any step. For example, female infertility may be due to one or more reasons such as, polycystic ovary syndrome (5), hormonal disorders (6), premature ovarian failure (7), genital infections (8), endometriosis (9), fallopian tube obstruction (10), congenital uterine anomalies (11), uterine synechiae (12), or other medical complications (diabetes and thyroid disorders) (13-14).

Nevertheless, male infertility is due to hormonal imbalances, and sperm abnormalities (3-15). Other main causes of infertility could be age of a couple (16), occupation, and socio-economic status (17). Few studies were dedicated to determine the prevalence and associated risk factors for infertility in Morocco. Therefore, our aim was to determine the difference between primary and secondary infertilities and to better understand the main infertility associated factors in the Moroccan population.

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Materials and Methods

This cross-sectional study was conducted with the approval of the Ethic of the Moroccan health authorities in the region of Marrakech-Safi. This region is located in the middle of Morocco and consists of one state and eight administrative provinces. This study was conducted at different public and private health centers in the region of Marrakech-Safi. A sample of 619 infertile women referring to these health centers was selected by a simple random sampling method, between 1 October 2013 and 31 December 2015. The subjects were chosen without any previous appointments.

The study protocol was explained and the informed consent was obtained from all participants before enrolment. In this study, all data was collected through a questionnaire and the information provided by health booklets for each married woman who had difficulty becoming a mother after at least 12 months of regular unprotected sexual intercourse. The questionnaire contained different elements: socio-economic data, demographic characteristics, age of the couple, and their nutritional status. Also, searching health booklets provided history and clinical information [urogenital infections, medical complications, diabetes, thyroidism, menstrual disorders, polycystic ovary syndrome (PCOS), tubal dysfunction, varicocele and congenital uterine anomalies] and methods of diagnosis, particularly the results of ultrasounds, hysterosalpingographies, hysteroscopies, hormone levels and semen analyses.

Other data associated with male and female reproductive organs were included such as menstruation disorders, uterine malformations, hormonal imbalances, varicocele, the quality of sperms, and other medical complications.

The subjects who had met our inclusion criteria were married women during their childbearing ages, who had referred to health centers for infertility problems after 12 months of trying for pregnancy. Prior to this research study, the collection tool was tested with a pilot group of women similar to those wishing to participate voluntarily in this survey. All women were entered into the study and their associated data was collected by trained research nursing students.

Statistical analyses

A one-sample Kolmogorov-Smirnov test was used to analyze normality for continuous variables. A Chi-square test and Fisher's exact test were used for categorical variables.

Student's t test was used to estimate the observed differences between the means. The multivariate data analysis was used to allow for the elimination of the confounding factors and entering the weight of the associated variables with the type of infertility in the bivariate analysis set at 0.2. These variables were used to identify factors that were independently associated with secondary infertility. Associations were measured in odds ratio (OR) with 95% confidence intervals (CI). Data analyses were carried out using SPSS (SPSS Inc. for Windows version 10.0, Chicago). For all analyses the differences were considered significant when $P<0.05$.

Results

A total of 619 infertile women were included in this study, 417 (67.37%) with primary infertility and 202 (32.63%) with secondary infertility.

Table 1: Comparison of socio-economic and demographic characteristics between primary and secondary infertilities

Variables and modalities	Primary infertility n (%) or Mean ± SD	Secondary infertility n (%) or Mean ± SD	P value
Women's age (Y)	28.7 ± 5.7	31.95 ± 5.6	0.0001
Socioeconomic status			
Low	28 (6.70)	26 (12.9)	0.003
Average and high	389 (93.3)	176 (87.1)	
Nutritional status			
Normal weight	351 (84.2)	172 (85.1)	0.490
Excess weight	66 (15.80)	30 (14.90)	
Duration of marriage			
>5 Y	145 (34.8)	146 (72.3)	0.0001
≤5 Y	272 (65.2)	56 (27.7)	
Period of infertility			
≤3.8 Y	269 (64.5)	112 (55.4)	0.075
>3.8 Y	148 (35.5)	90 (44.6)	
Imaging tests (women)			
Hysterosalpingography (HSG)	163 (39.08)	60 (29,70)	0.023
Pelvic ultrasonography	394 (94.48)	182 (90,09)	0.033
Hysteroscopy/ laparoscopy	45 (10.8)	15 (7.4)	0.183
Biological tests (women)			
Hormonal tests	177 (42.44)	70 (34.65)	0.063
Post-coital test	14 (3.14)	00 (00)	0.008
Partner age (Y)	35.8 ± 7.7	38.8 ± 6.8	0.001
Partner consultation	239 (57.3)	60 (29.7)	0.001
Semen analysis	237 (56.8)	59 (29.2)	0.001

Table 2: Comparison of clinical characteristics between primary and secondary infertilities

Variables and modalities	Primary infertility n (%)	Secondary infertility n (%)	P value
Ovulation disorder			
Menstrual disorders	213 (51.1)	92 (45.5)	0.191
Hormone disorder (FSH, LH, AMH)	65 (36.7)	27 (38.6)	0.782
Endocrine diseases (diabetes, thyroid)	22 (5.3)	15 (7.4)	0.290
Polycystic ovary syndrome	107 (27.2)	53 (29.1)	0.620
Tubal factors			
Obstruction and tubal dysfunction	105 (64.40)	41 (68.30)	0.581
Endometriosis/pelvic adhesion			
Endometriosis	14 (8.6)	8 (13.3)	0.290
Uterine synechiae	6 (3.7)	6 (10.0)	0.065
Uterine and cervical factors			
Congenital uterine anomaly	27 (6.9)	15 (8.2)	0.554
Fibroids	25 (6.3)	13 (7.1)	0.721
Polyps	12 (3.0)	4 (2.2)	0.760
Genital infections	65 (15.6)	33 (16.3)	0.810
Male factors			
Varicocele	31 (13.00)	4 (6.7)	0.263
Abnormal sperm	107 (45.1)	12 (20.3)	0.001
Origin of infertility			
Unexplained infertility	20 (8.33)	07 (11.30)	
Male infertility	54 (22.5)	04 (6.50)	0.003
Female infertility	106 (44.20)	41 (66.10)	
Mixed infertility	60 (25)	10 (16.10)	

FSH; Follicle stimulating hormone, LH; Luteinizing hormone, and AMH; Anti-mullerian hormone.

Table 3: Variables independently associated with primary (n=202) and secondary (n=417) infertilities, according to the multiple logistic regression model

Variables and modalities	β	χ^2	P value	OR	95%	CI
Women age	0.237	5.393	0.020	1.268	1.038	1.549
Partner age	-0.129	2.562	0.109	0.879	0.751	1.029
Socio-economic status	1.342	3.848	0.048	3.83	1.011	14.70
Hysterosalpingography	-0.340	0.264	0.608	0.712	0.194	2.606
Post-coital test	-18.499	0.000	0.999	0.000	0.000	-
Partner consultation	-1.817	1.021	0.312	0.162	0.005	5.516
Menstrual disorders	-0.117	0.047	0.829	0.889	0.307	2.574
Uterine synechiae	1.862	2.788	0.095	6.439	0.723	57.314
Abnormal sperm	-0.328	0.321	0.571	0.721	0.232	2.238
Hormone disorder	0.079	0.025	0.875	1.082	0.403	2.904
Duration of marriage: ≥ 5 Y	2.507	8.569	0.003	12.263	2.289	65.685
Period of infertility: ≥3, 8 Y	-1.560	3.628	0.057	0.210	0.042	1.046

OR; Odds ratio and CI; Confidence intervals.

The socio-economic and demographic characteristics between primary and secondary infertilities are presented in Table 1. The average of women's ages were 28.7 ± 5.7 years and 31.95 ± 5.6 years in primary and secondary infertility, respectively. The average ages of their husbands were 35.8 ± 7.7 years and 38.8 ± 6.8 years in primary and secondary infertility, respectively. The difference between their ages was significant. Also, a good socio-economic situation was reported by primary infertile women (93.3%) compared to those with secondary infertility (P=0.003). After that, the majority of these women reported that their weights were normal and only 15% had excess weight. Furthermore, a longer duration of marriage was reported in secondary infertility compared with primary infertility (P=0.001).

However, the duration of infertility was not different in the two groups of infertility. In comparison to secondary infertility, primary infertile women showed enthusiasm for medical diagnosis such as hysteronsalpingography (39.08%), and pelvic ultrasound (94.48%). However, in secondary infertility, 70.3% of spouses refused to see a specialist compared with the primary infertility group (P=0.001). The semen analysis was mainly practiced to evaluate primary infertility (56.8%) with a significant difference.

The clinical characteristics between the two groups of infertility were presented in Table 2. With the exception of

menstrual disorders, the major causes of primary infertility were entirely due to male reproductive organs, particularly varicocele and abnormalities of semen, with a significant difference. The main causes of secondary infertility were observed mostly among women; such causes include hormonal disturbance, medical complications, polycystic ovary syndrome, tubal dysfunction, genital infections, uterine anomalies, endometriosis and adhesions without significant difference. However, according to multiple logistic regression models, variables independently associated with primary and secondary infertility, are presented in Table 3. In this model, the duration of marriage (OR= 12.263: 2.289-65.685), the age of the woman (OR=1.268: 1.038-1.549), and the socio-economic status (OR=3.83: 1.011-14.70) were relatively independent predictive variables associated to secondary infertility.

Discussion

To our knowledge, this is the first study able to determine the difference between primary and secondary infertility in Morocco and to distinguish among the associated factors. The overall rates of primary and secondary infertility were 67.37 and 32.63%, respectively. This result is similar to those published in other areas of Africa (18). In secondary infertility, the couple's average age was higher when compared to those who had primary infertility. Previously, this age difference has been highlighted by other researchers to some degree (17).

In this study, this result can be explained partly by a major change in the age for marriage in Morocco (from 17.3 years old in 1960 to 26.6 years old in 2010) (19). The age of couple is clearly an important factor in reproduction, as a woman's fertility is strictly dependent on age. The peak of ability to reproduce usually around the age of 20 years for women. Indeed, it starts to decline from the age of 30, and reduce severely from the age of 40 (16).

In secondary infertility, an overly long duration of marriage and an advanced age of the couple could decrease their chances of having a new child. This finding was close to the one recorded by Keskin et al. (17). Also, the socio-economic level of a couple can influence the type of their infertility.

In primary infertility, the majority of women with relatively high-to-moderate socio-economic status are able to resolve their infertility problems. This status can provide fast and easy access to several diagnostic methods and infertility treatments (21). Moreover, excess body weight of women was a powerful determinant of infertility risk by ovulation disorders (22).

According to the perception of the participants, however, their body weights were normal in both primary and secondary infertility groups. This perception is not compatible with that observed among Moroccan population (23). Also, the apparent weight does not always reflect actual weight status based on the body mass index (24). In fact, the diagnosis of male infertility is an essential step for a quick and effective treatment. However, the sperm abnormality is the major cause for infertility (45.1%) among primary infertile men compared to secondary infertility (20.3%).

This result can be explained by the advanced age and also exposure to urogenital infections affecting the quantity and the quality of sperms. In Africa, the sperm abnormalities were estimated at 68% for the age 31-40 years (15). In this investigation, the rate of female infertility was significantly higher in secondary infertility than primary (66.10 vs. 44.20%). Also, the most common causes of female infertility were ovulation disorders, which manifest themselves by sparse or absent menstrual periods (22). Furthermore, certain studies have demonstrated that 40 to 50% of infertilities were due to female reproductive organs (21).

Finally, the difference between primary and secondary infertilities in Morocco was relatively associated with three independent variables, particularly the duration of marriage (OR=12.263: 2.289-65.685, the woman's age (OR=1.268) and socio-economic position (OR 3.83). Furthermore, the bounds of this interval were very far from the value 1, which means that the result was positive and therefore the duration of infertility was strongly related to secondary infertility.

To reduce the limitations in our study, further investigations should be undertaken with control groups of fertile women to provide additional information on risk factors for male and female infertility. Furthermore, a high-quality dialogue between all participants will be recommended for better management of infertility.

Conclusion

In this study, primary and secondary infertilities were due to the intersection of several demographic characteristics and medical factors. However, woman's age, duration of marriage and socio-economic status had a significant impact to accentuate the severity of secondary infertility.

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Author's Contribution

A.B., N.E., R.A.A, A.B., M.C.; Contributed to conception and design. A.B., N.E., R.A.; Contributed to all experimental work, data and statistical analysis, and interpretation of data. A.B., N.E., M.C.; Were responsible for overall supervision. A.B., N.E.; Drafted the manuscript, which was revised by M.C. and N.E. All authors read and approved the final manuscript.

References

1. Blaževičienė A, Jakušovaitė I, Vaškelytė A. Attitudes of fertile and infertile woman towards new reproductive technologies: a case study of Lithuania. Reprod Health. 2014; 11(1): 26.
2. Gurunath S, Pandian Z, Anderson RA, Bhattacharya S. Defining infertility-a systematic review of prevalence studies. Hum Reprod Update. 2011; 17(5): 575-588.
3. Smith JF, Walsh TJ, Shindel AW, Turek PJ, Wing H, Pasch L, et al. Sexual, marital, and social impact of a man's perceived infertility diagnosis. J Sex Med. 2009; 6(9): 2505-2515.
4. Tabong PT, Adongo PB. Infertility and childlessness: a qualitative study of the experiences of infertile couples in North Ghana. BMC Pregnancy Childbirth. 2013; 13: 72.
5. Lash MM, Yaghamee A, Strohsnitter W, Lalwani S. Association between secondary infertility and fallopian tube obstruction on hysterosalpingography. J Reprod Med. 2008; 53(9): 677-680.
6. Kazerooni T, Dehghan-Kooshkghazi M. Effects of metformin therapy on hyperandrogenism in women with polycystic ovarian syndrome. Gynecol Endocrinol. 2003; 17(1): 51-56.
7. Shen SY, Huang SY, Hsieh CH, Hsu MI, Cheng CY, Hsu CS. Clinical and biochemical characteristics of women with menstrual disturbance. Taiwan J Obstet Gynecol. 2014; 53(2): 178-182.
8. Goundry AL, Finlay ER, Llewellyn CD. Talking about links between sexually transmitted infections and infertility with college and university students from SE England, UK: a qualitative study. Reprod Health. 2013; 10: 47.
9. Wang PH, Su WH, Sheu BC, Liu WM. Adenomyosis and its variance: adenomyoma and female fertility. Taiwan J Obstet Gynecol. 2009; 48(3): 232-238.
10. Lavy G, Diamond MP, DeCherney AH. Ectopic pregnancy: its relationship to tubal reconstructive surgery. Fertil Steril. 1987; 47(4): 543-556.
11. Celik NY, Mulayim B. A mullerian anomaly "without classification": septate uterus with double cervix and longitudinal vaginal septum. Taiwan J Obstet Gynecol. 2012; 51(4): 649-650
12. Umdagas HA, Kawuwa BM, Hajara US, Mohammed S. Prevalence of uterine synechiae among infertile females in a Nigerian teaching hospital. J Obstet Gynaecol. 2006; 26(4): 351-352.
13. Codner E, Merino PM, Tena-Sempere M. Female reproduction and type 1 diabetes: from mechanisms to clinical findings. Hum Reprod Update. 2012; 18(5): 568-585.
14. Menif O, Omar S, Feki M, Kaabachi N. Hypothyroidism and pregnancy: impact on mother and child health. Ann Biol Clin. 2008; 66(1): 43-51.
15. Ugwuja EI, Ugwu NC, Ejikeme BN. Prevalence of low sperm count and abnormal semen parameters in male partners of women consulting at infertility clinic in Abakaliki, Nigeria. Afr J Reprod Health. 2008; 12(1): 67-73.
16. Dunson DB, Baird DD, Colombo B. Increased infertility with age in men and women. Obstet Gynecol. 2004; 103(1): 51-56.
17. Keskin U, Coksuer H, Gungor S, Ercan CM, Karasahin KE, Baser I. Differences in prevalence of sexual dysfunction between primary and secondary infertile women. Fertil Steril. 2011; 96(5): 1213-1217.
18. Ikechebelu JI, Adinma JI, Orie EF, Ikegwuonu SO. High prevalence of male infertility in southeastern Nigeria. J Obstet Gynaecol. 2003; 23(6): 657-659.
19. Elkhoudri N, Amor H, Baali A. Self-reported postpartum morbidity: prevalence and determinants among women in Marrakesh, Morocco. Reprod Health. 2015; 12(1): 75.
20. Akhondi MM, Kamali K, Ranjbar F, Shirzad M, Shafeghati S, Ardakani ZB, et al. Prevalence of Primary Infertility in Iran in 2010. Iran J Public Health. 2013; 42(12): 1398-1404.
21. Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: a review of literature. J Hum Reprod Sci. 2015; 8(4): 191.
22. Ramlau-Hansen CH, Thulstrup AM, Nohr EA, Bonde JP, Sorensen TI, Olsen J. Subfecundity in overweight and obese couples. Hum Reprod. 2007; 22(6): 1634-1637.
23. Benghanem GM, Elseviers M, Zamd M, Alaoui AB, Benahadi N, Trabelssi EH, et al. Chronic kidney disease, hypertension, diabetes, and obesity in the adult population of Morocco: how to avoid "over"- and "under"-diagnosis of CKD. Kidney Int. 2016; 89(6): 1363-1371.
24. Mikolajczyk RT, Maxwell AE, El Ansari W, Stock C, Petkeviciene J, Guillen-Grima F. Relationship between perceived body weight and body mass index based on self-reported height and weight among university students: a cross-sectional study in seven European countries. BMC Public Health. 2010; 10(1): 40.

Original Article

The Association of *PPARγ Pro12Ala* and *C161T* Polymorphisms with Polycystic Ovary Syndrome and Their Influence on Lipid and Lipoprotein Profiles

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Abstract

Background: The aim of present study was to clarify the role of the peroxisome proliferator-activated receptor *(PPAR) γ Pro12Ala* and *C161T* polymorphisms in the pathogenesis of polycystic ovary syndrome (PCOS) and their influence on lipid and lipoprotein profiles of patients.

Materials and Methods: The present cross-sectional study consisted of 50 women with PCOS, who referred to the Kermanshah University of Medical Sciences Clinic between April and October 2015, and 233 unrelated age-matched healthy women from the same region (West Iran). The *PPARγ Pro12Ala* and *PPARγ C161T* polymorphisms were genotyped using the polymerase chain reaction-restriction fragment length polymorphism method. Fasting blood sugar (FBS), serum triglycerides (TG), cholesterol, low density lipoprotein- cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C) and estradiol levels were measured.

Results: The serum level of estradiol was significantly lower in PCOS patients compared to healthy women. The *PPARγ Pro12Ala* (CG) genotype increased the risk of PCOS 2.96-fold. The frequency of the *PPARγ* T allele (at C161T) was 21% in patients and 17.2% in controls with no significant difference (P=0.52). In all studied individuals, the *PPARγ* CG genotype was associated with significantly higher levels of TG. However, significantly lower levels of total cholesterol and LDL-C were observed in *PPARγ TT* individuals compared with those with the CC genotype. Within the PCOS group, the *PPARγ CG* genotype was significantly associated with lower levels of estradiol compared with the CC genotype. Also, the CG genotype was significantly associated with higher levels of TG when compared with the CC genotype.

Conclusion: Our study shows that, unlike *PPARγ C161T*, *PPARγ Pro12Ala* is associated with the risk of PCOS. Also, we found that the lipid and lipoprotein profiles significantly vary based on *PPARγ Pro12Ala* and *C161T* genotypes.

Keywords: Estradiol, Lipid, Lipoprotein, Peroxisome Proliferator-Activated Receptor, Polycystic Ovary Syndrome

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Introduction

Polycystic ovary syndrome (PCOS) is one of the most frequent endocrine-related gynecological disorders among women of reproductive age (1). PCOS, a leading cause of female infertility, is characterized by hyperandrogenism, menstrual irregularity, chronic anovulation and multiple small sub-capsular ovarian cystic follicles (2). Around 50 to 70% of patients with PCOS are diagnosed with dyslipidemia (3).

The peroxisome proliferator-activated receptors (PPARs) belong to the nuclear hormone receptors that regulate the transcription of a variety of genes such as those involved in the metabolism of lipids in adipose tissue, liver and skin (4). The isoform PPARγ, which participates in lipid and glucose metabolism, is mainly expressed in adipose tissue (5).

The common *PPARγ* single nucleotide polymorphism (SNP) Pro12Ala (C/G; rs1801282) modulates its transcriptional activity, resulting in reduced transcriptional activity of PPARγ (4). The association of this SNP with PCOS has been investigated, however, there are inconsistent reports about the role of this polymorphism in susceptibility to PCOS, and its influence on lipid and lipoprotein profiles (5-9).

The *PPARγ* SNP C161T (rs3856806, His447His) in exon 6 is also associated with decreased transcription of *PPARγ* (10). The role of this polymorphism in susceptibil-

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ity to PCOS has also been studied but remains controversial (5, 8, 11, 12).

The aim of this study was to assess the association of *PPARγ Pro12Ala* and *C161T* variants with the risk of PCOS, and with lipid and lipoprotein profiles. In addition, we examined the association of both SNPs with the levels of estradiol and sex hormone binding globulin (SHBG) in a population from West Iran with a Kurdish ethnic background.

Materials and Methods

The present cross-sectional study consisted of 50 women with confirmed PCOS according to the Rotterdam criteria (13), who referred to the Kermanshah University of Medical Sciences Clinic between April and October 2015. The mean age of PCOS women was 23.6 ± 5.3 years (ranging between 14 and 43 years). A total of 233 unrelated age-matched healthy individuals without PCOS were also included in this study with the mean age of 22.2 ± 4.2 years, (ranging between 18 and 33 years, P=0.09). Controls were volunteers from students and staff of Kermanshah University of Medical Sciences without any history of hyperandrogenism reflected by the presence of hirsutism, acne or alopecia and menstrual irregularity.

Two out of three criteria of clinical and/or biochemical signs of PCOS, namely hyperandrogenism (the presence of hirsutism), acne or alopecia and ovarian dysfunction (oligo- and/or anovulation and/or polycystic ovaries detected by ultrasound scans) were sufficient to diagnose PCOS. Exclusion criteria were congenital adrenal hyperplasia, androgen-secreting tumors, and intake of any medication that may affect the endocrinal parameters along with the glucose and lipid profiles for at least 3 months prior to enrolment.

Height and weight were obtained from each individual and the body mass index (BMI) was calculated. All women in this study were from the Kermanshah province in West Iran, belonging to the Kurdish ethnicity.

All individuals agreed to participate in the study and signed a written informed consent before participation. The Ethics Committee of Kermanshah University of Medical Sciences approved the study. The study was in accordance with the principles of the Declaration of Helsinki II.

Biochemical analysis

From each individual, a sample of 10 milliliters of venous blood was collected at 9 am under standard conditions. The sample was divided to two portions of six milliliters; portion one was centrifuged for 10 minutes at 1600 g in the absence of any anticoagulant and the obtained serum was used for biochemical analysis according to the standard protocol. The second portion (4 ml) was treated with EDTA and used for DNA extraction and further genetic analysis.

The levels of fasting blood sugar (FBS), triglycerides (TG), cholesterol, low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) were measured using the Bionic Diagnostic Kits (Iran) on Mindray BS-480 Chemistry Analyzer (China). Serum estradiol level in the mid-follicular phase of the menstrual cycle and SHBG were measured using the chemiluminescent method by using the Abbott Architect i1000 (Abbott Laboratory, USA).

Genotyping

DNA was extracted from venous blood using the standard phenol-chloroform method (14). The polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) was used to genotype the *PPARγ Pro12Ala* (C/G) SNP by using specific.

F: 5′-GCCAATTCAAGCCCAGTC-3′
R: 5′-GATATGTTTGCAGACAGTGTATCAGTGAAGGAATCGCTTTCCG-3′ primers.

The PCR reaction in a final volume of 25 µl contained 20 pmol of each primer, 100-200 ng DNA, 200 µM dNTPs, 1.5 mM $MgCl_2$, 1 U Taq polymerase and 2.5 µl of 10X PCR buffer (SinaClon, Iran). The PCR conditions were an initial denaturation at 94°C for 5 minutes followed by 30 cycles of 94°C for 60 seconds, 55°C for 60 seconds and 72°C for 60 seconds, with a final extension for 5 minutes at 72°C. Five microliters of the resulting 270 bp PCR product was examined using electrophoresis on a 1% agarose gel containing the Gel Red (Kawsar Biotech Company , Iran) stain and was visualized under a UV Gel Documentation System (Quantum ST4). Fifteen microliters of the PCR product was treated with 5 U of the restriction enzyme *BstU I* at 37°C overnight and the RFLP products were electrophoresed on a 2% agarose gel (7). The C allele (ancestral) was not digested by the *BstU I* while the C to G substitution resulted in digestion of the PCR product into two fragments of 227 bp and 43 bp (Fig.1).

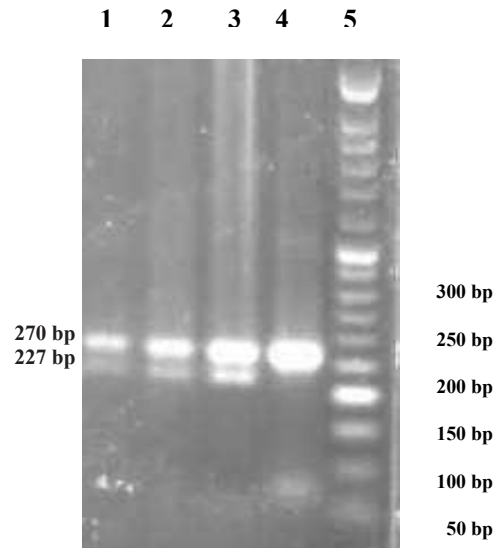

Fig.1: Agarose gel electrophoresis (2%) pattern of digested polymerase chain reaction (PCR) products by the *BstU* I restriction enzyme. From left to right, lanes 1, 2, and 3 represent the *PPARγ* CG genotype, lane 4 indicates the CC genotype and lane 5 shows the 50 bp DNA molecular weight marker.

The *PPARγ C161T SNP* was detected by PCR-RFLP using specific

F: 5′-CAA GAC AAC CTG CTA CAA GC-3′
R: 5′ -TCC TTG TAG ATC TCC TGC AG -3′ primers.

The PCR reaction consisted of 20 pmol of each primer, 100-200 ng DNA, 200 µM dNTPs, 1.5 mM $MgCl_2$, 1 U Taq polymerase and 2.5 µl of 10X PCR buffer in a final volume of 25 µl. The PCR thermal cycling conditions were an initial denaturation at 94°C for 5 minutes, followed by 35 cycles by 94°C for 60 seconds, 55°C for 60 seconds and 72°C for 60 seconds, with a final extension for 5 minutes at 72°C. Five microliters of the resulting 200 bp PCR product was examined using electrophoresis on a 1% agarose gel containing Gel Red stain and visualized under a UV Gel Documentation System (Quantum ST4). Fifteen microliters of the PCR product were treated with 5 U of the restriction enzyme *Pml1* at 37°C overnight and the RFLP products were electrophoresed on a 2% agarose gel (10). The ancestral allele fragment was digested into two fragments of 120 bp and 80 bp, while the derived allele remained intact (Fig.2).

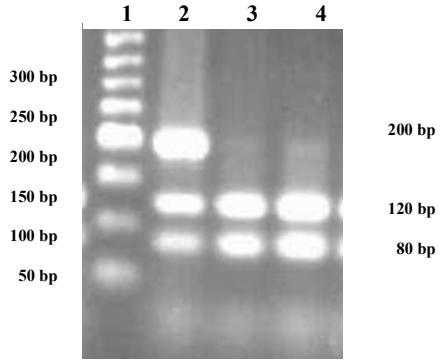

Fig.2: The agarose gel electrophoresis of restriction fragment length polymorphism (RFLP) products obtained by digestion of polymerase chain reaction (PCR) products by the *Pml1* restriction enzyme. From left to right, lanes 1, 2, and 3 and 4 represents the 50 bp DNA molecular weight marker, the CT genotype of *PPARγ C161T* and the wild type genotype of CC.

Statistical analysis

The frequency of alleles was calculated by the chromosome counting method and deviation from the Hardy-Weinberg equilibrium (HWE) was calculated using the Chi-square test. Comparison of genotype and allele frequencies of the two SNPs between PCOS patients and controls was undertaken using the Chi-square test. The SPSS logistic regression was used to calculate odds ratio (OR) as an estimate of relative risk for the disease and its 95% confidence interval (CI). The association between biochemical data and SNPs was calculated using the independent-sample t test and ANOVA. The P<0.05 was considered as statistically significant. The statistical package for social sciences (SPSS, SPSS Inc., Chicago, IL) version 16.0 was used for the statistical analysis.

Results

Demographic and biochemical characteristics of the participants are presented in Table 1. Patients were age-matched with controls (P=0.09). Also, the two groups were BMI-matched (P=0.25, Table 1). A significantly lower serum level of estradiol was observed in PCOS women compared with controls (70 ± 45.5 vs. 109.7 ± 91.2 pg/ml respectively, P<0.001). However, a lower level of SHBG was observed in patients (52.2 ± 24.5) compared with controls (58.6 ± 33.9) but was not statistically significant (Table 1).

Table 1: Characteristics of PCOS patients and controls

Variable	Patient n=50 Mean ± SD	Control n=233 Mean ± SD	P value
Age (Y)	23.6 ± 5.3	22.2 ± 4.2	0.09
BMI (Kg/m^2)	23.7 ± 4.9	22.8 ± 5.8	0.25
FBS (mg/dl)	78.6 ± 13.2	78.5 ± 14.8	0.97
Cholesterol (mg/dl)	131.1 ± 32.8	129.7 ± 30.6	0.78
TG (mg/dl)	78.8 ± 43.2	88 ± 51.5	0.25
HDL-C (mg/dl)	45.6 ± 11.7	46.5 ± 12.8	0.61
LDL-C (mg/dl)	74 ± 26.6	74.8 ± 24.5	0.82
Estradiol (pg/ml)	70 ± 45.5	109.7 ± 91.2	<0.001
SHBG (nmol/l)	52.2 ± 24.5	58.6 ± 33.9	0.13

PCOS; Polycystic ovary syndrome, BMI; Body mass index, FBS; Fasting blood sugar, TG; Triglycerides, HDL-C; High density lipoprotein-cholesterol, LDL-C; Low density lipoprotein-cholesterol, and SHBG; Sex hormone binding globulin.

The genotypic distribution of *PPARγ Pro12Ala* was in HWE in both patients and controls (P>0.1). However, the genotypic distribution of *PPARγ C161T* significantly deviated only in the control group (χ^2=5.03, P<0.05).

The genotype and allele frequencies of both SNPs are given in Tables 2, 3. The frequency of the CG genotype in patients was 32% and significantly higher than that in controls (13.7%, P=0.002, OR=2.96 (95% CI of 1.46-5.96) (Table 2). Given that the control group deviated from Hardy-Weinberg equilibrium for the C161T SNP, no further analysis was undertaken on the potential association of this SNP with PCOS.

Table 2: The frequency of *PPARγ Pro12Ala* (C/G) genotypes and alleles in patients and controls

Parameter	Patient n=50 (%)	Control n=233 (%)
Genotypes		
CC	34 (68)	201 (86.3)
CG	16 (32)	32 (13.7)
	χ^2=9.75, P=0.002, OR=2.96 , (95% CI: 1.46-5.96, P=0.002)	
Alleles		
C	84 (84)	434 (93.1)
G	16 (16)	32 (6.9)
	χ^2=9.75, P=0.002, OR=2.96 (95% CI: 1.46-5.96, P=0.002)	

OR; Odds ratio and CI; Confidence interval.

The effect of both polymorphisms on lipid and lipoprotein profiles along with estradiol and SHBG levels in all studied individuals is shown in Table 4. A significantly higher level of TG was detected in the presence of the *PPARγ CG* (101.1 ± 59.4 mg/dl) genotype compared to the CC genotype (76.0 ± 40 mg/dl). Considering the effect

of the *PPARγ C161T* polymorphism on lipid and lipoprotein profiles along with the estradiol level, we observed significantly lower levels of total cholesterol (85.5 ± 24.4 mg/dl, P=0.011) and LDL-C (42.5 ± 12.8 mg/dl, P=0.023) in homozygote TT individuals compared to those with the CC genotype (130.6 ± 30.6 and 75.4 ± 24.5 mg/dl respectively). The SHBG level was not significantly different between different genotypes of the two SNPs (Table 4).

Table 3: The genotype and allele frequencies of *PPARγ C161T* in the patient and control groups

Parameter	Patient n=50 (%)	Control n=233 (%)
Genotypes		
CC	31 (62)	155 (66.5)
CT	17 (34)	76 (32.6)
TT	2 (4)	2 (0.9)
	χ^2=3.05, P=0.21	
Alleles		
C	79 (79)	386 (82.8)
T	21 (21)	80 (17.2)
	χ^2=0.4, P=0.52	

When each group was studied separately, the association of the *PPARγ CG* genotype, compared with the CC genotype, with significantly lower level of estradiol was only observed in the PCOS group (54.3 ± 28.9 pg/ml vs.77.9 ± 50.5 pg/ml, P=0.045). Also, a significantly higher level of TG was associated with the CG genotyped compared to the CC genotype (115.6 ± 62.4 and 74.6 ± 39.9 mg/dl respectively, P=0.026).

Discussion

We identified an association between the *PPARγ Pro12Ala CG* genotype and the risk of PCOS in our population. We did not detect the GG genotype among our studied individuals because the homozygote Ala genotype is rare in the overall population (7).

There are inconsistent reports on the association of *PPARγ* SNPs with susceptibility to PCOS. This may be due to different frequencies of this SNP among different populations, but also different lifestyle, effects of environmental factors and also the influence of sample size.

In a study from Germany, the frequency of the *PPARγ Pro12Ala* SNP was not significantly different between PCOS and healthy women (7). Also, among Italians, the Pro12Ala SNP was unrelated to the risk of PCOS (5). However, among PCOS patients of Indian origin, the *PPARγ Pro12Ala* was associated with decreased PCOS susceptibility. However, the *PPARγ C161T* (His44His) did not affect the risk of PCOS among Indian (8) Caucasian (11) and Greek (12) women. In contrast, among the Italians, there was a significantly higher frequency of *PPARγ T* allele in PCOS patients than in controls (5). Meta-analysis by Zhang et al. (15) indicated that the Pro12Ala polymorphism reduced the risk of PCOS only in European but not in Asian populations.

Table 4: Mean number of primordial, primary, growing, atretic graafian follicles, graafian follicles and corpora lutea in the ovaries of rats in the experimental and control groups

Variable	*PPAR Pro12Ala* (C/G)		*PPAR C161T*		
	CC (n=235)	CG (n=48)	CC (n=186)	CT (n=93)	TT (n=4)
FBS (mg/dl)	78.7 ± 15	77.3 ± 11.6	79.4 ± 15.8	77.3 ± 11.3	63.3 ± 9.1
	P=0.61			P=0.47	P=0.06
Cholesterol (mg/dl)	129.0 ± 30.5	134.1 ± 32.9	130.6 ± 30.6	130.6 ± 30.7	85.5 ± 24.4
	P=0.33			P=1	P=0.011*
					P=0.012**
TG (mg/dl)	76.0 ± 40	101.1 ± 59.4	81.7 ± 46.4	79.8 ± 42.1	40.3 ± 18.4
	P=0.007			P=0.94	P=0.16
HDL-C (mg/dl)	46.8 ± 13	44.3 ± 10.6	46.9 ± 13.1	45.7 ± 11.7	36.0 ± 10.4
	P=0.16			P=0.71	P=0.2
LDL-C (mg/dl)	74.3 ± 24.4	76.1 ± 26.9	75.4 ± 24.5	74.5 ± 25.1	42.5 ± 12.8
	P=0.67			P=0.95	P=0.023*
					P=0.031**
Estradiol (pg/ml)	103.8 ± 86.6	96.8 ± 84.1	102.3 ± 86.5	103.6 ± 87	91.6 ± 57.2
	P=0.61			P=0.99	P=0.96
SHBG (nmol/l)	58.3 ± 33.8	53.2 ± 24.6	58.3 ± 34.2	55.2 ± 28.9	72.9 ± 32.1
	P=0.24			P=0.44	P=0.4

Data are presented as mean ± SD.*; Compared with the CC genotype, **; Compared with the CT genotype, FBS; Fasting blood sugar, TG; Triglycerides, HDL-C; High density lipoprotein-cholesterol, LDL-C; Low density lipoprotein-cholesterol, and SHBG; Sex hormone binding globulin

The PPARγ is a critical transcription factor involved in regulating glucose and lipid metabolism (16). The PPARγ is involved in energy regulation and fat deposition, and is recognized as an important gene contributing to obesity, obesity induced insulin resistance and dyslipidemia (8). The natural ligands of PPARs are unsaturated fatty acids, eicosanoids, oxidized LDL and VLDL, and linoleic acid derivatives. Fibrates and thiazolidinediones are pharmacological agonists of PPARs (17). Although we showed significant associations between the *PPARγ Pro12Ala* SNP and the lipid and lipoprotein profiles in a Kurdish population, this was not observed in a German population (7). Also, in a PCOS patient group of Italian origin, no significant difference in adiponectin, HDL-C, LDL-C and TG levels was observed between ancestral and variant genotypes of this SNP (5). In contrast, in PCOS women from Korea, a significantly increased HDL-C level was detected in individuals carrying the variant allele (9).

The small sample size of the studied PCOS patient group is the main limitation of the present study which may affect the association observed between *PPARγ* genotypes and PCOS, lipid and lipoprotein profiles, and estradiol and SHBG levels.

Conclusion

Our study showed an association between *PPARγ Pro12Ala* and the risk of PCOS while no influence of *PPARγ C161T* on susceptibility to PCOS was observed. Also, we found that the lipid and lipoprotein profiles are affected by the presence of *PPARγ Pro12Ala* and *C161T* polymorphisms. The ancestral CC genotype of C161T had a lowering effect on the TG level and the minor T allele had a beneficial effect in lowering cholesterol and LDL-C. In PCOS patients the variant CG genotype of Pro12Ala was associated with a lower level of estradiol and a higher concentration of TG.

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Author's Contributions

Z.R.; Designed the study, interpreted the results and critically revised the manuscript. F.C.-N., S.S., Z.R., A.E.; Provided the samples and analyzed the data. E.S.; Wrote the preliminary draft of manuscript. A.V.-R.; Performed the statistical analysis. All authors read and approved the final manuscript.

References

1. Kauffman RP, Baker TE, Baker VM, DiMarino P, Castra-cane VD. Endocrine and metabolic differences among phenotypic expressions of polycystic ovary syndrome ac-cording to the 2003 Rotterdam consensus criteria. Am J Obstet Gynecol. 2008; 198(6): 670. e1-e7
2. Kiranmayee D, Kavya K, Himabindu Y, Sriharibabu M, Madhuri GLJ, Venu S. Correlations between anthropometry and lipid profile in women with PCOS. J Hum Reprod Sci. 2017; 10(3): 167-172.
3. Diamanti-Kandarakis E, Papavassiliou AG. Molecular mechanisms of insulin resistance in polycystic ovary syndrome. Trends Mol Med. 2006; 12(7): 324-332.
4. Amr K, Abdel-Hameed M, Sayed K, Nour-Edin F, Abdel Hay R. The Pro12Ala polymorphism of the gene for peroxisome proliferator activated receptor-gamma is associated with a lower global acne grading System score in patients with acne vulgaris. Clin Exp Dermatol. 2014; 39 (6): 741-745.
5. Orio F Jr, Matarese G, Di Biase S, Palomba S, Labella D, Sanna V, et al. Exon 6 and 2 peroxisome proliferator-activated receptor-gamma polymorphisms in polycystic ovary syndrome. J Clin Endocrinol Metab. 2003; 88 (12): 5887-5892.
6. Korhonen S , Heinonen S, Hiltunen M, Helisalmi S, Hippelainen M, Koivunen R, et al. Polymorphism in the peroxisome proliferator-activated receptor-g gene in women with polycystic ovary syndrome. Hum Reprod. 2003; 18 (3): 540-543.
7. Hahn S, Fingerhut A, Khomtsiv U, Khomtsiv L, Tan S, Quadbeck B, et al. The peroxisome proliferator activated receptor gamma Pro12Ala polymorphism is associated with a lower hirsutism score and increased insulin sensitivity in women with polycystic ovary syndrome. Clin Endocrinol (Oxf). 2005; 62 (5): 573-579.
8. Shaikh N, Mukherjee A, Shah N, Meherji P, Mukherjee S. Peroxisome proliferator activated receptor gamma gene variants influence susceptibility and insulin related traits in Indian women with polycystic ovary syndrome. J Assist Reprod Genet. 2013; 30 (7): 913-921.
9. Chae SJ, Kim JJ, Choi YM, Kim JM, Cho YM, Moon SY. Peroxisome proliferator-activated receptor-gamma and its coactivator-1alpha gene polymorphisms in Korean women with polycystic ovary syndrome. Gynecol Obstet Invest. 2010; 70(1): 1-7.
10. Krasznai M, Szaniszlo K, Kraxner H, Vargha E, Kovacs M, Kaszas E, et al. Association of PPAR polymorphisms with cytokine levels in allergic rhinitis. Eur Arch Otorhinolaryngol. 2012; 269 (2): 513-521.
11. Antoine HJ, Pall M, Trader BC, Chen YD, Azziz R, Goodarzi MO. Genetic variants in peroxisome proliferator-activated receptor gamma influence insulin resistance and testosterone levels in normal women, but not those with polycystic ovary syndrome. Fertil Steril. 2007; 87(4): 862-869.
12. Christopoulos P, Mastorakos G, Gazouli M, Deligeoroglou E, Katsikis I, Diamanti-Kandarakis E, et al. Peroxisome proliferator-activated receptor-γ and -δ polymorphisms in women with polycystic ovary syndrome. Ann N Y Acad Sci. 2010; 1205: 185-191.
13. The Rotterdam ESHRE/ASRM-Sponsored Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004; 19(1): 41-47.
14. Rahimi Z, Merat A, Gerard N, Krishnamoorthy R, Nagel RL. Implications of the genetic epidemiology of globin haplotypes linked to the sickle gene in Southern Iran. Hum Biol. 2006; 78 (6): 719-731.
15. Zhang H, Bi Y, Hu C, Lu W, Zhu D. Association between the Pro12Ala polymorphism of PPARγ gene and the polycystic ovary syndrome: a meta-analysis of case-control studies. Gene. 2012; 503(1): 12-17.
16. Wan J, Xiong S, Chao S, Xiao J, Ma Y, Wang J, et al. PPAR gamma gene C161T substitution alters lipid profile in Chinese patients with coronary artery disease and type 2 diabetes mellitus. Cardiovasc Diabetol. 2010; 9: 13.
17. Gupta M, Mahajan VK, Mehta KS, Chauhan PS, Rawat R. Peroxisome proliferator-activated receptors (PPARs) and PPAR agonists: the 'future' in dermatology therapeutics? Arch Dermatol Res. 2015; 307 (9): 767-780.

Original Article

Molecular Evidence of *Chlamydia trachomatis* Infection and Its Relation to Miscarriage

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Abstract

Background: *Chlamydia trachomatis* (CT) infection is the most common sexually transmitted disease in the world that can persist and also ascend in the genital tract. This intracellular and silent infection is related to some adverse pregnancy outcomes, such as miscarriage. The aims of this study were to explore the best CT screening tests using blood and vaginal samples and to investigate the correlation between CT infection and the incidence of miscarriage.

Materials and Methods: This case-control study was done in October 2013 through June 2014, using purposive sampling from 157 female participants with or without a history of miscarriage. The samples were taken after each participant had signed a letter of consent and had completed a questionnaire. To achieve the objectives of this study, polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) tests were performed on vaginal swabs and blood samples, respectively.

Results: PCR results showed a significantly higher CT infection rate in the miscarriage group compared to the control group (11.3 vs. 0%, P=0.007). Anti-CT IgG and IgA antibodies were found in 4.2 and 2.1% of cases in the miscarriage group, and in 1.7 and 6.7% of cases in the control group, respectively (P>0.05). Despite lower humoral responses in this study, positive samples were detected only by one of the following techniques; PCR, ELISA IgA and ELISA IgG. It also should be noted that PCR worked best in terms of detection.

Conclusion: Based on the obtained data, there is a strong association between molecular evidence of CT infection and miscarriage. A higher rate of CT detection in molecular tests compared to serological assays suggests that PCR could be used as the first-choice assay for detection of *C. trachomatis*. However, the importance of serological tests in detecting potential past CT infection or upper genital infection not amenable to sampling is undeniable.

Keywords: *Chlamydia trachomatis*, Enzyme-Linked Immunosorbent Assay, Miscarriage, Polymerase Chain Reaction

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Introduction

Although *chlamydiae* were discovered in 1907, chlamydial disease was known for centuries before that. *Chlamydia trachomatis* (CT) is a Gram-negative, non-motile and obligate intracellular bacterium, which causes one of the most prevalent sexually transmitted diseases called chlamydia (1). The World Health Organisation estimated that compared to the year 2005, 131 million new cases of urogenital CT infection have occurred in women and men aged 15-49 years globally in the year 2012 (2).

This genital infection can result in adverse reproductive outcomes such as infertility, premature delivery, ectopic pregnancy, low birth weight, and miscarriage (3). Despite significant progress in medical sciences, many miscarriages still occur. Miscarriage is the most common sequela of pregnancy, which is defined as pregnancy loss before the 24th week of gestation (4). Nonetheless, in most cases, the causes of miscarriage are unknown. Nonaka et al. (5) have reported that prevalence of chromosomal aberrations in patients with a history of recurrent spontaneous abortion is less than those who have sporadic miscarriage. Also, in women with recurrent spontaneous abortion, 25-32% of conception products have abnormal karyotypes. On the one hand, chromosomal aberrations are observed in approximately 50% of early miscarriages. On the other hand, infections have been attributed to 15% of early miscarriages and 66% of late miscarriages (6).

Enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) are two common methods for detection of CT. In 2003-2006, a group in Poland evaluated the frequency of CT infection in wom-

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en suffering from spontaneous miscarriage by PCR and ELISA IgG and IgA (7). Also, the same serologic and molecular tests were used to investigate whether CT is related to miscarriage in Switzerland (8). Results of a serologic study for diagnosis of CT in sub fertile women suggests that MOMP-based (major outer membrane protein of CT used as antigen) ELISA is equally suitable, if not slightly better, than micro-immunofluorescence assays in terms of sensitivity and specificity (9). For detection of CT in laboratories, Nucleic Acid Amplification tests have been reported to function better than other available tests because of their sensitivity and specificity (8). Since the method of choice for detection of CT is ELISA in most clinics, this study was done to evaluate the validity of this method to improve the screening program for detection of this infection. Moreover, the correlation between CT infection and the incidence of miscarriage was investigated.

Materials and Methods

This study was a case-control study and samples were collected starting in October 2013 through June 2014. The sample size was calculated with regard to the reported prevalence of CT (10), 95% confidence interval and 80% power. The control group comprised 60 pregnant women without any miscarriage history, ranging from 20 to 40 years of age (mean 27.85 ± 5.14 years), who attended a pregnancy assessment unit. The miscarriage groups included 55 women with 1-2 and 42 women with ≥3 miscarriages, ranging from 19 to 45 years of age (mean 30.88 ± 5.9 years), who were referred to a Fertility Centre in Isfahan, Iran. In the miscarriage group, samples were taken after the last miscarriage (4-24 weeks of gestation) and termination of bleeding.

All participants were married and had one sex partner. Local Ethical Committee approval and participants' consent were obtained. A questionnaire containing demographic information, anti-biotherapy history, and previous adverse pregnancy outcome was completed by participants. The criteria for participant selection were no use of any chlamydia-related antibiotics during the last three months, no bleeding, and submitting a completed questionnaire. In order to exclude cases who most likely had genetic problems, questions regarding possible products of conception with congenital malformation and developmental delay, past karyotype tests, and a history of infertility and genetic disorders in family members were asked in the questionnaires.

Sample collection

Vaginal samples were collected using sterile cotton swabs and were conserved in phosphate buffer saline (PBS) at -70°C until tested. Blood samples were collected in 5-ml volumes and the sera were separated by centrifugation at 2500 rpm (1090×g). All the sera were aliquoted into several tubes to avoid excessive freeze-thaw cycles and were stored at -20°C prior to analysis.

DNA extraction

We used boiling method to extract DNA from vaginal samples, since it has been reported as a rapid and cost-effective method with a high DNA efficiency (11). Briefly, after removing vaginal swabs from Falcon centrifuge tubes (Aratebfan, Tehran), the remaining PBS solution was centrifuged at 2000 rpm (700×g) for 15 minutes. The supernatant was then discarded and the pellet was vortexed and transferred to a 1.5 ml micro-tube. To fully remove the PBS, the micro-tube was also micro-centrifuged at 2000 rpm (295×g) for 15 minutes. After draining the supernatant fluid from the tubes, 400 µl of Tris base-EDTA (TE) buffer containing 1 mol l-1 Tris base (pH=8.0) and 0.5 mol l-1 EDTA was added to each sample. The suspension was boiled in a water bath for 10 minutes and then centrifuged at 10000 rpm (7378×g) for 10 minutes. Subsequently, the supernatant containing extracted DNA was harvested and stored at -20°C.

Beta-globin polymerase chain reaction

The presence of human cells and the absence of inhibitory elements in the extracted DNA were evaluated by amplification of a 268-bp fragment of the *beta-globin* gene. Primers used in this step were:

PCO4: 5′-CAACTTCATCCACGTTCACC-3′
GH20: 5′-GAAGAGCCAAGGACAGGTAC-3′ (11).

PCR was carried out on 2 µl of the extracted DNA samples in a 25 µl reaction volume consist of 20 pmol of each primer, 2 mM $MgCl_2$, 0.3 mM dNTP and 1 U of Taq DNA polymerase. All PCR reagents were purchased from Cinna Gene Company (Tehran, Iran). The PCR protocol was as follows: an initial step 10 minutes at 95°C; 30 cycles of 1 minute at 94°C, 1 minute at 58°C, and 1 minute at 72°C; and a final step 8 minutes at 72°C.

C. trachomatis plasmid polymerase chain reaction

To detect *Chlamydia trachomatis* in the validated DNA samples, a 241-bp fragment of chlamydial cryptic plasmid was amplified. Relevant primers for this PCR were:

KL1: 5′-TCCGGAGCGAGTTACGAAGA-3′
KL2: 5′-AATCAATGCCCGGGATTGGT-3′ (12).

PCR was performed on a final volume of 25 µl containing 5 µl DNA, 6 pmol of each primer (Genfanavaran, Iran), 3 mM $MgCl_2$, 0.2 mM dNTP and 1 U of Taq DNA polymerase were used for each experiment. The PCR protocol was as follows: an initial step 2 minutes at 95°C; 30 cycles of 30 seconds at 95°C, 30 seconds at 58.3°C, and 30 seconds at 72°C; and a final step 5 minutes at 72°C.

Serological assays

Serum samples were tested by MOMP-based ELISA kits (Euroimmun, Germany) to detect anti-CT IgA and IgG antibodies. All steps were performed according to the manufacturer's instructions. The IgA kit used in this experiment had 100% sensitivity and 97.4% specificity and

the IgG kit had 78.2% sensitivity and 97.1% specificity. There was no cross reactivity with other *Chlamydia pneumoniae* positive samples for the kits.

Statistical analysis

This was a case-control study and data analysis was carried out using GraphPad Prism version 6.07 for Windows. The Chi-square and Fishers exact tests were used for analysing diagnostic findings (PCR, IgA and IgG). Student's t test was used to determine the mean and the standard deviations for comparing ages among participants with and without miscarriage. P<0.05 were considered statistically significant.

Results

PCR and ELISA were performed on vaginal swabs and blood samples of 157 participants, respectively. Then the relationship between the number of previous miscarriages and the prevalence of CT infection was evaluated. The number of miscarriages was given under three categories (0, 1-2, and ≥ 3 miscarriages).

Detection of *Chlamydia trachomatis* by polymerase chain reaction

Internal control PCR showed that all samples were free of inhibitory elements (Fig.1). In both miscarriage groups together 11.3% of the patients were positive for CT infection, where all of the 60 women in the control group were tested negative (Fig.2). Detailed data with statistical analysis are shown in Table 1.

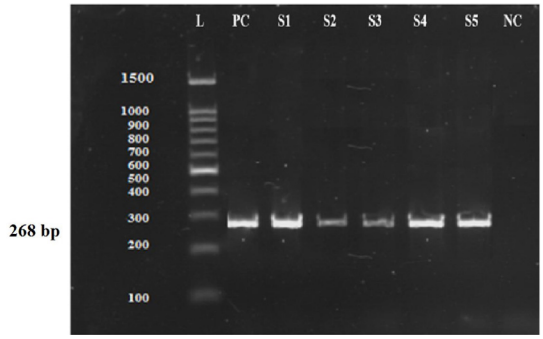

Fig.1: Human beta globin polymerase chain reaction (PCR) as internal control. Gel electrophoresis of amplified human *beta globin* gene presenting 268 bp amplicons in 157 extracted samples. L; 100 bp ladder, PC; Positive control, S1-S5; Samples, and NC; Negative control.

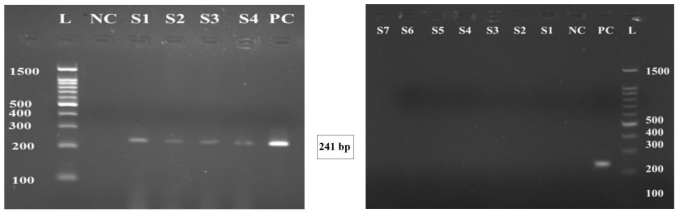

Fig.2: *Chlamydia trachomatis* (CT) plasmid polymerase chain reaction (PCR). Gel electrophoresis of amplified CT plasmid presenting 241 bp amplicons. The gel electrophoresis results on the left show the presence of CT infection in women in the miscarriage group. The gel electrophoresis results on the right present the absence of CT infection in the participants. L; 100 bp ladder, PC; Positive control, S1-S6; Samples, and NC; Negative control.

Table 1: The number of *C. trachomatis* positive cases in the control and both miscarriage groups together

Diagnostic tools	Miscarriage group n=97	Control group n=60	P value
PCR+	11	0	0.007
IgG+	4	1	0.649
IgA+	2	4	0.203

The results of the relationship between the number of previous miscarriages and prevalence of CT infection are shown in Table 2. According to the PCR data, none of the participants without a history of miscarriage were positive for CT infection. On the other hand, 5 out of 55 women with 1-2 miscarriages and 6 out of 42 women with three or more miscarriages were positive for CT as indicated by PCR. The difference between these three categories was statistically significant.

Table 2: Results reported for *C. trachomatis* infection by three diagnostic tools in women regarding their history of previous miscarriages

Number of miscarriages	Count	PCR^{+} (%)	IgG^{+} (%)	IgA^{+} (%)
0*	60	0	1.7	6.7
1-2	55	9.1	5.4	0
≥3	42	14.3	2.4	4.8
P value**		0.004	0.744	0.494

*; Pregnant women with no miscarriage and **; χ^2 for trend for each diagnostic method.

Three out of 38 women (7.9%) in ≤25-year age group, 4 out of 97 women (4.1%) in 26-35-year age group, and four out of 22 women (18%) in 36-45-year age group were positive for CT by PCR. Evaluation of association of mother's age with CT infection revealed that there was a significantly higher correlation between 36-45-year age group and CT infection compared to other age groups, as indicated by PCR (P=0.042).

Detection of *Chlamydia trachomatis* in women by ELISA IgA and IgG

In the miscarriage groups, 4.1 and 2.1% of women were positive for CT IgG and IgA antibodies, respectively. However, in the control group, these ratios were 1.7 and 6.7% of the cases (Table 1). The statistical analysis did not show any significant relationship between miscarriage and the detection of IgG or IgA chlamydial antibodies compared to the control group.

The relationship between the number of previous miscarriages and the prevalence of anti-CT antibodies was evaluated by ELISA IgA and IgG as well (Table 2). In terms of the prevalence of CT IgA antibodies, 4 out of 60 women without miscarriage history, and 2 out of 42 women with three or more previous miscarriages were CT positive. However, CT IgA antibody was not found in the women with 1-2 miscarriages. By comparing these three categories, we did not obtain any statistical significance.

CT IgG antibodies were detected in 1 out of 60 women without miscarriage history, 3 out of 55 women with 1-2 miscarriages and 1 out of 42 women with three or more miscarriages. The difference among these three categories was not statistically significant.

Discussion

According to our PCR results, there is a positive relationship between miscarriage and underlying CT infection. The association between molecular evidence of CT infection and miscarriage has been reported by previous studies (8, 13). Also, in another study in Australia, miscarriage was attributed to the presence of chlamydia and gonorrhoea detected by PCR before pregnancy (14).

Furthermore, in our study a significant molecular relationship was shown between the 36-45-year age group and the incidence of chlamydia positivity, which was in agreement with other studies. In 2010 Jenab et al. (11), reported a correlation between CT infection and 35-45-year age group, in a study on asymptomatic and symptomatic women in Isfahan, Iran. In a study in West Midlands, UK, it was found that there is a remarkable increase in the rate of STIs even in older adults, aged ≥45 years old (15). Also, Parish et al. (16) found that CT infection is concentrated in the 25-44-year age range in China. It has been reported that in China and other Asian societies, onset of sexually transmitted diseases can be late due to sexual activity beginning after reaching adolescence. Nonetheless, it has been observed in some researches that CT infection is more frequent in younger ages (17, 18). To the best of our knowledge, there is not a very clear reason for the incidence of CT infection in older ages.

Our ELISA results showed no significant relationship between the number of previous miscarriages and CT infection, which was in accordance with earlier serologic studies on women suffering from recurrent spontaneous abortion (19-21). However, in two other studies it was reported that there was an association between experienced miscarriages and IgG antibodies to CT (7, 22). We observed low prevalence of CT IgG and IgA antibodies in this studied population. Its reason might be CT serotype replacement with fewer immunogenic types leading to lower antibody levels over time (23). Likewise, a 20-year long timed study in Finland showed decreased CT sero-prevalence and increased current infection prevalence detected by nucleic acid amplification tests over time (24). The reason can be reinfection due to untreated sex partner. In addition, immunity to CT is serovar-specific, partial and short-term (25), which can raise the rate of acute infection in women.

Despite the accuracy of the tests, the CT-positive samples were surprisingly confirmed by only one of our three diagnostic tools (PCR, ELISA IgG and ELISA IgA). For example, all PCR-positive samples were IgG/IgA-negative or IgG-positive samples were PCR/IgA-negative. This contradiction may happen due to different reasons. Positive serologic and negative molecular detection of CT may be due to an old infection or resolution of CT (26, 27) or relocation of CT from the lower to the upper genital tract (28, 29). Moreover, positive molecular and negative serologic detection of CT can be due to further lower genital infection, very low organism concentration in the upper genital tract and below the immune system detection level (30), delayed or even absent CT humoral responses in serum in spite of clinical symptoms (31, 32), early antibiotic consumption leading to persistent or chronic infection before recognizing the bacterium by the immune system (arrested immunity) (33), decrease in the anti-CT antibodies titre below the ELISA detection level (34), or primary infection. Also, in another study there were pregnant women who were positive for endo-cervical CT IgA, but negative for CT DNA, possibly due to a recently cleared CT infection, upper genital infection or a positive cervical serology caused by blood contamination containing CT antibodies (35). Therefore, IgA antibodies do not indicate recent CT infection. Instead, according to several studies, they have been attributed to chronic or persistent CT infections (28).

At present, these alternative explanations for the discrepancy between molecular and serological results are not evident at this point, as they are case-dependent. This reflects the unique adaptive immunity in the genital tract compared to other mucosal sites. There is an association between specific host immune responses and susceptibility to or protection from CT infection (36). In fact, individual's immune system defines that CT resolves, enters the resistance phase or reinfection occurs. The pregnancy itself is a reason of changes in the host immune responses (37). Also, person-to-person variation in responding to CT infection is due to the women's genital tract specific microbiota (38). Perhaps a long-term follow up with a larger number of participants will lead to more definitive explanations for the discrepancy between the test results.

Conclusion

Taken together, to improve the precision and the efficiency of chlamydia detection in the current CT screening tests in clinical laboratories (usually ELISA), it is recommended that molecular tests, such as PCR, be performed as gold standard tests. Moreover, serological tests are helpful in evaluating disease conditions to differentiate ongoing from past damages caused by CT. In cases of past CT infections or upper genital infections not amenable to sampling, a serological test is an effective method to detect the infection and its importance has not faded. However, PCR is the test of choice to detect current CT infection, CT infection at the earliest days of transmission, and persistent CT infection in arrested-immunity cases. Thus, the inclusion of CT molecular and serological screening tests to other pregnancy and prenatal tests, which could allow for early detection and treatment of this infection, would decrease adverse reproductive outcomes.

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Author's Contributions

R.R.; Contributed to the conception, design, supervision, and revision of the manuscript. S.B.; Contributed to all experimental work, data acquisition, data analysis, interpretation, and writing the manuscript. N.G.; Contributed to critically reviewing the article. P.G., M.H.N.E.; Contributed to the sample collection. All authors read and approved the final manuscript.

References

1. Igietseme JU, Black CM. Chlamydial infection: a clinical and public health perspective. Chlamydial infection: a clinical and public health perspective. Switzerland: Karger Medical and Scientific Publishers; 2013.
2. Newman L, Rowley J, Vander Hoorn S, Wijesooriya NS, Unemo M, Low N, et al. Global estimates of the prevalence and incidence of four curable sexually transmitted infections in 2012 based on systematic review and global reporting. PLoS One. 2015; 10(12): 1-17.
3. Mylonas I. Female genital Chlamydia trachomatis infection: where are we heading? Arch Gynecol Obstet. 2012; 285(5): 1271-1285.
4. Rai R, Regan L. Recurrent miscarriage. Lancet. 2006; 368(9535): 601-611.
5. Nonaka T, Ooki I, Enomoto T, Takakuwa K. Complex chromosomal rearrangements in couples affected by recurrent spontaneous abortion. Int J Gynaecol Obstet. 2015; 128(1): 36-39.
6. Giakoumelou S, Wheelhouse N, Cuschieri K, Entrican G, Howie SE, Horne AW. The role of infection in miscarriage. Hum Reprod Update. 2016; 22(1): 116-133.
7. Wilkowska-Trojniel M, Zdrodowska-Stefanow B, Ostaszewska-Puchalska I, Redźko S, Przepieść J, Zdrodowski M. The influence of Chlamydia trachomatis infection on spontaneous abortions. Adv Med Sci. 2009; 54(1): 86-90.
8. Baud D, Goy G, Jaton K, Osterheld MC, Blumer S, Borel N, et al. Role of Chlamydia trachomatis in miscarriage. Emerg Infect Dis. 2011; 17(9): 1630-1635.
9. Muvunyi MC, Claeys L, De Sutter T, De Sutter P, Temmerman M, Van Renterghem L, et al. Comparison of four serological assays for the diagnosis of Chlamydia trachomatis in subfertile women. J Infect Dev Ctries. 2011; 6(5): 396-402.
10. Sotoodeh Jahromi A, Farjam MR, Mogharrab F, Amiryan M, Jamshidi Makiani M, Madani A, et al. Chlamydia trachomatis in women with full-term deliveries and women with abortion. Am J Infect Dis. 2010; 6(3): 66-69.
11. Jenab A, Roghanian R, Golbang N, Golbang P, Chamani-Tabriz L. Comparison of three methods of DNA extraction in endocervical specimens for Chlamydia trachomatis infection by spectrophotometry, agarose gel, and PCR. Arch Immunol Ther Exp. 2010; 58(3): 227-234.
12. Fatholahzadeh B, Bahador A, Haghighi Hasanabad M, Bazarjani F, Haghighi F. Comparative screening of Chlamydia trachomatis infection in women population in Tehran, Iran. Iran Red Crescent Med J. 2012; 14(5): 289-293.
13. Ahmadi A, Khodabandehloo M, Ramazanzadeh R, Farhadifar F, Roshani D, Ghaderi E, Farhangi N. The relationship between Chlamydia trachomatis genital infection and spontaneous abortion. J Reprod Fertil. 2016; 17(2): 110.
14. Campbell S, Lynch J, Esterman A, McDermott R. Pre-pregnancy predictors linked to miscarriage among Aboriginal and Torres Strait Islander women in North Queensland. Aust N Z J Public Health. 2011; 35(4): 343-351.
15. Bodley-Tickell AT, Olowokure B, Bhaduri S, White DJ, Ward D, Ross JD, et al. Trends in sexually transmitted infections (other than HIV) in older people: analysis of data from an enhanced surveillance system. Sex Transm Infect. 2008; 84(4): 312-317.
16. Parish WL, Laumann EO, Cohen MS, Pan S, Zheng H, Hoffman I, et al. Population-based study of chlamydial infection in China: a hidden epidemic. JAMA. 2003; 289(10): 1265-1273.
17. Baud D, Zufferey J, Hohlfeld P, Greub G. Performance of an automated multiplex immunofluorescence assay for detection of Chlamydia trachomatis immunoglobulin G. Diagn Microbiol Infect Dis. 2014; 78(3): 217-219.
18. Rours GI, Duijts L, Moll HA, Arends LR, de Groot R, Jaddoe VW, et al. Chlamydia trachomatis infection during pregnancy associated with preterm delivery: a population-based prospective cohort study. Eur J Epidemiol. 2011; 26(6): 493-502.
19. Paukku M, Tulppala M, Puolakkainen M, Anttila T, Paavonen J. Lack of association between serum antibodies to Chlamydia trachomatis and a history of recurrent pregnancy loss. Fertil Steril. 1999; 72(3): 427-430.
20. Rae R, Smith IW, Liston WA, Kilpatrick DC. Chlamydial serologic studies and recurrent spontaneous abortion. Am J Obstet Gynecol. 1994; 170(3): 782-785.
21. Sugiura-Ogasawara M, Ozaki Y, Nakanishi T, Kumamoto Y, Suzumori K. Pregnancy outcome in recurrent aborters is not influenced by chlamydia IgA and/or G. Am J Reprod Immunol. 2005; 53(1): 50-53.
22. Witkin SS, Ledger WJ. Antibodies to Chlamydia trachomatis in sera of women with recurrent spontaneous abortions. Am J Obstet Gynecol. 1992; 167(1): 135-139.
23. Wikström E, Surcel HM, Merikukka M, Ohman H, Namujju PB, Tasanen K, et al. Changes over time in the Chlamydia trachomatis serotype distribution in Finnish women. Scand J Infect Dis. 2014; 46(5): 397-400.
24. Lyytikäinen E, Kaasila M, Hiltunen-Back E, Lehtinen M, Tasanen K, Surcel HM, et al. A discrepancy of Chlamydia trachomatis incidence and prevalence trends in Finland 1983-2003. BMC Infect Dis. 2008; 8: 169.
25. Batteiger BE, Xu F, Johnson RE, Rekart ML. Protective immunity to Chlamydia trachomatis genital infection: evidence from human studies. J Infect Dis. 2010; 201 Suppl 2: S178-S189.
26. Chavez JM, Vicetti Miguel RD, Cherpes TL. Chlamydia trachomatis infection control programs: lessons learned and implications for vaccine development. Infect Dis Obstet Gynecol. 2011; 2011: 754060.
27. Geisler WM. Duration of untreated, uncomplicated Chlamydia trachomatis genital infection and factors associated with Chlamydia resolution: a review of human studies. J Infect Dis. 2010; 201 Suppl 2: S104-S113.
28. Muvunyi CM, Dhont N, Verhelst R, Temmerman M, Claeys G, Padalko E. Chlamydia trachomatis infection in fertile and subfertile women in Rwanda: prevalence and diagnostic significance of IgG and IgA antibodies testing. Hum Reprod. 2011; 26(12): 3319-3326.
29. Taylor BD, Haggerty CL. Management of Chlamydia trachomatis genital tract infection: screening and treatment challenges. Infect Drug Resist. 2011; 4: 19-29.
30. Hamdad-Daoudi F, Petit J, Eb F. Assessment of Chlamydia trachomatis infection in asymptomatic male partners of infertile couples. J Med Microbiol. 2004; 53(Pt 10): 985-990.
31. Domeika M, Savicheva A, Sokolovskiy E, Frigo N, Brilene T, Hallén A, et al. Guidelines for the laboratory diagnosis of Chlamydia trachomatis infections in East European countries. J Eur Acad Dermatol Venereol. 2009; 23(12): 1353-1363.
32. Mylonas I, Kirschner W, Weissenbacher T, Gingelmaier A, Weissenbacher ER, Friese K. Chlamydia trachomatis infections-a time for action? Dtsch Med Wochenschr. 2007; 132(21): 1170-1176.
33. Brunham RC, Rekart ML. The arrested immunity hypothesis and the epidemiology of chlamydia control. Sex Transm Dis. 2008; 35(1): 53-54.
34. Gopalkrishna V, Aggarwal N, Malhotra VL, Koranne RV, Mohan VP, Mittal A, et al. Chlamydia trachomatis and human papillomavirus infection in Indian women with sexually transmitted diseases and cervical precancerous and cancerous lesions. Clin Microbial Infect. 2000; 6(2): 88-93.
35. Witkin SS, Bongiovanni AM, Inglis SR. Detection of endocervical anti-Chlamydia trachomatis immunoglobulin A in pregnant women by a rapid, 6-minute enzyme-linked immunosorbent assay: comparison with PCR and chlamydial antigen detection methods. J Clin Microbiol. 1997; 35(7): 1781-1783.
36. Darville T, Hiltke TJ. Pathogenesis of genital tract disease due to Chlamydia trachomatis. J Infect Dis. 2010; 201 Suppl 2: S114-S125.
37. Ahmadi A, Khodabandehloo M, Ramazanzadeh R, Farhadifar F, Roshani D, Ghaderi E, et al. The relationship between Chlamydia trachomatis genital infection and spontaneous abortion. J Reprod Fertil. 2016; 17(2): 110-116.
38. Ziklo N, Huston WM, Hocking JS, Timms P. Chlamydia trachomatis genital tract infections: When host immune response and the microbiome collide. Trends Microbiol. 2016; 24(9): 750-765.

Original Article

Melatonin Improves The Developmental Competence of Goat Oocytes

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Abstract

Background: DNA methylation is one the epigenetic mechanisms, which is critically involved in gene expression. This phenomenon is mediated by DNA methyl-transferases and is affected by environmental stress, including *in vitro* maturation (IVM) of oocytes. Melatonin, as an antioxidant, may theoretically be involved in epigenetic regulation via reductions of reactive oxygen species. This study was performed to investigate DNA methylation and the possibility of goat oocyte development after treatment with different concentrations of melatonin.

Materials and Methods: This experimental study was performed to investigate DNA methylation and the possibility of goat oocyte development after treatment with different concentrations of melatonin. For this purpose, oocytes with granulated cytoplasm were selected and co-cultured with at least two layers of cumulus cells in maturation medium with 10^{-6} M, 10^{-9} M, 10^{-12} M and 0-M (as control group) of melatonin. Nucleus status, glutathione content and developmental competence of the oocytes in each experimental group were assessed. Also, expression of genes associated with DNA methylation, including DNA methyltransferase 1 (*DNMT1*), DNA methyltransferase 3b (*DNMT3b*) and DNA methyltransferase 3a (*DNMT3a*) was evaluated by quantitative real time-polymerase chain reaction (RT-PCR).

Results: According to our findings, the percentage of oocytes that reached the M-II stage significantly increased in the 10-12 M group ($P<0.05$). Also, a significant elevation of glutathione content was observed in melatonin-treated oocytes ($P<0.05$). Analysis of blastocyst formation revealed that developmental competence of the oocytes was higher than the control group ($P<0.05$). It was observed that melatonin treatment decreased expression levels of DNA methyltransferases (*DNMTs*) and global DNA methylation ($P<0.05$). In addition, the expression of melatonin receptor1A (*MTNR1A*) was detected in both cumulus and oocyte by RT-PCR.

Conclusion: The results suggested that in goat model melatonin affects DNA methylation pattern, leading to an improvement in the developmental competence of the oocytes.

Keywords: Glutathione, Melatonin, Methylation

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Introduction

A good number of experiments have been designed to improve the in vitro production of goat embryos by adding numerous factors such as growth factors and antioxidants to maturation media (1, 2).

Maturation of oocytes with a high level of competence is essential to obtain more high quality blastocysts (3). A study demonstrated that supplementation of MM with cysteine as an antioxidant enhances the level of intracellular glutathione (GSH) during *in vitro* maturation (IVM) and is maintained even after *in vitro* fertilization (IVF) (4). Another study suggested that addition of brain-derived neurotrophic factors as a growth factors increases GSH and improves developmental competence in ovine oocytes (5, 6). Nonetheless, the percentage of embryos that successfully develop into blastocysts is low (7).

During *in vitro* embryo production, various reacive oxygen species (ROS) scavengers such as l-ascorbic acid (vitamin c) and cysteine are used to protect oocytes and embryos from harmful effects of oxidative stress (OS) (8, 9). ROS has adverse effects on mitochondrial functions and epigenetic outcomes. OS strongly alters the expression of ten-eleven translocation (TET1), which is responsible for changing 5-methylcytosine to 5-hydroxymethylcytosine in bovine embryo (10).

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Melatonin (N-acetyl-5-methoxytryptamine) is a potential antioxidant (11), which is produced from tryptophan and is secreted by the pineal gland. Melatonin is a well-known multifunctional molecule, as it mediates circadian rhythm, enhances immune-function, and regulates seasonal reproductive activity (12). It has been previously suggested that melatonin affects gene expression of several antioxidant enzymes such as glutathione peroxidase and superoxide dismutase (13). Melatonin can pass through cellular membrane and reach cytosol, inner mitochondria and nuclei, where it protects cells from signals that trigger apoptosis (6, 14). Several recent studies have shown that melatonin has beneficial effects on developmental competence of sheep, porcine, cattle, and mouse embryos, which is related to anti-oxidant capability of melatonin (15-20).

According to a previous study, hydrolazine has an effect on methylation level by inhibition of methyltransferases. A review by Korkmaz et. al. (21) indicated that melatonin, like hydrolazine, can change methylation levels, which affects activation of genes without any changes in DNA sequencing.

Taken together, melatonin may have an effect on DNA methylation of goat oocytes as well as their developmental competence. Therefore, in this study the effects of different concentrations of melatonin on developmental competence, methylation dynamics and GSH level in goat oocytes were evaluated.

Materials and Methods

In this experimental study, unless otherwise specified, all chemicals and media were obtained from Sigma-Aldrich (St. Louis, Mo, USA) and Gibco (Grand Island, NY, USA), respectively. Similarly, all plastic dishes and tubes were obtained from Nunc (Roskilde, Denmark).

Oocyte collection and *in vitro* maturation

Abattoir ovaries were obtained from goat and transferred in physiological saline at 35°C within 4 hours after collection. Cumulus oocyte complexes (COCs) were aspirated from follicles with 2-6 mm diameters. The procedure of in vitro oocyte maturation was performed as described previously (22). Briefly, selected COCs with more than two layers of cumulus were washed three times in HEPES-buffered tissue culture medium-199 (HTCM-199) containing 10% fetal bovine serum (FBS). After washing, COCs (n=10) were transferred into 50 µL micro-drops of HTCM-199 supplemented with 10% FBS, 1µg/mL 17β-estradiol, 5.0 µg/mL luteinizing hormone (LH), 0.5 µg/mL follicle-stimulating hormone (FSH), 100 IU/mL penicillin, 100 µg/mL streptomycin and melatonin under mineral oil. All cultures were incubated in maximum humidity with 5% CO_2 at 38.5°C for 24 hours.

Experimental design

After 24 hours of culturing the cells, treatment groups with 10^{-6} M melatonin (M-10^{-6}), 10^{-9} M melatonin (M-10^{-9}) and 10^{-12} M melatonin (M-10^{-12}), and the control group (without melatonin) were used in the designed experiments. The following were analyzed for each treatment and control group: nuclear maturation, GSH content, ROS levels, global DNA methylation, gene expression and developmental competence after parthenogenetic activation (PA). For each condition three to five replicates were used.

Evaluation of nuclear maturation rate

For evaluating the transition from germinal vesicle (GV) to metaphase II (M-II) stage, COCs were striped from the cumulus cells mechanically in the presence of hyaloronidase and were fixed in 4% paraformaldehyde. Then, oocytes were washed in phosphate buffered saline (PBS) and stained with 5 µg/mL bisbenzimide (Hoechst 33342, excitation: 346 emission: 460) for 5 minutes. Stained oocytes were evaluated using epifluorescence microscope (Nikon Eclipse-600) for first polar body extrusion (23).

Assessment of glutathione concentration

Glutathione content of oocytes was measured as described previously (24). Briefly, denuded oocytes were incubated in tyrodes medium (TLH) containing 5 mg/mL polyvinylalcohol (PVA) and 10µM CellTracker Blue (excitation: 371 emission: 464) for 30 minutes at 38.5°C. After incubation, oocytes were washed in PBS and observed using epi-fluorescence microscope (Nikon clips-300). Digital images were captured and analyzed by Image J software.

Analysis of reactive oxygen species level in maturation medium

The ROS production in centrifuged culture medium following IVM was measured by the chemiluminescence. One microliter of luminal (50 mM) dissolved in dimethyl sulfoxide was added to 400 µl of the supernatant. The global ROS levels were evaluated by measuring chemiluminescence with a luminometer (LKB 953, Wallac, Gaithersburg, MD) for 15 minutes, and the results were expressed in relative light units (RLU)/s (25, 26).

Parthenogenetic activation and embryo development

Parthenogenetic activation method was described earlier (22). Briefly, after the maturation period, oocytes were stripped from cumulus cells by vortexing. Denuded oocytes were exposed to 5 mM inomycine for 5 minutes in HTCM and then washed three times in Charles Rosenkrans 1 with amino acid (CR1aa) medium. Afterward, oocytes were incubated for 2 hours in CR1aa medium contain 2 mM 6-dimethylaminopurine. Finally, activated oocytes (n=6) were transferred to 20 µl droplets of CR1aa plus 3 mg/mL bovine serum albumin (BSA) under mineral oil at 38.5°C, 5% O_2, 5% CO_2 and maximum humidify for 3 days, and then the medium was refreshed with 10% FBS. The cleavage and blastocyst rate were determined on day 3 and 8 post activation, respectively.

Immunostaining of 5-methylcytosine

After fixation in 4% paraformaldehyde, the oocytes were permeabilised with 1% Triton X-100 in PBS for 1 hour, then washed in Tween-20 in 1% PBS/BSA and treated with

2 N HCl for 1 hour at room temperature. After washing in Tween-20 in PBS, the samples were blocked in 0.5% Triton X-100 in 1% PBS/BSA for 1 hour. After blocking, the oocytes were incubated with primary anti-5-methyl cytosine antibody (mouse monoclonal, Abcam, Cambridge, UK) at 1:200 in the blocking buffer for 1 hour at room temprature. After incubation with the primary antibody, the samples were washed in PBS/BSA and incubated with phycoerythrin-conjugated secondary antibody (Molecular Probes, Invitrogen, Carlsbad, CA, USA). After the final wash in PBS/BSA, the DNA of oocytes was stained with 1 µg/mL Hoechst 33342 for 15 minutes. Oocytes were mounted on slides and observed with a Nikon (Eclips-300) fluorescence microscope and the fluorescence intensity of the oocytes was analyzed by Image J software (27).

RNA isolation and reverse transcriptase-polymerase chain reaction

For each group, three pools of biological replicates containing (n=10) mature oocytes and their surrounding cumulus cells were used for total RNA isolation. RNA pellets were dissolved in sterile water and cDNA was synthesized using M-MULVE Reverse transcriptase. Briefly, 2 µg total RNA was mixed with 5 mM Random Hexamer. Five µL water was added to 2 µL of oocytes and incubated at 75ºC for 5 minutes for the reaction to take occur. Then 10 µL RT buffer, 10 mM dNTPs, 10 µL RNase inhibitor and 200 U reverse transcriptase were added to reach a total volume of 20 µL. Reverse transcriptase-polymerase chain reaction (RT-PCR) was done in an applied Bio Rad thermocycler. After the reverse transcriptase reaction was finished, the samples were maintained at 4ºC overnight. PCR reaction was performed in total volumes of 26 µL that included 2 µL cDNA, 2 µL of each primer and 1.25 µL tag polymerase, 20.75 µL Master Mix (Takara, Japan). The PCR primers for each gene are listed in Table 1. The endogenous control (YWHAZ) and the three investigated genes were amplified with PCR cycle program at 94ºC for 3 minutes followed by 40 cycles of 94ºC for 30 seconds and 72ºC for 45 seconds. The number of cycles varied between 30 and 40, depending on the abundance of a particular mRNA. Ten microliters of PCR product were mixed with 1 mL loading buffer and electrophoreses was carried out on a 2% agarose gel in TAE for 25 minutes. The ovary was used as a positive control for melatonin receptors (28).

Quantitative real time-polymerase chain reaction analysis

Real-time quantitative RT-PCR was performed to assess the expression of the investigated genes by using Rotor Gene Q instrument (QIAGEN, Germany). Real time PCR reactions were carried out in a total volume of 13 µL according to the manufacturer's manuals for DNA Master SYBR Green I Mix (Takara, Japan). The primer concentrations were adjusted to 1 µM for each gene. The cycling parameters were 5 seconds at 95ºC, 3 minutes at 95ºC for denaturation, 15 seconds at 60ºC, 10 seconds at 72ºC for amplification and 40 cycles of extension. Expression of YWHAZ transcript was used as the internal housekeeping gene. Three replications were performed and the mRNA level of each sample was normalized to that of YWHAZ mRNA level. The relative levels of mRNA were analyzed by the REST software (Qiagen, Germany) (6).

Statistical analysis

The nuclear maturation of oocytes, cleavage and blastocyst rates were compared by x^2 analysis. The intracellular GSH content and ROS levels were analyzed by one-way ANOVA followed by Tukey's test via SPSS 22 for windows (SPSS, Chicago, IL, USA). Relative gene expression levels of different genes were evaluated by REST software. A $P<0.05$ was considered statistically significant. The data are expressed as mean ± SD.

Results

Nuclear maturation of goat oocytes

The effect of melatonin on nuclear maturation of goat oocytes was examined. Supplementing the IVM medium with melatonin significantly increased the rate of M-II oocytes at M-10-12 group (88%) when compared to the control group (76.1%) (Table 2). No significant difference ($P>0.05$) was observed between the other groups.

Table 1: Primer sequences used for gene expression

Gene name	Primer sequence (5´-3´)	Annealing temperature (c)	Product size (bp)	Accession number
MTNR1A	F: TCGCCTCCATCCTC R: AACACATTCCCTGCGT	60	106	XM _005698759.1
DNMT3b	F: GAAGATCCTACAAAGACAG R: AATTTTCCCCTCCTTCTCCTGC	60	115	NM_18181302
DNMT1	F: CGGAACTTCGTCTCCTTC R: CACGCCGTACTGACCAG	60	114	XM_015471996.1
DNMT3a	F: AGCACAA CGGAGAAGCC R: TTCCAGGAAGCAGTTCTTG	60	192	NM_001206502
YWHAZ	F: ATCTTGT GTCGTGTGGGG R: CTCGG AGAACTTGCCATC	60	140	XM_005689196.2

Table 2: The effect of melatonin treatment on nuclear maturation in goat oocytes

Group	Number of COC's	n (MII %)	P value	Odd ratio
Control	67	51 (76.1)	-	-
M-10^{-6}	64	54 (84.3)	0.27	1.16
M-10^{-9}	75	65 (86.6)	0.14	1.12
M-10^{-12}	92	81 (88)	0.04*	1.23

*; Significant difference and COC; Cumulus oocyte complexes.

Glutathione level in goat oocyte

The obtained results from fluorescence intensity experiment (Fig.1A) indicate that GSH content was significantly higher in melatonin-treated groups compared to the control (P<0.05).

Oocyte developmental competence

Our findings demonstrated that different concentrations of melatonin (i.e. M-10^{-6}, M-10^{-9} and M-10^{-12}) had no effect on cleavage rate after PA when compared to the control group. However, the blastocyst formation were higher (P<0.05) in M-10^{-6} (55.4%), M-10^{-9} (49.2%) and M-10^{-12} (51%) groups as compared to control group (34.7%). Moreover, in terms of blastocyst formation, no difference (P>0.05) was observed among melatonin-treated groups (Table 3).

Effect of melatonin on the reactive oxygen species level of maturation medium

The present data indicate that M-10^{-12} group had a significant effect on ROS levels in comparison to the control groups (Fig.1B).

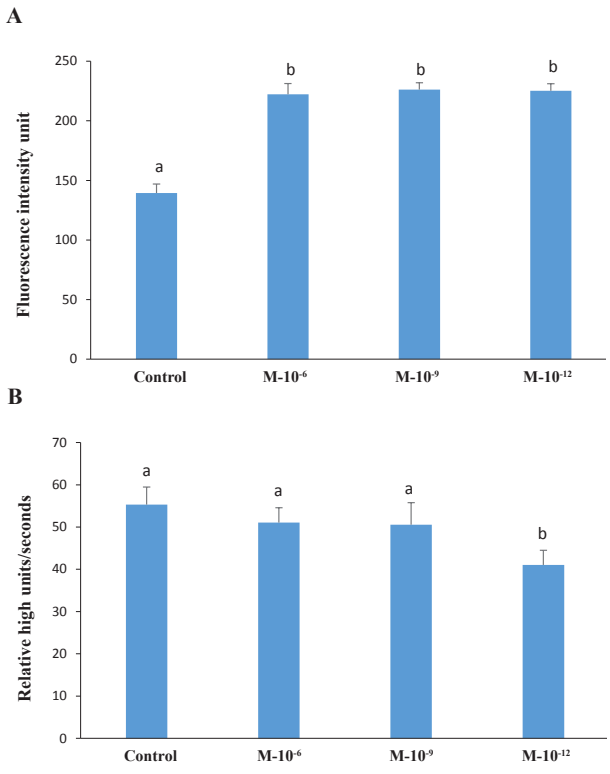

Fig.1: Glutathione (GSH) and reactive oxygen species (ROS) levels. The effect of melatonin on intracellular **A.** GSH and **B.** ROS levels in goat oocytes after *in vitro* maturation. Different letters (a, b) indicate a significant difference (P<0.05).

Table 3: The effect of different concentrations of melatonin during *in vitro* maturation on cleavage and blastocyst rates of goat oocytes after parthenogenesis activation

Group	Number of COC's	Cleavage rate n (%)	P value	Blastocyst rate n (%)	P value
Control	92	72 (78)	-	25 (34.7)	-
M-10^{-6}	95	74 (77.8)	0.99	41 (55.4)	0.01*
M-10^{-9}	89	65 (73)	0.55	32 (49.2)	0.05*
M-10^{-12}	66	45 (68.1)	0.13	23 (51)	0.04*

*; Significant difference and COC; Cumulus oocyte complexes.

Changes in DNA methylation in goat oocytes treated with melatonin

Representative images of labeling for 5-methyl cytosine in goat oocytes are shown in Figure 2A-D. Results from quantitative analysis of these images by Image J software showed significantly different methylation levels between the control and M-10^{12} groups (Fig.2E).

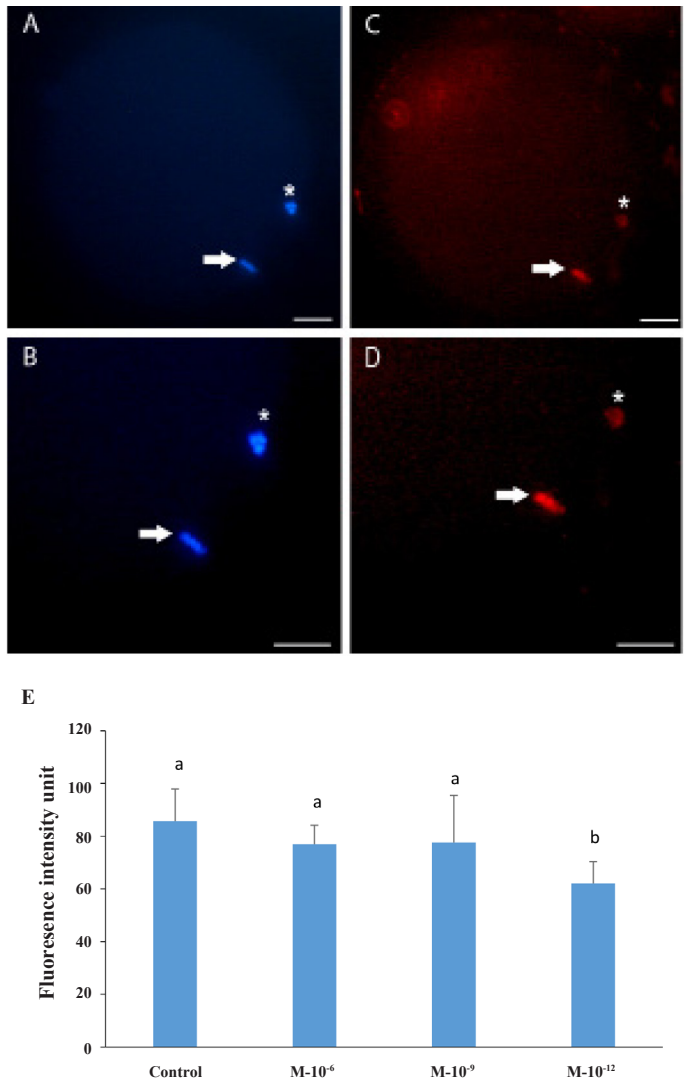

Fig.2: Immunocytochemical staining of oocyte. Oocytes stained with **A, B.** Hoechst followed by **C**, **D.** Methyl cytosin labeling in goat oocytes. *; Indicated polar body and arrow indicated M-II plate (scale bar: 20 µm), and **E.** Changes in methylation levels in goat oocytes from the experimental and control groups, as estimated by immunostaining. Different letters (a, b) indicate a significant difference (P<0.05).

The effect of melatonin on the expression of DNA methyltransferase genes

The expression of DNMTs genes were analyzed by quantitative real-time PCR in mature oocytes (Fig.3A). The expression of *DNMT1* in M-10^{-12} group was significantly lower ($P<0.05$) in comparison to the control group. Our observations indicated that the expression of *DNMT3a* was lower significantly in all melatonin-treated groups compared to the control group. The expression of *DNMT3b* was significantly lowered in the oocytes with melatonin 10^{-6} treatment compared to the control groups ($P<0.05$).

The effect of exogenous melatonin on the expression of melatonin receptor

The expression of *MTNR1A* gene was detected via RT-PCR in both oocytes and cumulus cells, in response to melatonin addition to the IVM medium (Fig.3B, C). This result shows that *MTNR1A* exists in both oocytes and cumulus cells independent from the presence of melatonin in the maturation medium.

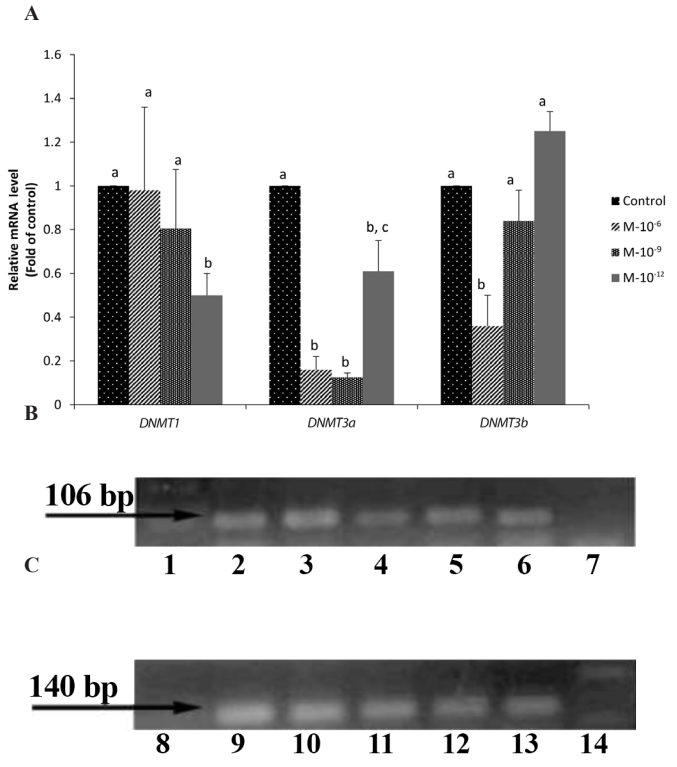

Fig.3: Gene expression following oocyte *in vitro* maturation. **A.** The expression of DNMTs genes in goat matured oocytes treated and un-treated with melatonin. Different letters in each gene group indicate significant difference in gene expression, **B.** The expression of melatonin receptor (*MTNR1A*) in mature goat oocytes treated and un-treated with melatonin (lanes 2 and 3) and cumulus cells from matured oocyte treated and un-treated (lanes 4 and 5). Lane 1 shows the DNA molecular weight marker (100 bp ladder). Lane 7 shows the polymerase chain reaction (PCR) reaction without cDNA substrate as the negative control, and **C.** The expression of *YWHAZ* in goat matured oocytes treated and un-treated with melatonin (lanes 10 and 11) and cumulus cells from matured oocyte treated and un-treated (lanes 12 and 13). Lane 8 shows the DNA molecular weight marker (100 bp ladder). Lane 14 shows the PCR reaction without cDNA substrate. Lane 6 and 9 in both pictures show the expression of *MTNR1A* and *YWHAZ* in ovary tissue as a positive control.

Discussion

A considerable amount of studies on melatonin indicates that it is a multifunctional antioxidant molecule, mediating several circadian and seasonal reproductive processes, as well as acting as a radical scavenger (29, 30). Therefore, in this study we investigated the effects of melatonin on oocyte maturation and embryo development in goats, which are important farm animals. Also, the expression of *MTNR1A* and DNMT-related genes in goat oocytes were analyzed.

Our results indicated that melatonin at 10^{-12} M has a significant effect on first polar body extrusion. These results are consistent with previous findings, in which melatonin was shown to be an essential factor for first polar body extrusion in porcine, bovine and mouse (15, 20, 31).

In some studies, GSH level in oocytes is used to evaluate cytoplasmic maturation of the oocytes; in fact, GSH is known to be an important intra-oocyte factor for developmental competence (32). Data from bovine and mouse shows that GSH level in embryos treated with melatonin increases significantly after IVM and vitrification (6, 31), which is consistent with our results.

In this study, we used parthenogenesis technique, because in this method developmental competence of the oocytes is completely independent from sperm effects. Our results indicated that after activation, melatonin increases blastocyst rate but does not have any significant effects on cleavage rate. These data are consistent with data from porcine (14), bovine and mice, (6, 33), but in contrast with results from ovine (34). This discrepancy may be due to the species specific effect of melatonin or technical factors that can influence developmental competence of the oocytes (35, 36).

According to our results from GSH and blastocyst formation, we can argue that supplementation of melatonin in the maturation medium improves cytoplasmic maturation of the oocytes, which has a beneficial effect on developmental competence of the oocytes following parthenogenesis. Therefore, melatonin, like other antioxidants including resveratrol, can be used in maturation medium and protect oocytes from harmful effects of ROS (35).

This study revealed that melatonin treatment during goat oocyte maturation decreases the expression level of *DNMT1* and *DNMT3a*, which have vital roles in increasing transcription and expression of other genes (36). In addition, our results from immunofluorescence assay indicated that melatonin lowers global methylation level in goat oocytes.

It has been clearly established that *in vitro* production of an embryo has adverse effects on DNA methylation (37). Other studies have shown that porcine embryo, which was produced *in vitro*, has higher levels of DNA methylation in comparison to those produced *in vivo*. For this reason, researcher have used drugs for manipulating epigenetic outcomes after nuclear transfer. However,

some of them were toxic and their usage requires further experiments. For example, 5-Aza-2-deoxycytidine had an effect on DNA hypomethylation with no effect on H3K9 hyperacetylation (38). Recent have indicated that melatonin, as a natural antioxidant, can be used in cancer research, similar to procaine and hydralazine, which are known as methyltransferase inhibitors (39). So, based on our results melatonin can be used in IVM for regulation of DNA methylation levels.

In this work, we also examined the expression of *MTNR1A* in oocytes and cumulus cells. Our results confirmed the expression of *MTNR1A* in both cumulus and oocytes in goat model by using RT-PCR. This is an important aspect of our report, as it is presenting data on a different kind of reproduction-regulating receptor compared to previous studies (31).

Conclusion

Supplementation of melatonin at different concentrations during IVM of oocytes improved the potential development of parthenogenetic embryos. This improvement is due to increased amount of intracellular GSH, decreased ROS levels and decreased abundance of DNMTs gene transcripts in mature oocytes, which are all important in nuclear methylation and gene expression. In addition, *MTNR1A* expression was detected in both cumulus cells and oocytes of the goat.

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Author's Contributions

K.P., M.S.; Participated in study design, data collection and evaluation, drafting and statistical analysis. S.S., A.H.A.-K., H.R.; Performed laboratory experiments. All authors performed editing and approving the final version of this paper for submission, also participated in the finalization of the manuscript and approved the final draft.

References

1. Souza-Fabjan JM, Locatelli Y, Duffard N, Corbin E, Touzé JL, Perreau C, et al. In vitro embryo production in goats: Slaughterhouse and laparoscopic ovum pick up–derived oocytes have different kinetics and requirements regarding maturation media. Theriogenology. 2014; 81(8): 1021-1031.
2. Russell DF, Baqir S, Bordignon J, Betts DH. The impact of oocyte maturation media on early bovine embryonic development. Mol Reprod Dev. 2006; 73(10): 1255-1270.
3. Ikeda S, Ichihara-Tanaka K, Azuma T, Muramatsu T, Yamada M. Effects of midkine during in vitro maturation of bovine oocytes on subsequent developmental competence. Biol Reprod. 2000; 63(4): 1067-1074.
4. De Matos DG, Furnus CC. The importance of having high glutathione (GSH) level after bovine in vitro maturation on embryo development: effect of β-mercaptoethanol, cysteine and cystine. Theriogenology. 2000; 53(3): 761-771.
5. Abazari-Kia AH, Dehghani-Mohammadabadi M, Mohammadi-Sangcheshmeh A, Zhandi M, Salehi M. Regulation of embryonic development and apoptotic-related gene expression by brain-derived neurotrophic factor in two different culture conditions in ovine. Theriogenology. 2015; 84(1): 62-69.
6. Dehghani-Mohammadabadi M, Salehi M, Farifteh F, Nematollahi S, Arefian E, Hajjarizadeh A, et al. Melatonin modulates the expression of BCL-xl and improve the development of vitrified embryos obtained by IVF in mice. J Assist Reprod Genet. 2014; 31(4): 453-461.
7. Rizos D, Ward F, Duffy P, Boland MP, Lonergan P. Consequences of bovine oocyte maturation, fertilization or early embryo development in vitro versus in vivo: implications for blastocyst yield and blastocyst quality. Mol Reprod Dev. 2002; 61(2): 234-248.
8. Guerin P, El Mouatassim S, Menezo Y. Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. Hum Reprod Update. 2001; 7(2): 175-189.
9. Nabenishi H, Ohta H, Nishimoto T, Morita T, Ashizawa K, Tsuzuki Y. The effects of cysteine addition during in vitro maturation on the developmental competence, ROS, GSH and apoptosis level of bovine oocytes exposed to heat stress. Zygote. 2012; 20(03): 249-259.
10. Dzitoyeva S, Chen H, Manev H. Effect of aging on 5-hydroxymethylcytosine in brain mitochondria. Neurobiol Aging. 2012; 33(12): 2881-2891.
11. Galano A, Tan DX, Reiter RJ. Melatonin as a natural ally against oxidative stress: a physicochemical examination. J Pineal Res. 2011; 51(1): 1-16.
12. Arendt J. Melatonin and the pineal gland: influence on mammalian seasonal and circadian physiology. Rev Reprod. 1998; 3(1): 13-22.
13. Kotler M, Rodríguez C, Sáinz RM, Antolin I, Menéndez-Peláez A. Melatonin increases gene expression for antioxidant enzymes in rat brain cortex. J Pineal Res. 1998; 24(2): 83-89.
14. Choi J, Park SM, Lee E, Kim JH, Jeong YI, Lee JY, et al. Antiapoptotic effect of melatonin on preimplantation development of porcine parthenogenetic embryos. Mol Reprod Dev. 2008; 75(7): 1127-1135.
15. Kang JT, Koo OJ, Kwon DK, Park HJ, Jang G, Kang SK, et al. Effects of melatonin on in vitro maturation of porcine oocyte and expression of melatonin receptor RNA in cumulus and granulosa cells. J Pineal Res. 2009; 46(1): 22-28.
16. Papis K, Poleszczuk O, Wenta-Muchalska E, Modlinski JA. Melatonin effect on bovine embryo development in vitro in relation to oxygen concentration. J Pineal Res. 2007; 43(4): 321-326.
17. Abecia J, Forcada F, Zuniga O. The effect of melatonin on the secretion of progesterone in sheep and on the development of ovine embryos in vitro. Vet Res Commun. 2002; 26(2): 151-158.
18. Ishizuka B, Kuribayashi Y, Murai K, Amemiya A, Itoh MT. The effect of melatonin on in vitro fertilization and embryo development in mice. J Pineal Res. 2000; 28(1): 48-51.
19. Salehi M, Kato Y, Tsunoda Y. Effect of melatonin treatment on developmental potential of somatic cell nuclear-transferred mouse oocytes in vitro. Zygote. 2014; 22(2): 213-217.
20. Salimi M, Salehi M, Masteri Farahani R, Dehghani M, Abadi M, Novin MG, et al. The effect of melatonin on maturation, glutathione level and expression of HMGB1 gene in Brilliant Cresyl Blue (BCB) stained immature oocyte. Cell J. 2014; 15(4): 294-301.
21. Korkmaz A, Reiter RJ, Topal T, Manchester LC, Oter S, Tan DX. Melatonin: an established antioxidant worthy of use in clinical trials. Mol Med. 2009; 15(1-2): 43-50.
22. Abazari-Kia AH, Mohammadi-Sangcheshmeh A, Dehghani-Mohammadabadi M, Jamshidi-Adegani F, Veshkini A, Zhandi M, et al. Intracellular glutathione content, developmental competence and expression of apoptosis-related genes associated with G6PDH-activity in goat oocyte. J Assist Reprod Genet. 2014; 31(3): 313-321.
23. Hong SG, Jang G, Oh HJ, Koo OJ, Park JE, Park HJ, et al. The effects of brain-derived neurotrophic factor and metformin on in vitro developmental competence of bovine oocytes. Zygote. 2009; 17(03): 187-193.
24. You J, Kim J, Lim J, Lee E. Anthocyanin stimulates in vitro development of cloned pig embryos by increasing the intracellular glutathione level and inhibiting reactive oxygen species. Theriogenology. 2010; 74(5): 777-785.
25. Bedaiwy MA, Falcone T, Mohamed MS, Aleem AA, Sharma RK, Worley SE, et al. Differential growth of human embryos in vitro: role of reactive oxygen species. Fertil Steril. 2004; 82(3): 593-600.
26. Zare Z, Farahani RM, Salehi M, Piryaei A, Novin MG, Fathabadi FF, et al. Effect of L-carnitine supplementation on maturation and early embryo development of immature mouse oocytes selected by brilliant cresyle blue staining. J Assist Reprod Genet. 2015; 32(4): 635-643.
27. Fulka H, Barnetova I, Mosko T, Fulka J. Epigenetic analysis of hu-

man spermatozoa after their injection into ovulated mouse oocytes. Hum Reprod. 2008; 23(3): 627-634.
28. Zhao XM, Min JT, Du WH, Hao HS, Liu Y, Qin T, et al. Melatonin enhances the in vitro maturation and developmental potential of bovine oocytes denuded of the cumulus oophorus. Zygote. 2015; 23(4): 525-536.
29. Tan DX, Chen L, Poeggeler B, Manchester L, Reiter R. Melatonin: a potent, endogenous hydroxyl radical scavenger. Endocr J. 1993; 1(4): 57-60.
30. Reiter RJ. The pineal and its hormones in the control of reproduction in mammals. Endocr Rev. 1980; 1(2): 109-131.
31. El-Raey M, Geshi M, Somfai T, Kaneda M, Hirako M, Abdel-Ghaffar AE, et al. Evidence of melatonin synthesis in the cumulus oocyte complexes and its role in enhancing oocyte maturation in vitro in cattle. Mol Reprod Dev. 2011; 78(4): 250-262.
32. Yoshida M, Ishigaki K, Nagai T, Chikyu M, Pursel VG. Glutathione concentration during maturation and after fertilization in pig oocytes: relevance to the ability of oocytes to form male pronucleus. Biol Reprod. 1993; 49(1): 89-94.
33. Zhao XM, Min JT, Du WH, Hao HS, Liu Y, Qin T, et al. Melatonin enhances the in vitro maturation and developmental potential of bovine oocytes denuded of the cumulus oophorus. Zygote. 2014:23(4): 525-536.
34. Casao A, Abecia JA, Pérez JC, Blanco TM, Vázquez M, Forcada F. The effects of melatonin on in vitro oocyte competence and embryo development in sheep. Span J Agri Res. 2010; 8(1): 35-41.
35. Mukherjee A, Malik H, Saha AP, Dubey A, Singhal DK, Boateng S, et al. Resveratrol treatment during goat oocytes maturation enhances developmental competence of parthenogenetic and handmade cloned blastocysts by modulating intracellular glutathione level and embryonic gene expression. J Assist Reprod Genet. 2014; 31(2): 229-239.
36. Lyko F, Brown R. DNA methyltransferase inhibitors and the development of epigenetic cancer therapies. Natl Cancer Inst. 2005; 97(20): 1498-1506.
37. Deshmukh RS, Østrup O, Østrup E, Vejlsted M, Niemann H, Lucas-Hahn A, et al. DNA methylation in porcine preimplantation embryos developed in vivo and produced by in vitro fertilization, parthenogenetic activation and somatic cell nuclear transfer. Epigenetics. 2011; 6(2): 177-187.
38. Jafarpour F, Hosseini SM, Hajian M, Forouzanfar M, Ostadhosseini S, Abedi P, et al. Somatic cell-induced hyperacetylation, but not hypomethylation, positively and reversibly affects the efficiency of in vitro cloned blastocyst production in cattle. Cell Reprogram. 2011;13(6): 483-493.
39. Iager AE, Ragina NP, Ross PJ, Beyhan Z, Cunniff K, Rodriguez RM, et al. Trichostatin A improves histone acetylation in bovine somatic cell nuclear transfer early embryos. Cloning Stem Cells. 2008; 10(3): 371-380.

Original Article

Do Serum Vitamin D Levels Have Any Effect on Intrauterine Insemination Success?

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Abstract

Background: Recent studies have shown that vitamin D has an essential role in the reproductive system. In this study, we aimed to investigate the effect of vitamin D levels in patients undergoing ovulation induction (OI), and subsequent intrauterine insemination (IUI) procedure.

Materials and Methods: One hundred and four infertile and one hundred and three fertile women were recruited in this cross-sectional study which was conducted in a tertiary level maternity hospital. Infertile patients were divided into pregnant and non-pregnant subgroups after treatment. Individual characteristics and 25-hydroxyvitamin D_3 [25 (OH) D_3] levels were compared between the groups.

Results: The vast majority of our study population consisted of women who had vitamin D deficiency (96.6%). There was no statistically significant difference between infertile and fertile groups in terms of serum 25 (OH) D_3 levels (P=0.512). Similarly, no significant difference was observed between the pregnant and non-pregnant subgroups of infertile patients regarding 25 (OH) D_3 levels (P=0.267).

Conclusion: There is no association between female infertility and serum vitamin D levels. Vitamin D does not predict pregnancy in infertile women undergoing OI with IUI. Further research which will provide a comparison between much more women who have deficient and sufficient 25 (OH) D_3 levels is warranted.

Keywords: Infertility, Intrauterine Insemination, Ovulation Induction, Vitamin D

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Introduction

Intrauterine insemination (IUI) is used to transport sperm directly into the uterus. It is a simple, non-invasive, and cost-effective technique used for assisted reproduction. The most common indication for IUI is cervical infertility, and it is also used in male subfertility, anovulation, endometriosis cases in which at least one tube is healthy, as well as unexplained infertility (1, 2). Although there may be a trend towards higher pregnancy rates when the number of IUIs per cycle is increased, a recent meta-analysis has shown that increased IUI numbers do not increase pregnancy (3). Previous investigations reported that IUI had a success rate of 10-20% for clinical pregnancies (4).

Recently, the effect of vitamin D (VD) has been investigated on not only the musculoskeletal system, but also in the reproductive and other systems (5). The biologic actions of VD are mediated through the vitamin D receptor (VDR). VDR was found to be in the ovary (particularly the granulosa cells), uterus, placenta, and testis, suggesting VD may have a significant role in human reproduction (6). Two studies supporting this data indicated that VD deficiency is responsible for reduced fertility and reproductive capacity in female rats (7, 8). Research conducted on human subjects also supports this role as in experimental animal studies (9).

Calcitriol (1, 25 dihydroxyvitamin D_3) which is the active form of VD stimulates CYP19 expression (CYP19 encodes the aromatase enzyme) that results in increased estrogen production, when it was bound to VDR (10). Furthermore, it has been reported that decidua secretes calcitriol during blastocyst implantation, and calcitriol has been reported to regulate the immune response in the maternal-fetal interface during pregnancy (11).

There are several studies which presented controversial results on the differences in 25-hydroxyvitamin D_3 [25 (OH) D_3] levels of the patients undergoing different infertility treatment modalities (12, 13). The aim of our study was to investigate the 25 (OH) D_3 levels in patients who underwent ovulation induction with IUI and then to

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determine the relationship between 25 (OH) D_3 levels and the occurrence of pregnancy.

Materials and Methods

This case-control study was conducted between March 2014 and June 2014 in the infertility outpatient clinics of Zekai Tahir Burak Women's Health Education and Research Hospital. This is a government supported tertiary level maternity hospital located in the capital city of Turkey. The institutional review board approved the study and informed consent was obtained from each patient (approval number: 23.09.2013/9). All of the study protocols were carried out in accordance with the Helsinki Decleration (14).

We defined the infertile patients as those reproductive age couples who were unable to become pregnant in the absence of contraception. For the women below 35 years of age, infertility was diagnosed as a minimum of 1 year of trying to become pregnant, whilst for the women above 35 years of age, the diagnosis was limited to 6 months of unprotected sexual intercourse. After we obtained detailed information about age, duration of infertility, infertility type, previous history of surgery, and any systemic disturbances (such as diabetes mellitus, hypertension, and thyroidal disease), a complete physical and gynaecological examination was performed on all of the women. We confirmed tubal patency in the women using hysterosalpingography (HSG) and if there was bilateral tubal occlusion detected with HSG, we applied laparoscopy and hysteroscopy to define any pathology such as pelvic adhesions or endometriosis. When we suspected an intracavitary lesion in the uterus after HSG, or transvaginal ultrasound, we performed hysteroscopy.

We included women with mild male factor infertility, unexplained infertility, and polycystic ovary syndrome (PCOS). We excluded patients who had advanced age (above 40 years of age), any systemic or endocrine diseases, stage 3-4 endometriosis, or intracavitary lesions in uterus (such as endometrial polyp, submucous myoma, and uterine septum), smokers and women who used of any kinds of drugs or substances likely to affect levels of VD. We also excluded patients whose partner had a motile sperm count lower than 5 million/mL. The fertile group consisted of patients who applied to the family planning unit of our hospital for contraceptions. These patients had given birth in the previous 12 months, has not breastfed their neonate, and had no history of infertility.

After initial clinical assessment, infertile patients were evaluated for clomiphene citrate (CC) or gonadotropins (Gn) and IUI use. Those patients who had used CC with IUI treatments for three times or were above 35 years of age were directed into the Gn with IUI regimen (n=63), whilst the other infertile patients were directed into the CC and IUI regimen (n=41). When 18-20 mm (dominant follicles) were found through ultrasound, 2 human chorionic gonadotropin (hCG, Pregnyl, MSD, Netherlands) ampoules containing 5,000 units each, were injected intramuscularly, and IUI applied 36 hours after the injection. When there were 3 or more dominant follicles, or endometrial thickness was less than 6 mm, hCG was not administered. Then 2 weeks later, a blood sample was obtained from patients for β-hCG measurement. Clinical pregnancy was diagnosed 5 weeks after IUI, when the evidence of fetal heart activity or presence of the gestational sac in the uterine cavity was detected.

The concentration of serum 25 (OH) D_3 was used to determine the status of VD in the body for this study since it has been proven to be the best biomarker for VD insufficiency. It also reflects VD levels from both dietary intake and in-skin synthesis (6). The two groups were matched in term of veiling habits, daily exposure to sunlight, and dietary intake of VD-rich foods which was determined by a dietician.

The serum levels of 25 (OH) D_3 levels and baseline hormones including estradiol, follicle stimulating hormone (FSH), luteinizing hormone, prolactin, and thyroid stimulating hormone were measured on the third day of the menstrual cycle when ovulation induction was started. We performed the recruitment of study volunteers in a single season, because the blood levels of VD have seasonal variabilities (15, 16). In addition, patients living in the same geographical region were selected for the study (17).

After overnight fasting, venous blood samples were obtained early in the morning and transferred to the laboratory in a non-transparant box to avoid exposure to light, and then serum was separated by centrifugation at 5,000 rpm (2,236 g) for 10 minutes. The serum 25 (OH) D_3 levels were measured using an enzyme linked immunosorbent assay kit (Immunodiagnostic AG, Leverkusen, Germany), and presented in ng/mL. The intra-assay and inter-assay coefficients of variation for serum 25 (OH) D_3, were 8.9 and 10.6% respectively. Serum 25 (OH) D_3 concentrations <20 ng/mL was considered as VD deficiency. Types of VD deficiency were also classified as mild (10-20 ng/mL), moderate (5-10 ng/mL), and severe (<5 ng/mL). Serum 25 (OH) D3 concentrations between 20 and 30 ng/mL was accepted as VD insufficiency whereas a threshold value of ≥30 ng/mL was considered sufficient serum VD levels. Basal hormone levels were measured using an Immulite 2000 analyzer (EURO/DPC Ltd., Gwynedd, UK). Body mass index (BMI) was defined as the weight in kilograms divided by the square of the height in meters.

We examined the women who had a positive result for β-hCG using transvaginal ultrasound at at least weeks 6-7 of gestation to detect fetal cardiac activity. The difference between the two subgroups (pregnant and non-pregnant) of infertile patients in terms of 25 (OH) D_3 levels was the primary outcome measured of this study. The secondary outcome was the comparison of serum 25 (OH) D_3 levels between infertile and fertile groups.

Data were recorded and analysed using the Statistical Package for the Social Sciences program for Windows version 17.0 (SPSS Inc, Chicago, IL, USA). The normal distribution of the variables was assessed using the Shapiro-Wilk's test. Continuous variables were presented as

the mean with standard deviation (SD) or median (range), and categorical variables were presented as the number (percentage) of subjects. Continuous variables were compared using independent samples t-test if they were normally distributed or with the Mann-Whitney U test if they were non-normally distributed. Categorical variables were analyzed using the Chi-square (χ^2) test or Fisher's exact test. Correlations were calculated using Spearman's correlation analysis. In all analyses, two-tailed P<0.05 were considered as statistically significant. Post-hoc power analysis demonstrated that we achieved a power of 0.95 with a 5% level of significance and a 0.5 effect size by using a two sample comparison (18). Power analysis was carried out on G-power software (G-power v3.1.9.2, Universitat Kiel, Kiel, Germany).

Results

One hundred and four infertile and one hundred and three fertile women were included into this cross-sectional, case-control study. Examination of the infertile and fertile patients showed that there was no statistically significant difference between the groups regarding their mean age and BMI. Obstetric history characteristics were statistically significantly different between the two groups (P<0.001 for all). The mean FSH levels were higher in the infertile patients than in the fertile patients (7.4 ± 2.1 mU/mL vs 6.2 ± 1.6 mU/mL, P=0.001), but it was within the normal range in either group. Mean prolactin levels of the fertile group (14.4 ± 5.4 ng/mL) were higher than the infertile group's (12.2 ± 4.6 ng/mL). This difference was statistically meaningful (P=0.002), but those values were in the normal range as with mean FSH levels. There were no statistically significant differences in 25 (OH) D_3 levels between the 2 groups [7.3 (3-25.5) ng/mL vs. 6.8 (3.4-37.1) ng/mL, P=0.512], as seen in Table 1. No significant correlation between serum 25 (OH) D_3 and FSH levels was observed either in the entire study population (Spearman's r=0.051, P=0.466).

Table 1: Descriptive characteristics and serum 25 (OH) D_3 levels of infertile and fertile patients

Characteristic	Infertile group n=104	Fertile group n=103	P value
Age (Y)**	28.1 (4.7)	29.4 (5.4)	0.088[a]
BMI (kg/m²)**	25.1 (3.6)	25.7 (3.8)	0.234[a]
Gravida*	0 (0-6)	2 (1-6)	<0.001[b]
Parity*	0 (0-1)	2 (0-5)	<0.001[b]
Alive*	0 (0-1)	2 (0-5)	<0.001[b]
Abortion*	0 (0-5)	0 (0-4)	<0.001[b]
FSH (mIU/mL)**	7.4 (2.1)	6.2 (1.6)	0.001[a]
LH (mIU/mL)**	5.3 (2.5)	4.9 (1.9)	0.154[a]
Estradiol (pg/mL)*	44.0 (12-148)	42.7 (21-99)	0.791[b]
TSH (µlU/mL)**	1.9 (0.8)	2 (0.9)	0.859[a]
Prolactin (ng/mL)**	12.2 (4.6)	14.4 (5.4)	0.002[a]
25 (OH) D_3 (ng/mL)*	7.3 (3-25.5)	6.8 (3.4-37.1)	0.512[b]

BMI; Body mass index, FSH; Follicle-stimulating hormone, LH; Luteinizing hormone, TSH; Thyroid stimulating hormone, *; Median (minimum-maximum), **; Mean (SD), [a]; Student t Test, and [b]; Mann Whitney U test. P<0.05 is considered as statistically significant.

The severity of VD deficiency in the infertile and fertile groups showed that most of the participants were deficient for VD (96.2 vs. 97.1%) and only 1 (1%) participant in the fertile group had 25 (OH) D_3 levels ≥30 ng/mL. 19 (18.3%) patients were in the severe deficiency group, 56 (53.8%) cases were in the moderate deficiency group, and lastly 25 (24%) women were in the mild deficiency group among the infertile group. The number of fertile patients in the same groups was 19 (18.4%), 58 (56.3%), and 23 (22.3%), respectively (P=0.776).

After IUI treatment, the numbers of clinical pregnancies and live births among 104 infertile patients were 14 (13.3%) and 10 (9.61%), respectively. When infertile patients were divided into two subgroups (pregnant and non-pregnant), there was no statistically significant difference between these subgroups regarding age, BMI, obstetrical history, baseline hormone levels, or ovulation induction agent used. Similarly, no significant difference was observed between the pregnant and non-pregnant subgroups of infertile patients in terms of serum 25 (OH) D_3 levels (P=0.267). Ten (71.4%) patients out of the 14 clinical pregnancies had moderately deficient VD levels. The only significant parameter that may predict pregnancy was the age of the patients, namely the pregnant group was statistically significantly younger than the non-pregnant group (Table 2).

Table 2: Individual characteristics, ovulation induction type and vitamin D levels in pregnant and non-pregnant patients after IUI

Characteristic	Non-pregnant group n=90	Pregnant group n=14	P value
Age (Y)**	28.5 (4.7)	25.5 (4.4)	0.027[a]
BMI (kg/m²)**	25 (3.5)	25.3 (4)	0.784[a]
Gravida*	0 (0-6)	0 (0-2)	0.745[b]
Parity*	0 (0-1)	0 (0-1)	0.459[b]
Alive*	0 (0-1)	0 (0-1)	0.459[b]
Miscarriage*	0 (0-5)	0 (0-2)	0.335[b]
FSH (mIU/ml)**	7.1 (3.5-13.6)	6.8 (3.4-13.5)	0.378[a]
LH (mIU/ml)**	4.9 (2.1-14)	5.9 (2.2-10.3)	0.247[a]
Estradiol (pg/ml)**	44.5 (12-148)	42.5 (20-82)	0.398[a]
TSH (µlU/ml)**	1.8 (0.4-5.3)	2.0 (1.3-3.5)	0.160[a]
Prolactin (ng/ml)**	12.3 (4.9-28.4)	12.9 (7.6-20.9)	0.788[a]
25 (OH) D_3 (ng/mL)*	7.3 (3-25.5)	8.1 (4.7-22.1)	0.267[b]
Ovulation induction type***			0.777[c]
CC	55 (61.1)	8 (57.1)	
Gn	35 (38.9)	6 (42.9)	

IUI; Intrauterine insemination, BMI; Body mass index, FSH; Follicle-stimulating hormone, LH; Luteinizing hormone, TSH; Thyroid stimulating hormone, CC; Ovulation induction with clomiphene citrate, Gn; Ovulation induction with gonadotropin, *; Median (minimum-maximum), **; Mean (SD), *; n (%), [a]; Student's t test, [b]; Mann-Whitney U test, and [c]; Fisher's exact test. P<0.05 is considered as statistically significant.**

Discussion

Our study showed that infertile and fertile patients had similar serum VD levels and that there was no statistically significant difference in serum VD measurements between the pregnant and non-pregnant groups after IUI.

VD has an essential role in both male and female reproductive system (19). It was found that its deficiency is highly prevalent among women undergoing ovarian stimulation (9). Considering the previous data, we designed such a study assuming that VD could be lower in infertile patients, but we found no relationship between them. This result may be due to the fact that VD deficiency is very common in our study population, because 200 of the 207 patients also including women with no fertility problem had VD deficiency at the initial examination. This was a surprise and suggests that fertile patients who had a delivery in the preceding 12 months may have exhausted their VD stores during the most recent pregnancy and they had not been able to replace it yet. VD deficiency is one of the general public health matters in our country, with similar inferences having been suggested in other studies from our country (20, 21).

Ovulation induction with IUI is the most utilized method of infertility treatment in our unit. The success of IUI treatment is multifactorial, and pregnancy rates per cycle have been estimated as 10.2% in a IUI cycle with controlled ovarian stimulation (22).

A study by Ott et al. (23) demonstrated that 25 (OH) D_3 levels may predict ovarian response to ovarian stimulation. This suggestion is consistent with another study showing that VDR exists in human ovaries and is important for sex steroid synthesis (10). However, when we compared pregnant patients with non pregnant patients in terms of serum VD levels, there was no statistically significant difference between them. This may be associated to the differences of individual VDR receptivity and VDR polymorphism (24). Although VDR polymorphism has been reported as not being related to infertility in an endometriosis study, there is a need for further research to clarify this particular issue (25). Another noteworthy result of our study is that patients who became pregnant after IUI treatment were younger than those who did not. It has already been shown that age is one of the most important factors in infertility management (26).

In two rat studies, VD deficiency was shown to significantly increase infertility, decrease probability of viable births and healthy full-grown individuals (8, 27). Although the exact mechanism remains to be elucidated, compromised ovarian folliculogenesis and infertility were found in two studies conducted on VD deficient mice (28, 29). A recent human study supporting these inferences showed that there is a negative correlation between serum levels of 25 (OH) D_3 and FSH (30). However, we found no correlation between them.

VD has been found to be related with the activation of key enzymes in steroidogenesis such as 3-beta-hydroxysteroid dehydrogenase, and it has been shown to induce the production of progesterone that consequently leads to uterine quiescence (5). Thus, VD may play a protective role for ongoing pregnancies through this mechanism.

A recent randomized controlled trial by Asadi et al. (31) showed that endometrial thickness was enhanced by the administration of VD in patients undergoing Gn and IUI treatment. Another study found that higher serum and follicular fluid 25 (OH) D_3 levels were associated with higher pregnancy rates in women undergoing IVF (32). Similarly, a Greek study group found that follicular fluid VD levels significantly correlated with the quality of embryos (18). However, the same authors suggested that excess serum and follicular fluid vitamin levels may have a detrimental effect on IVF outcomes. These findings led us to think that an optimal level of VD is necessary for ovulation, fertilization, and implantation.

The strength of this study is that our data is single-centered and reliable. The data were obtained prospectively from the patients living in the same geographical region during the same season. There is a limitation to our study; VD deficiency was wide-spread in our study population, consistent with the results of previous studies on this issue (19, 20). The similarity of the groups in terms of high prevalence of VD deficiency may have caused 25 (OH) D_3 levels to not be distinguishable between the groups.

Conclusion

No significant difference was observed between pregnant and nonpregnant women who underwent ovulation induction with IUI treatment with regard to serum 25 (OH) D_3 levels. No association was found between infertility and serum 25 (OH) D_3 levels either. Further research which compares women who have deficient and sufficient serum VD levels is warranted.

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Author's Contributions

N.Y., E.E., A.T.; Participated in study design, data collection and evaluation, drafting and statistical analysis. A.S., A.S.O.-E.; Contributed to data collection, evaluation and interpretation. S.E., H.I.Y.; Contributed extensively in interpretation of the data, critical revision of the article. All authors performed editing and approving the final version of this paper for submission, also participated in the finalization of the manuscript and approved the final draft.

References

1. Cantineau AE, Cohlen BJ, Al-Inany H, Heineman MJ. Intrauterine insemination versus fallopian tube sperm perfusion for non tubal infertility. Cochrane Database Syst Rev. 2014; 12: CD006942.
2. Koçak M, Demir B, Yalvaç S, Haberal A. Effectiveness of ovulation induction and intrauterine insemination in heterogenous subfertile couples. Gynecol Obstet and Reprod Med. 2001; 7(2): 99-102.
3. Zavos A, Daponte A, Garas A, Verykouki C, Papanikolaou E, Anifandis G, et al. Double versus single homologous intrauterine insemination for male factor infertility: a systematic review and meta-analysis. Asian J Androl. 2013;15(4): 533-538.
4. Duran HE, Morshedi M, Kruger T, Oehninger S. Intrauterine insemination: a systematic review on determinants of success. Hum Reprod Update. 2002; 8(4): 373-384.

5. Irani M, Merhi Z. Role of vitamin D in ovarian physiology and its implication in reproduction: a systematic review. Fertil Steril. 2014; 102: 460-468.
6. Johnson LE, DeLuca HF. Vitamin D receptor null mutant mice fed high levels of calcium are fertile. J Nutr. 2001; 131: 1787–1791.
7. Johnson LE, DeLuca HF. Reproductive defects are corrected in vitamin D-deficient female rats fed high calcium, phosphorus and lactose diet. J Nutr. 2002; 132(8): 2270–2273
8. Kwiecinksi GG, Petrie GI, DeLuca HF. 1, 25-Dihydroxyvitamin D3 restores fertility of vitamin D-deficient female rats. Am J Physiol Endocrinol Metab. 1989; 256(4): 483–487
9. Vanni VS, Vigano P, Somigliana E, Papaleo E, Paffoni A, Pagliardini L, et al. Vitamin D and assisted reproduction technologies: current concepts. Reprod Biol Endocrinol. 2014; 12: 47.
10. Kinuta K, Tanaka H, Moriwake T, Aya K, Kato S, Seino Y. Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads. Endocrinology. 2000; 141: 1317–1324.
11. Vigano P, Lattuada D, Mangioni S, Ermellino L, Vignali M, Caporizzo E, et al. Cycling and early pregnant endometrium as a site of regulated expression of the vitamin D system. J Mol Endocrinol. 2006; 36: 415–424.
12. Franasiak JM, Molinaro TA, Dubell EK, Scott KL, Ruiz AR, Forman EJ, et al. Vitamin D levels do not affect IVF outcomes following the transfer of euploid blastocysts. Am J Obstet Gynecol. 2015; 212(3): 315. e1-6.
13. Paffoni A, Ferrari S, Viganò P, Pagliardini L, Papaleo E, Candiani M, et al. Vitamin D deficiency and infertility: insights from in vitro fertilization cycles. J Clin Endocrinol Metab. 2014; 99(11): 2372-2376.
14. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. J Postgrad Med. 2002; 48(3): 206-208.
15. Farrar MD, Zulf Mughal M, Adams JE, Wilkinson J, Berry JL, Edwards L, et al. Sun exposure behavior, seasonal vitamin D deficiency and relationship to bone health in adolescents. J Clin Endocrinol Metab. 2016; 101(8): 3105-3113.
16. Eloi M, Horvath DV, Szejnfeld VL, Ortega JC, Rocha DA, Szejnfeld J, et al. Vitamin D deficiency and seasonal variation over the years in São Paulo, Brazil. Osteoporos Int. 2016; 27(12): 3449-3456.
17. Turkish State Meteorological Service. www.mgm.gov.tr. Official statistics of Ankara. Available from: www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler istatistik.aspx?m¼ANKARA#sfB [last accessed on 16 June 2017].
18. Anifandis GM, Dafopoulos K, Messini CI, Chalvatzas N, Liakos N, Pournaras S, et al. Prognostic value of follicular fluid 25-OH vitamin D and glucose levels in the IVF outcome. Reprod Biol Endocrinol. 2010; 8: 91.
19. Luk J, Torrealday S, Neal Perry G, Pal L. Relevance of vitamin D in reproduction. Hum Reprod. 2012; 27(10): 3015-3027.
20. Hekimsoy Z, Dinç G, Kafesçiler S, Onur E, Güvenç Y, Pala T, et al. Vitamin D status among adults in the Aegean region of Turkey. MC Public Health 2010; 10: 782.
21. Aydogmus S, Kelekci S, Aydogmus H, Eriş S, Desdicioğlu R, Yilmaz B, et al. High prevalence of vitamin D deficiency among pregnant women in a Turkish population and impact on perinatal outcomes. J Matern Fetal Neonatal Med. 2015; 28: 1828-1832.
22. Soria M, Pradillo G, García J, Ramón P, Castillo A, Jordana C, et al. Pregnancy predictors after intrauterine insemination: analysis of 3012 cycles in 1201 couples. J Reprod Infertil. 2012; 13: 158–166.
23. Ott J, Wattar L, Kurz C, Seemann R, Huber JC, Mayerhofer K, et al. Parameters for calcium metabolism in women with polycystic ovary syndrome who undergo clomiphene citrate stimulation: a prospective cohort study. Eur J Endocrinol. 2012; 166: 897-902.
24. Colonese F, Laganà AS, Colonese E, Sofo V, Salmeri FM, Granese R, et al. The pleiotropic effects of vitamin D in gynaecological and obstetric diseases: an overview on a hot topic. Biomed Res Int. 2015; 2015: 986281.
25. Vilarino FL, Bianco B, Lerner TG, Teles JS, Mafra FA, Christofolini DM, et al. Analysis of vitamin D receptor gene polymorphisms in women with and without endometriosis. Hum Immunol. 2011; 72(4): 359-363.
26. Nuojua-Huttunen S, Tomas C, Bloigu R, Tuomivaara L, Martikaine H. Intrauterine insemination treatment in subfertility: an analysis of factors affecting outcome. Hum Reprod. 1999; 14: 698–703.
27. Halloran BP, DeLuca HF. Effect of vitamin D deficiency on fertility and reproductive capacity in the female rat. J Nutr. 1980; 110(8): 1573-1580.
28. Yoshizawa T, Handa Y, Uematsu Y, Takeda S, Sekine K, Yoshihara Y, et al. Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. Nat Genet. 1997; 16(4): 391-396.
29. Panda DK, Miao D, Tremblay ML, Sirois J, Farookhi R, Hendy GN, et al. Targeted ablation of the 25-hydroxyvitamin D 1alpha -hydroxylase enzyme: evidence for skeletal, reproductive, and immune dysfunction. Proc Natl Acad Sci USA. 2001; 98(13): 7498-7503.
30. Jukic AM, Steiner AZ, Baird DD. Association between serum 25-hydroxyvitamin D and ovarian reserve in premenopausal women. Menopause. 2015; 22: 312-316.
31. Asadi M, Matin N, Frootan M, Mohamadpour J, Qorbani M, Tanha FD. Vitamin D improves endometrial thickness in PCOS women who need intrauterine insemination: a randomized double-blind placebo-controlled trial. Arch Gynecol Obstet. 2014; 289(4): 865-870.
32. Ozkan S, Jindal S, Greenseid K, Shu J, Zeitlian G, Hickmon C, et al. Replete vitamin D stores predict reproductive success following in vitro fertilization. Fertil Steril. 2010; 94(4): 1314–1319.

Original Article

Effects of Varicocelectomy on Serum Testosterone Levels among Infertile Men with Varicocele

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Abstract

Background: The main purpose of this study is to evaluate the effects of varicocelectomy on serum testosterone levels and semen quality in infertile men who suffer from varicocele.

Materials and Methods: This prospective study enrolled 115 subjects with clinical varicocele grades II and III and 240 fertile men as the control group. Total volume of testosterone serum level (ng/dl) and semen quality were compared before and after microscopic varicocelectomy. We normalized testosterone serum levels for age, grade, and testis size basis. SPSS 20 software was used to analyze the data. All results of continuous variables were reported as mean ± SD. Statistical significance was set at a $P<0.05$.

Results: The mean ages of individuals who participated in the treatment (32.2 ± 5.23) and control (32.8 ± 5.27) groups were similar. There were similar mean values for adjusted testosterone levels between the varicocele (567 ± 222 ng/ml) and control (583 ± 263 ng/ml) groups. In the varicocele group, the adjusted testosterone levels insignificantly increased to 594 ± 243 ng/ml. Among semen parameters, only mean sperm concentration significantly increased after varicocelectomy.

Conclusion: Despite increases in sperm concentration, adjusted testosterone levels did not significantly improve after varicocelectomy.

Keywords: Infertility, Testosterone, Varicocele, Varicocelectomy

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Introduction

The relationship between varicocele and male infertility was first noted in the late 1800s when Bennet reported an improvement in semen quality after correction for bilateral varicoceles in a patient (1, 2). Varicocele is an abnormal dilatation of the pampiniform plexus of the veins that drain the testis. Restoration of this abnormality has been shown to cause positive effects on the spermatogenesis process (2-4). According to a number of studies, varicocelectomy improves semen parameters, hormonal profiles, and pregnancy rates (5-8). However, the process by which varicocele and its repair affects testicular Leydig cell function, semen quality, and the resultant changes in testosterone production levels are less understood and intensely debated. Many studies have reported that varicocelectomy promotes Leydig cell function based on testosterone levels. In addition, research indicates that ageing in men can induce a reduction in serum testosterone levels (2, 9, 10).

Among mechanisms involved in controlling testicular testosterone level, temperature has been highlighted. Animal models showed that both varicocele and increased testicular temperatures impede sperm production (5, 11). Disruption in the cooling system in veins of the scrotum during varicocele results in an increase in temperature of the scrotum. This phenomenon can be overcome by varicocelectomy (5, 8, 11). High temperatures can reduce the activity of the 17-α hydroxyl progesterone aldolase enzyme, which results in decreased testosterone production. Thus, it is believed that treatment of varicocele may improve the function of Leydig cells, reactivate this enzyme, and increase testosterone production (12-14). In light of this understanding, we aim to assess the effects of varico-

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celectomy on serum testosterone levels and semen quality in infertile men with varicocele.

Materials and Methods

Patients and group design

We conducted this prospective research on 115 infertile men with clinical varicocele grades II and III and 240 fertile men as the control group. The study received approval from the Ethical Committee (number: EC/91/1114) of Royan Institute (Tehran, Iran) and was conducted from August, 2012 to February, 2015. The subjects were men, ages 21-46 years, who were not affected by diabetes and did not take medications known to elicit imbalanced androgen levels. The control group included men who had one or more children, did not suffer from varicocele and diabetes, and did not take medications known to elicit changes in androgen levels. Prior to performing the study, consent letters were received from the patients which informed them of all the study procedures. We included another control group, called the witness group, as the positive control that compared testosterone hormone levels between non-varicocele treated fertile men (had at least one child in the recent year or had more children during their coupling life) against infertile men diagnosed with varicocele.

Blood sample collection and testosterone assay

Patients and fertile males provided blood samples and we compared their serum testosterone levels. The blood samples of infertile men were taken 3-6 months after surgery in order to reassess the changes in serum testosterone levels. Semen parameters (concentration, motility, and morphology) were assessed according to WHO guidelines. In infertile men, prior to varicocelectomy, we assessed the effects of age, testis size (left-right), and grade on the mean total testosterone level. Semen samples were obtained by masturbation after 3-5 days of sexual abstinence. Accordingly, the patient's samples were taken before and after varicocelectomy to evaluate the effects of varicocele repair upon the quality of the sperm parameters.

Blood samples were taken from fertile and infertile men. The level of total testosterone was evaluated by an Elisa Kit (AccuBind® Microwell ELISA Kit, Monobind Inc., Lake Forest, CA, USA) before and after (3-6) varicocelectomy. The sample group (individuals with varicocele) was categorized into two groups according to testis volume of the patients with the volume $\leq$16 ml (2). We also characterized the study group members into two groups based on age less than 35 years old and more than 35 years old.

Statistical analysis

The Pearson correlation was applied to specify the relationship between continuous variables, and the independent t test was used to compare testosterone levels, age, and semen parameters between infertile men with varicocele and fertile men. The unit of testosterone is ng/dl. SPSS 16 software was used to analyze the data. The paired t test was performed to compare the pre- and post-operative testosterone levels, semen volumes, sperm concentrations, and motility. All results of the continuous variables were reported as mean standard deviation. Statistical significance was set at a $P<0.05$. Multiple linear regression analysis was applied to identify potential factors that affected the changes in mean testosterone levels before surgery.

Results

A total of 355 men participated in the study-240 control and 115 infertile men with varicocele. Fertile men had higher mean testosterone levels (583 ± 263 ng/dl) compared to infertile men (567 ± 222 ng/dl) before the operation, however this was not a statistically significant difference (P=0.558). The mean ages of infertile (32.2 ± 5.23 years) and fertile men (32.8 ± 5.27 years) were not significantly different (P=0.328). There was a significant linear relationship observed between age and testosterone level among the control group (Fig.1, r=-0.28, P<0.0001), but we did not observe this in the varicocele group (r=-0.17, P=0.07). The mean size of the left testes (18.58 ± 4.98) was statistically lower than the right testes (19.01 ± 4.75, P=0.017). Pearson correlation showed a significant correlation between total testosterone and right testis size (r=0.21, P=0.026) in infertile men with varicocele before surgery (Fig.2). There was no relationship between grade of varicocele and testosterone level (r=-0.05, P=0.58). Varicocelectomy resulted in an insignificant rise in testosterone levels from 567 ± 222 ng/dl to 594 ± 243 ng/dl (P=0.27, Table 1).

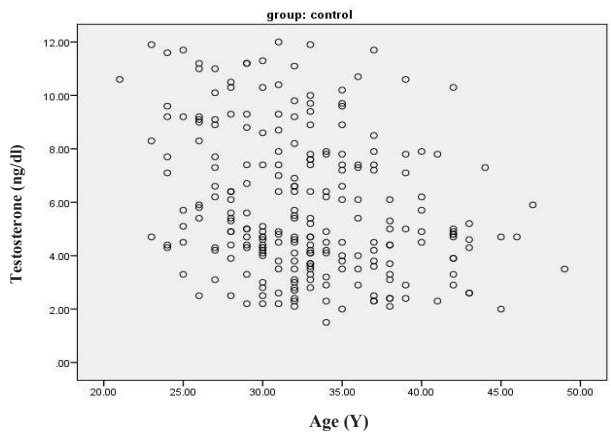

Fig.1: Scatter plot that demonstrates the relationship between testosterone and age in the control group (P<0.05).

Table 1: Comparison of testosterone and semen parameters before and after varicocelectomys

Variable	Before surgery Mean ± SD	After surgery Mean ± SD	P value
Testosterone (ng/dl)	567 ± 223	594 ± 243	0.27
Volume	3.29 ± 1.67	3.39 ± 1.80	0.47
Sperm concentration ($\times 10^6$/ml)	19.10 ± 23.50	28.90 ± 31.90	0.00
Sperm motility (%)	31.60 ± 24.60	32.30 ± 25.60	0.66

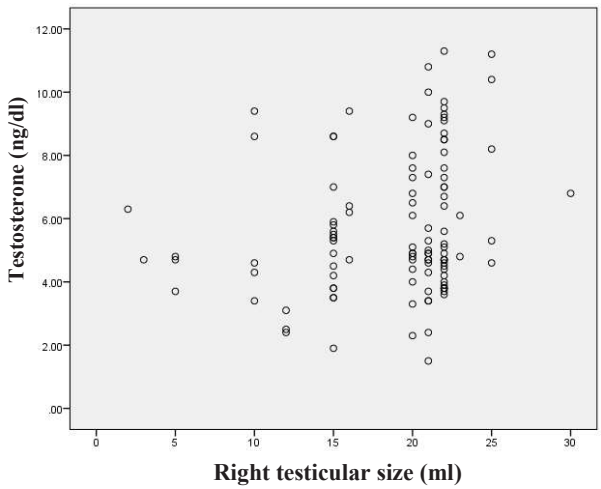

Fig.2: Scatter plot that demonstrates the relationship between testosterone and right testis size in infertile men with varicocele before surgery (P<0.05).

Semen parameters that included including: volume, motility, and concentration were assessed before and after surgery. Both volume and motility of the sperm nonsignificantly increased after surgery. However, sperm concentration significantly (P<0.001) increased after surgery. Linear regression was used to show the effects of the variables (age, grade, and testis size) on total testosterone before surgery (Table 2). The other model was selected using the backward method (Table 3). The regression coefficient for age and right testis size compared to testosterone as the dependent variable was significant (P=0.036).

Table 2: Correlation between testosterone concentrations before surgery with age, grade of varicocele, and left and right testis sizes

Variable	Coefficient	P value
Age	-0.166	0.077
Left testis size	0.177	0.063
Right testis size	0.211	0.026
Varicocele grade	-0.052	0.579

Table 3: Multivariable linear regression coefficients for testosterone before surgerys

Variable	Coefficient	Standard error	P value
Age	-0.067	0.039	0.092
Right testis size	0.087	0.041	0.036

Discussion

The relationship between varicocele and disorder in the function of testosterone production was not clearly understood in that work. To the best of our knowledge, few or no studies have assessed the effect of varicocelectomy upon Leydig cell function and testosterone production. Treatment of varicocele may lead to a suitable condition on total testosterone levels (2). As shown in our research, despite the increased testosterone level in infertile men after varicocelectomy, the difference was not significant.

Other researchers reported the negative impact of varicocele on spermatogenesis. In order to improve the quality of sperm parameters, varicocelectomy was used to treat male infertility. Therefore, we evaluated the other parameters that supposedly affect total testosterone levels. These parameters included age, grade, and testis size. We determined that the difference in the sizes of the left and right testes impacted total testosterone level in infertile men. According to previous studies, the probability of varicocele increased with increased age (15-17). Hsiao et al. (18) showed that the testosterone levels lower than 400 ng/dl improved considerably in individuals after varicocele treatment. However, it has been shown in earlier works that varicocelectomy may improve testosterone production even if it is not significant in addition to semen quality, particularly sperm concentration and motility (8, 9, 19). The present research has shown that the preoperative testosterone levels in infertile men were lower compared to fertile men. After surgery, testosterone levels increased in infertile men with varicocele. However, this increase was not significant. Other sperm parameters such as volume, motility and concentration were analyzed prepostoperative. Although all parameters increased, only the increase in sperm concentration was statistically significant.

In addition to statistical analysis of the mentioned components, we assessed multivariable linear regression coefficients for testosterone before surgery by taking into consideration age and right testis size. Although the coefficient regression related to age stood negative, it was not significant. There was a significant relation between right testis size and total serum testosterone level.

Reşorlu et al. (20) recently reported no changes in serum testosterone levels after varicocele repair. Notably, low-normal testosterone values were recorded both before and after the repair with no significant change in serum testosterone levels for any of their study groups. Preoperative and 6-month postoperative subjects were evaluated by Rodriguez Peña et al. (21), in which hormonal profiles and other data showed an increase in the serum testosterone after surgery, but this increase was not significant. Zohdy et al. (22) reported that patients who underwent varicocelectomy demonstrated a significant postoperative improvement in serum testosterone levels. Considering that varicocele has been universally accepted to negatively impact testis function, including paracrine and endocrine functions of the Leydig cells, the relationship between varicocele and diminished androgen levels appears to be reversed with varicocele repair. However, further studies are needed to better understand the multifactorial pathophysiology of varicocele-mediated Leydig cell dysfunction (23).

Conclusion

The results of this study show that varicocelectomy could improve sperm parameters such as sperm concentration and increase the testosterone level of blood serum

although the increase is statistically insignificant. Nevertheless, it appears that this treatment is necessary to improve function in testes afflicted with varicocele.

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Author's Contributions

M.J., F.F., M.A.S.G., S.J.H., F.D., R.S.; Contributed to conception and design. M.A.S.G., S.J.H., F.D., R.S.; Contributed to all experimental work. M.C.; Data and statistical analysis and interpretation of data. F.F., M.J.; Were responsible for overall supervision. M.J.; Drafted the manuscript. All authors read and approved the final manuscript.

References

1. Bennet WH. Varicocele, particularly with reference to its radical cure. The Lancet Journal. 1889; 261-262.
2. Tanrikut C, Goldstein M, Rosoff JS, Lee RK, Nelson CJ, Mulhall JP. Varicocele as a risk factor for androgen deficiency and effect of repair. BJU Int. 2011; 108(9): 1480-1484.
3. The influence of varicocele on parameters of fertility in a large group of men presenting in infertility clinics. World Health Organization. Fertil Steril. 1992; 57(6): 1289-1293.
4. Gorelick JI, Goldstein M. Loss of fertility in men with varicocele. Fertil Steril. 1993; 59(3): 613-616.
5. Hsiao W, Rosoff JS, Pale JR, Greenwood EA, Goldstein M. Older age is associated with similar improvements in semen parameters and testosterone after subinguinal microsurgical varicocelectomy. J Urol. 2011; 185(2): 620-625.
6. Kirby EW, Wiener LE, Rajanahally S, Crowell K, Coward RM. Undergoing varicocele repair before assisted reproduction improves pregnancy rate and live birth rate in azoospermic and oligospermic men with a varicocele: a systematic review and meta-analysis. Fertil Steril. 2016; 106(6): 1338-1343.
7. Barekat F, Tavalaee M, Deemeh MR, Bahreinian M, Azadi L, Abbasi H, et al. A preliminary study: N-acetyl-L-cysteine improves semen quality following varicocelectomy. Int J Fertil Steril. 2016; 10(1): 120-126.
8. Tavalaee M, Bahreinian M, Barekat F, Abbasi H, Nasr-Esfahani MH. Effect of varicocelectomy on sperm functional characteristics and DNA methylation. Andrologia. 2015; 47(8): 904-909.
9. Damsgaard J, Joensen UN, Carlsen E, Erenpreiss J, Blomberg Jensen M, Matulevicius V, et al. Varicocele is associated with impaired semen quality and reproductive hormone levels: a study of 7035 healthy young men from six european countries. Eur Urol. 2016; 70(6): 1019-1029.
10. Christe N, Meier CA. Hypotestosteronaemia in the aging male: should we treat it? Swiss Med Wkly. 2015; 145: w14216.
11. Durairajanayagam D, Agarwal A, Ong C. Causes, effects and molecular mechanisms of testicular heat stress. Reprod Biomed Online. 2015; 30(1): 14-27.
12. Shiraishi K, Matsuyama H. Elevation of testicular temperature predicts testicular catch-up growth and hypotrophy after varicocelectomy and observation in adolescent varicocele. Urology. 2013; 82(1): 205-209.
13. Andò S, Giacchetto C, Colpi G, Panno ML, Beraldi E, Lombardi A, et al. Plasma levels of 17-OH-progesterone and testosterone in patients with varicoceles. Acta Endocrinol (Copenh). 1983; 102(3): 463-469.
14. Li F, Yue H, Yamaguchi K, Okada K, Matsushita K, Ando M, et al. Effect of surgical repair on testosterone production in infertile men with varicocele: a meta-analysis. Int J Urol. 2012; 19(2): 149-154.
15. Canales BK, Zapzalka DM, Ercole CJ, Carey P, Haus E, Aeppli D, et al. Prevalence and effect of varicoceles in an elderly population. Urology. 2005; 66(3): 627-631.
16. Chiba K, Fujisawa M. Clinical outcomes of varicocele repair in infertile men: a review. World J Mens Health. 2016; 34(2): 101-109.
17. Levinger U, Gornish M, Gat Y, Bachar GN. Is varicocele prevalence increasing with age? Andrologia. 2007; 39(3): 77-80.
18. Hsiao W, Rosoff JS, Pale JR, Powell JL, Goldstein M. Varicocelectomy is associated with increases in serum testosterone independent of clinical grade. Urology. 2013; 81(6): 1213-1217.
19. Tavalaee M, Bahreinian M, Barekat F, Abbasi H, Nasr-Esfahani MH. Effect of varicocelectomy on sperm functional characteristics and DNA methylation. Andrologia. 2015; 47(8): 904-909.
20. Reşorlu B, Kara C, Sahin E, Unsal A. The significance of age on success of surgery for patients with varicocele. Int Urol Nephrol. 2010; 42(2): 351-356.
21. Rodriguez Peña M, Alescio L, Russell A, Lourenco da Cunha J, Alzu G, Bardoneschi E. Predictors of improved seminal parameters and fertility after varicocele repair in young adults. Andrologia. 2009; 41(5): 277-281.
22. Zohdy W, Ghazi S, Arafa M. Impact of varicocelectomy on gonadal and erectile functions in men with hypogonadism and infertility. J Sex Med. 2011; 8(3): 885-893.
23. Hayden RP, Tanrikut C. Testosterone and varicocele. Urol Clin North Am. 2016; 43(2): 223-232.

Original Article

Detection of Y Chromosome Microdeletions and Hormonal Profile Analysis of Infertile Men undergoing Assisted Reproductive Technologies

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Abstract

Background: Y chromosome deletions (YCDs) in azoospermia factor (AZF) region are associated with abnormal spermatogenesis and may lead to azoospermia or severe oligozoospermia. Assisted reproductive technologies (ART) by intracytoplasmic sperm injection (ICSI) and testicular sperm extraction (TESE) are commonly required for infertility management of patients carrying YCDs. The aim of this study was to estimate the frequency of YCDs, to find the most frequent variant in infertile men candidate for ART and to compare YCD distribution with a control fertile group. The semen parameters, hormonal profiles and ART outcomes of the infertile group were studied.

Materials and Methods: This case-control study consisted of 97 oligozoospermic or non-obstructive azoospermic (NOA) infertile men, who had undergone ART, as the case group and 100 fertile men as the control group. DNA samples were extracted from blood samples taken from all 197 participants and YCDs were identified by multiplex polymerase chain reaction (PCR) of eight known sequence-tagged sites. The chi-square test was used to compare the mean values of hormone and sperm parameters between the two groups. $P<0.05$ was considered statistically significant.

Results: No YCD was detected in the control group. However, 20 out of 97 (20.6%) infertile men had a YCD. AZFc, AZFbc and AZFabc deletions were detected in 15 (75%), four (20%) and one (5%) YCD-positive patients. No fertilization or clinical pregnancy was seen following ICSI in this sub-group with YCD. The mean level of FSH was significantly higher in the group with YCD (28.45 ± 22.2 vs. 4.8 ± 3.17 and 10.83 ± 7.23 in YCD-negative patients with and without clinical pregnancy respectively).

Conclusion: YCD is frequent among NOA men and YCD screening before ART and patient counseling is thus strongly recommended.

Keywords: Assisted Reproductive Technologies, Non-obstructive Azoospermia, Y Chromosome Deletion

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Introduction

Approximately 15% of all couples of reproductive age have difficulty conceiving a child (1). Male-factor infertility accounts for about half of these cases (2). Varicocele, obstruction of spermatic duct, erectile dysfunction, failure of ejaculation and sex hormone imbalances have been identified as major causes of male infertility. However, 10% of male infertility is due to genetic factors including chromosomal aneuploidies and rearrangements, microdeletions and single gene defects (3, 4).

Proper spermatogenesis is dependent on numerous genes, many of which are located on the long arm of the Y chromosome (Yq11). A 10 Mb region on the long arm of the Y chromosome, namely the azoospermia factor (AZF) region, is frequently deleted in men with unexplained spermatogenic failure. AZF was first mapped in 1976 to Yq11 and has shown to play an important role in male germ cell proliferation and differentiation (5,

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6). Further studies, by analyzing sequence-tagged sites (STS), have revealed the genetic complexity of the AZF region. This region is structurally subdivided into three sections, namely AZFa, AZFb and AZFc from proximal to distal of the Yq region (7).

Yq has many palindrome repeats across the AZF. The homologous recombination between these repeats generates microdeletions in the AZF sub-regions, which in turn may lead to spermatogenic failure (8, 9). Y chromosome deletions (YCDs) may affect spermatogenesis at different progression steps. For example, deletion of AZFa causes Sertoli cell-only syndrome (SCOS). Identification of these deletions is thus highly important since isolating sperms by testicular sperm extraction (TESE) for intracytoplasmic sperm injection (ICSI) is improbable (10). Patients with AZFb microdeletions may have normal spermatogonia and primary spermatocytes in their tubules, however, they display pre-meiotic spermatogenic arrest or SCOS and, eventually, azoospermia. It is therefore difficult to recover mature sperms using TESE from AZFb-deleted patients (11, 12) . Complete deletion of AZFc, the most frequent type of YCD, presents a wide range of phenotypes from azoospermia to severe oligozoospermia (13, 14).

The worldwide incidence of YCD is approximately 1-55.5% in infertile men showing significant variation among different populations (9). The aim of this study was to estimate the frequency of YCDs in infertile males and identify the most frequent variant among those who had ART at our Infertility Center and compared the results with those of a fertile group. Also semen parameters, hormonal profiles and ART outcomes of the infertile group were studied.

Materials and Methods

Patient selection and DNA extraction

In this case-control study, a total of 140 infertile men, from couples who had undergone ART at the Infertility Center of Ghadir Mother and Child Hospital, and 100 fertile men without any history of primary or secondary infertility and with at least one phenotypically normal child, who were referred to the Genetics Research Center at Shahid Dastghaib Hospital, were enrolled in this case-control study.

The infertile men with known karyotype abnormalities, obstructive azoospermia, varicocele, testicular tumors and abnormal physical examinations were excluded, resulting in a case group consisting of 97 men. These individuals had either non-obstractive azoospermia (NOA) or oligozoospermia (defined as sperm counts less than 15×10^6 according to the World Health Organization 2010. This study was approved by the Institutional Ethics Committee of Shiraz University of Medical Sciences and all of the participants signed a written consent form before enrolment. All 197 participants consciuosly donated a 2 ml peripheral blood sample and DNA was extracted from these samples by a commercial DNA extraction kit (Qiagen, Germany) and YCD typing was undertaken.

The hospital charts of the 97 infertile men were checked for their semen analysis parameters, hormonal profiles and ART outcomes. Semen samples had been analyzed for standard sperm quality parameters (volume, count, rates of motility and morphology) according to the World Health Organization (2010) and the Kruger classification (15, 16). ART outcomes were defined as fertilization and clinical pregnancy (CP). Fertilization was considered as development of two pro-nucleus stage embryos at 16-18 hours after *in vitro* fertilization (IVF)/ICSI. CP was defined as detection of a gestational sac and a fetal heart at 4-5 weeks after embryo transfer by transvaginal ultrasound scan.

Polymerase chain reaction analysis

Samples were tested for classical YCD by typing six STSs, namely sY84, sY86, sY127, sY134, sY254 and sY255 by using the YChromStrip kit (Operon, Spain) for cases and a manual PCR method for all controls. Initially, the ZFX/ZFY was used to determine the presence of Y chromosome in all tested individuals. The detection of sY14 (SRY) was employed as an internal control of PCR.

To detect AZFa, AZFb and AZFc, sY86, sY127 and sY254 were used for Multiplex PCR I and sY84, sY134 and sY255 were used for Multiplex PCR II. Multiplex PCR reactions were carried out in a total volume of 50 µL. Amplifications were carried out on a thermocycler (Eppendorf, Germany) with cycling conditions of an initial denaturation at 94°C for 15 minutes followed by 35 cycles of 94°C for 30 seconds for denaturation, 57°C for 90 seconds for primer annealing and 72°C for 1 minute for extension. This program was followed by a final extension step at 72°C for 10 minutes. A clear amplified product of the expected site was considered as a positive result for that site.

The reaction products were then analyzed by electrophoresis on a 1.5% agarose gel (Sigma, USA). If a deletion was observed, a second identical reaction was run to confirm the deletion in the presence of a positive control for that deletion. All primer sequences, the location of markers and the size of PCR products are shown (Table 1).

Statistical analysis

The mean of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone and sperm parameters were compared between groups using the t test (one-way ANOVA test considering number of the groups). $P<0.05$ was considered statistically significant.

Table 1: Details of primers, sequence-tagged sites (STS) location and polymerase chain reaction product sizes

Primer name	Sequence (5´-3´)	Product size	Location	Acession number	Position
ZFY	F: GTCTTGTTGCAGCCCATGTA R: CAAAGGGAGAACTAGCAGGC	495 bp	sY1301	BV679198.1	Yp11.2
sY84	F: AGAAGGGTCTGAAAGCAGGT R: GCCTACTACCTGGAGGCTTC	326 bp	DYS273	G12019	Yq11.1
sY86	F: GTGACACACAGACTATGCTTC R: ACACACAGAGGGACAACCCT	320 bp	DYS148	G49207	Yq11.21
sY127	F: GGCTCACAAACGAAAAGAAA R: CTGCAGGCAGTAATAAGGGA	274 bp	DYS218	G11998	Yq11.222
sY134	F: GTCTGCCTCACCATAAAACG R: ACCACTGCCAAAACTTTCAA	301 bp	DYS224	G12001	Yq11.222
sY254	F: GGGTGTTACCAGAAGGCAAA R: GAACCGTATCTACCAAAGCAGC	380 bp	DAZ1	G38349	Yq11.223
sY255	F: GTTACAGCATTCGGCGTGAT R: CTCGTCATGTGCAGCCAC	126 bp	DAZ	G65827	Yq11.223
SRY	F: GAATATTCCCGCTCTCCGGA R: GCTGGTGCTCCATTCTTGAG	472 bp	SRY	G38356	Yp11.3

Results

Y chromosome deletion frequency

The 100 fertile men had a mean age of 29.67 ± 6.17 while the 97 infertile men with oligozoospermia or azoospermia had a mean age of 35.13 ± 7.7. No YCD was detected in the 100 fertile men. Twenty (20.6%) infertile men had YCD on their Yq. Of the observed YCD, AZFc was the most frequent (15 YCD-positive cases (75%), followed by AZFbc (four YCD-positive cases (20%) and AZFabc (singleton (5%).

Assisted reproductive technologies outcome

We classified the infertile men who had ART based on presence/absence of YCD and clinical pregnancies into three groups. Number of participants and ART outcome results are shown (Table 2).

Table 2: Classification of the infertile men based on presence/absence of YCD and clinical pregnancy after ART

Infertile men	Participants	Fertilization
CP in the absence of YCD	42	42
No CP in the absence of YCD	35	33
No CP in the presence of YCD	20	0

ART; Assisted reproductive technology, CP; Clinical pregnancy, and YCD; Y chromosome deletion. Values are presented as counts.

Sperm parameters, documented by semen analyses, of the three infertile groups and their hormonal profiles for the FSH, LH and testosterone are shown (Table 3). All patients carrying YCD were azoospermic. Moreover the mean level of FSH was significantly different between groups (P=0.023). The FSH level was 28.45 ± 22.2 in the group with YCD. However it was 4.8 ± 3.17 and 10.83 ± 7.23 in YCD-negative groups with or without clinical pregnancy respectively.

Discussion

Microdeletions of the AZF region on the long arm of Y chromosome are one of the most important genetic causes of male infertility, which is manifested commonly as severe oligozoospermia and NOA (17, 18). The incidence of YCD is estimated to be about 65-70% in azoospermic men (19). Nevertheless, YCD has been reported to be approximately 5-13% in infertile men with severe oligozoospermia and azoospermia (20, 21).

There have been several studies reporting the prevalence of YCD in Iran, however, some discrepancies exist (22, 23). Totonchi et al. (24) investigated AZF microdeletions in 3654 Iranian infertile men. They found 185 cases (5.06%) with AZF microdeletions. Among patients carrying YCD, 79.4% had azoospermia and 20.5% had severe oligozoospermia. AZFc microdeletions were found to be the most prevalent form among YCDs. Re-

Table 3: Sperm parameters and hormonal profiles of the infertile men in three groups

Infertile men	Volume (ml)	Count ($\times 10^6$/ml)	Motility (%)	Morph (%)	FSH (mIU/ml)	LH (mIU/ml)	Testosterone (ng/dl)
CP and no YCD	3.72 ± 1.9	9.59 ± 1.8	23.64 ± 18.8	9.23 ± 6.1	4.8 ± 3.17	3.76 ± 2.5	4.53 ± 1.3
No CP and no YCD	3.29 ± 1.67	7.35 ± 1.8	20.91 ± 19.3	9.55 ± 5.4	10.83 ± 7.23	8.29 ± 7.8	6.63 ± 5.5
No CP and presence of YCD	4.17 ± 1.3	0	0	0	28.45 ± 22.2	8.56 ± 3.71	4.4 ± 2.6
P value	0.476	0.064	0.003*	0.45	0.023*	0.31	0.53

CP; Clinical pregnancy, YCD; Y chromosome deletion, Morph; Morphology, FSH; Follicle stimulating hormone, LH; Luteinizing hormone, and Testost; Testosterone. Values are presented as mean ± SD. *P<0.05 is significant.

cently, a meta-analysis conducted by Yousefi-Razin et al. (25) affirmed the rate of YCD to be 12.1% in Iranian azoospermic or severe oligozoospermic individuals. However, they suggested that the frequency of YCDs is related to ethnic and territorial differences.

In our study, YCD was detected in 20.6% of infertile men, among which the AZFc region deletions was the most frequent, comprising 75% of all deletions. These results are in agreement with previous reports in Iran and other countries (8, 14, 24, 26). We noticed that ICSI cycles undertaken for all of the 15 men carrying AZFc deletions failed in this study. This finding does not affirm the available literature which indicates that men with AZFc deletions may have successful ICSI outcomes (27).

Previous studies have nevertheless shown that ICSI results are worse for men with NOA compared with men with obstructive azoospermia (20). Although the literature indicates that successful ART outcomes are possible after repeated ICSI cycles, the couple should be counseled about the inheritance of this fertility problem in their male offspring and sperm cryopreservation at a young age is strongly suggested for their male children.

It is reported that with complete deletion of AZFa and AZFb, TESE is usually not successful for sperm harvesting. Interestingly, one of our NOA patients who carried a complete AZFabc deletion had successful sperm retrieval by TESE. Although his retrieved sperm did not result in a successful fertilization, this finding shows that the results of YCD tests can not always predict the failure of sperm retrieval by TESE. We believe that ICSI failure in the YCD-positive sub-group in this study is likely due to their profound testicular failure which is reflected by their high mean FSH level (28.45 ± 22.2 mIU/ml).

All patients with YCD were azoospermic and had failed fertilization results after TESE and ICSI. There are several reports demonstrating a lower fertilization rate in patients with YCD compared with infertile men without any deletions (28-30). It should be taken into consideration that in the present study we did not select the patients according to presence/absence of YCD from the beginning. On the contrary, the infertile men who had ART were enrolled in this study. Although the study design here is different, the observation of no fertilization in the YCD-positive sub-group is in agreement with previous studies.

We observed a much higher FSH level in the group with YCDs compared with the other two groups. The appropriate induction and maintenance of sperm production is dependent on appropriate serum FSH levels. It has been shown that azoospermic men with FSH levels ≥20 IU/L have lower chances of having live-born children with the ICSI method (31, 32). YCDs cause impaired spermatogenesis and by inducing a positive feedback on FSH lead to higher FSH levels. Absence or severe reduction of spermatocytes has been known to cause high FSH levels. However, FSH levels are normal when there is normal sperm counts associated with maturation arrest. Till now, no cut-off value is identified for FSH that can accurately predict the failure of harvesting sperm during TESE.

Our second infertile group did not have any CP in spite of proceeding to the fertilization stage in 33 out of the 35 cases (94%) and being YCD-negative . Possibility of other concurrent causes of infertility such as poor oocyte quality in the two individuals who did not have fertilization should be considered. In the remaining 33 individuals, implantation issues may be a possible explanation for the failure of clinical pregnancy in fertilized oocytes.

Future studies regarding YCD frequencies and ART outcomes with larger sample sizes, in seprated ethnic groups are strongly recommended. Moreover, investigation of AZFc sub-mutations may lead to valuable insights. Also, evaluation of any probable correlation between YCD and histopathological results might add further insights.

Conclusion

YCD had a relatively high frequency among NOA men in our study. This result confirms the necessity of YCD screening for infertile men and appropriate patient counseling before ART.

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Author's Contributions

B.N.J., S.Z., Z.A.; Conceived and devised the study. M.D.; Was responsible for sample collection. A.B., S.M.; Carried out all experiments. A.B., S.M., N.M.V.; Analyzed the results. Z.A., B.N.J., M.E.P.; Assisted in defining the idea and writing the manuscript. The manuscript was revised by A.Z. and N.M.V. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

References

1. de Kretser DM. Male infertility. Lancet. 1997; 349(9054): 787-790.
2. Thonneau P, Marchand S, Tallec A, Ferial ML, Ducot B, Lansac J, et al. Incidence and main causes of infertility in a resident population (1,850,000) of three French regions (1988-1989). Hum Reprod. 1991; 6(6): 811-816.
3. Vogt PH. Molecular genetics of human male infertility: from genes to new therapeutic perspectives. Curr Pharm Des. 2004; 10(5): 471-500.
4. Ambulkar PS, Sigh R, Reddy M, Varma PS, Gupta DO, Shende MR, et al. Genetic risk of azoospermia factor (AZF) microdeletions in idiopathic cases of azoospermia and oligozoospermia in

central Indian population. J Clin Diagn Res. 2014; 8(3): 88-91.
5. Tiepolo L, Zuffardi O. Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. Hum Genet. 1976; 34(2): 119-124.
6. Vogt PH. Azoospermia factor (AZF) in Yq11: towards a molecular understanding of its function for human male fertility and spermatogenesis. Reprod Biomed Online. 2005; 10(1): 81-93.
7. Vogt PH, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, Kiesewetter F, et al. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. Hum Mol Genet. 1996; 5(7): 933-943.
8. Sheikhha MH, Zaimy MA, Soleimanian S, Kalantar SM, Rasti A, Golzade M, et al. Multiplex PCR screening of Y-chromosome microdeletions in azoospermic ICSI candidate men. Iran J Reprod Med. 2013; 11(4): 335-338.
9. Suganthi R, Vijesh VV, Jayachandran S, Fathima Benazir JA. Multiplex PCR based screening for microdeletions in azoospermia factor region of Y chromosome in azoospermic and severe oligozoospermic south Indian men. Iran J Reprod Med. 2013; 11(3): 219-226.
10. Kleiman SE, Yogev L, Lehavi O, Hauser R, Botchan A, Paz G, et al. The likelihood of finding mature sperm cells in men with AZFb or AZFb-c deletions: six new cases and a review of the literature (1994-2010). Fertil Steril. 2011; 95(6): 2005-2012.
11. Yu XW, Wei ZT, Jiang YT, Zhang SL. Y chromosome azoospermia factor region microdeletions and transmission characteristics in azoospermic and severe oligozoospermic patients. Int J Clin Exp Med. 2015; 8(9): 14634-14646.
12. Mau Kai C, Juul A, McElreavey K, Ottesen AM, Garn ID, Main KM, et al. Sons conceived by assisted reproduction techniques inherit deletions in the azoospermia factor (AZF) region of the Y chromosome and the DAZ gene copy number. Hum Reprod. 2008; 23(7): 1669-1678.
13. Krausz C, Hoefsloot L, Simoni M, Tuttelmann F, European Academy of Andrology, European Molecular Genetics Quality Network. EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: state-of-the-art 2013. Andrology. 2014; 2(1): 5-19.
14. Elfateh F, Rulin D, Xin Y, Linlin L, Haibo Z, Liu RZ. Prevalence and patterns of Y chromosome microdeletion in infertile men with azoospermia and oligzoospermia in Northeast China. Int J Reprod Med. 2014; 12(6): 383-388.
15. Check JH, Adelson HG, Schubert BR, Bollendorf A. Evaluation of sperm morphology using Kruger's strict criteria. Arch Androl. 1992; 28(1): 15-17.
16. World Health Organization. Laboratory manual of the WHO for the examination of human semen and sperm-cervical mucus interaction. Ann Ist Super Sanita. 2001; 37(1): I-XII, 1-123.
17. Zhu XB, Gong YH, He J, Guo AL, Zhi EL, Yao JE, et al. Multicentre study of Y chromosome microdeletions in 1,808 Chinese infertile males using multiplex and real-time polymerase chain reaction. Andrologia. 2017; 49(5).
18. Ferlin A, Arredi B, Speltra E, Cazzadore C, Selice R, Garolla A, et al. Molecular and clinical characterization of Y chromosome microdeletions in infertile men: a 10-year experience in Italy. J Clin Endocrinol Metab. 2007; 92(3): 762-770.
19. Jungwirth A, Giwercman A, Tournaye H, Diemer T, Kopa Z, Dohle G, et al. European association of urology guidelines on male infertility: the 2012 update. Eur Urol. 2015; 62(2): 324-332.
20. Reijo R, Alagappan RK, Patrizio P, Page DC. Severe oligozoospermia resulting from deletions of azoospermia factor gene on y chromosome. Lancet. 1996; 347(9011): 1290-1293.
21. Malekasgar AM, Mombaini H. Screening of Y chromosome microdeletions in iranian infertile males. J Hum Reprod Sci. 2008; 1(1): 2-9.
22. Saliminejad K, Khorshid HR. Discrepancy in the results of Y chromosome microdeletions in an Iranian population. J Hum Reprod Sci. 2011; 4(3): 157.
23. Saliminejad K, Khorshid HR. Methodological errors in screening of Yq microdeletion in Iranian azoospermic men. Indian J Med Res. 2012; 135: 137-138.
24. Totonchi M, Mohseni Meybodi A, Borjian Boroujeni P, Sedighi Gilani M, Almadani N, Gourabi H. Clinical data for 185 infertile Iranian men with Y-chromosome microdeletion. J Assist Reprod Genet. 2012; 29(8): 847-853.
25. Yousefi-Razin E, Nasiri MJ, Omrani MD. Frequency of Y chromosome microdeletions among Iranian infertile men with azoospermia and severe oligozoospermi: a meta-analysis. J Reprod Infertil. 2015; 17(4): 208-212.
26. Zaimy MA, Kalantar SM, Sheikhha MH, Jahaninejad T, Pashaiefar H, Ghasemzadeh J, et al. The frequency of Yq microdeletion in azoospermic and oligospermic Iranian infertile men. Int J Reprod Med. 2013; 11(6): 453-458.
27. Gardner D, Weissman A, Howles C, Shoham Z. Textbook of assisted reproductive technologies. 3rd ed. Geneva: Informa; 2012; 343-356.
28. van Golde RJ, Wetzels AM, de Graaf R, Tuerlings JH, Braat DD, Kremer JA. Decreased fertilization rate and embryo quality after ICSI in oligozoospermic men with microdeletions in the azoospermia factor c region of the Y chromosome. Hum Reprod. 2001; 16(2): 289-292.
29. Choi JM, Chung P, Veeck L, Mielnik A, Palermo GD, Schlegel PN. AZF microdeletions of the Y chromosome and in vitro fertilization outcome. Fertil Steril. 2004; 81(2): 337-341.
30. Liu XH, Qiao J, Li R, Yan LY, Chen LX. Y chromosome AZFc microdeletion may not affect the outcomes of ICSI for infertile males with fresh ejaculated sperm. J Assist Reprod Genet. 2013; 30(6): 813-819.
31. Ramasamy R, Lin K, Gosden LV, Rosenwaks Z, Palermo GD, Schlegel PN. High serum FSH levels in men with nonobstructive azoospermia does not affect success of microdissection testicular sperm extraction. Fertil Steril. 2009; 92(2): 590-593.
32. Zitzmann M, Nordhoff V, von Schonfeld V, Nordsiek-Mengede A, Kliesch S, Schuring AN, et al. Elevated follicle-stimulating hormone levels and the chances for azoospermic men to become fathers after retrieval of elongated spermatids from cryopreserved testicular tissue. Fertil Steril. 2006; 86(2): 339-347.

International Journal of Fertility and Sterility (Int J Fertil Steril)
Guide for Authors

Aims and Scope: International Journal of Fertility & Sterility is a quarterly English publication of Royan Institute of Iran. The aim of this journal is to disseminate information through publishing the most recent scientific research studies on Fertility and Sterility and other related topics. **Int J Fertil Steril** has been certified by Ministry of Culture and Islamic Guidance since 2007. It has also been accredited as a scientific and research journal by HBI (Health and Biomedical Information) Journal Accreditation Commission since 2008. **This open access journal holds the membership of the Committee on Publication Ethics (COPE).**

1. Types of articles

The articles in the field of Fertility and Sterility can be considered for publications in Int J Fertil Steril. These articles are as below:

A. Original articles are scientific reports of the original research studies. The article consists of English Abstract (structured), Introduction, Materials and Methods, Results, Discussion, Conclusion, Acknowledgements, Author's Contributions, and References **(Up to 40).**

B. Review articles are the articles written by well experienced authors and those who have excellence in the related fields. The corresponding author of the review article must be one of the authors of at least three published articles appearing in the references. The review article consists of English Abstract (unstructured), Introduction, Conclusion, Author's Contributions, and References **(Up to 70).**

C. Systematic Reviews

Systematic reviews are a type of literature review that collect and critically analyzes multiple research studies or papers. The Systematic reviews consist of English Abstract (unstructured), Introduction, Materials and Methods, Results, Discussion, Conclusion, Acknowledgements, Author's Contributions, and References **(Up to 70).**

D. Short communications are the articles containing new findings. Submissions should be brief reports of ongoing researches. The short communication consists of English Abstract (unstructured), the body of the manuscript (should not hold heading or subheading), Acknowledgements, Author's Contributions, and References **(Up to 30).**

E. Case reports are short discussions of a case or case series with unique features not previously described which make an important teaching point or scientific observation. They may describe novel techniques or use equipment, or new information on diseases of importance. It consists of English Abstracts (Unstructured), Introduction, Case Report, Discussion, Acknowledgements, Author's Contributions, and References **(Up to 30).**

F. Editorial should be written by either the editor in chief or the editorial board.

G. Imaging in reproductive medicine should focus on a single case with an interesting illustration such as a photograph, histological specimen or investigation. Color images are welcomed. The text should be brief and informative.

H. Letter to the editors are welcome in response to previously published **Int J Fertil Steril** articles, and may also include interesting cases that do not meet the requirement of being truly exceptional, as well as other brief technical or clinical notes of general interest.

I. Debate.

2. Submission Process

It is recommended to see the guidelines for reporting different kinds of manuscripts here. This guide explains how to prepare the manuscript for submission. Before submitting, we suggest authors familiarize themselves with **Int J Fertil Steril** format and content by reading the journal via the website (www.ijfs.ir). The corresponding author ensures that all authors are included in the author list and agree with its order, and they must be aware of the manuscript submission.

A. Author contributions statements

It is essential for authors to include a statement of responsibility in the manuscript that specifies the contribution of every one of them. This participation must include conception and design of the manuscript, data acquisition or data analysis and interpretation, drafting of the manuscript and/or revising it for critically important intellectual content, revision and final approval of the manuscript and statistical analysis, obtaining funding, administrative, technical, or material support, or supervision. Authors who do not meet the above criteria should be acknowledged in the **Acknowledgments Section**.

B. Cover letter

Each article should be accompanied by a cover letter, signed by all authors specifying the following statement: "The manuscript has been seen and approved by all authors and is not under active consideration for publication. It has neither been accepted for publication nor published in another journal fully or partially (except in abstract form). I hereby assign the copyright of the enclosed manuscript to **Int J Fertil Steril**. The corresponding author must confirm the proof of the manuscript before online publishing. Also, is it needed to suggest three peer reviewers in the field of their manuscript.

C. Manuscript preparation

Authors whose first language is not English encouraged to consult a native English speaker in order to confirm his manuscripts to US or British (not a mixture) English usage and grammar. The manuscript should be prepared in accordance with the "International Committee of Medical Journal Editors (ICMJE)". Please send your manuscript in two formats (word and Pdf). The abstract and

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It should be added that an essential step toward the integration and linking of scientific information reported in published literature is using standardized nomenclature in all fields of science and medicine. Species names must be italicized (e.g., Homo sapiens) and also the full genus and species written out in full, both in the title of the manuscript and at the first mention of an organism in a paper.

It is necessary to mention that genes, mutations, genotypes, and alleles must be indicated in italics. Please use the recommended name by consulting the appropriate genetic nomenclature database, e.g., HUGO for human genes. In another word; if it is a human gene, you must write all the letters in capital and italic (e.g., OCT4, c-MYC). If not, only write the first letter in capital and italic (e.g., Oct4, c-Myc). **In addition, protein designations are the same as the gene symbol but are not italicized.**

Of note, Int J Fertil Steril will only consider publishing genetic association study papers that are novel and statistically robust. Authors are advised to adhere to the recommendations outlined in the STREGA statement (http://www.strega-statement.org). The following criteria must be met for all submissions:

1. Hardy-Weinberg Equilibrium (HWE) calculations must be carried out and reported along with the P-values if applicable [see Namipashaki et al. 2015 (Cell J, Vol 17, N 2, Pages: 187-192) for a discussion].

2. Linkage disequilibrium (LD) structure between SNPs (if multiple SNPs are reported) must be presented.

3. Appropriate multiple testing correction (if multiple independent SNPs are reported) must be included.

Submissions that fail to meet the above criteria will be rejected before being sent out for review.

Each of the following manuscript components should begin in the following sequence:

Authors' names and order of them must be carefully considered (full name(s), highest awarded academic degree(s), email(s), and institutional affiliation(s) of all the authors in English. Also, you must send mobile number and full postal address of the corresponding author).

Changes to Authorship such as addition, deletion or rearrangement of author names must be made only before the manuscript has been accepted in the case of approving by the journal editor. In this case, the corresponding author must explain the reason of changing and confirm them (which has been signed by all authors of the manuscript). If the manuscript has already been published in an online issue, an erratum is needed.

Title is providing the full title of the research (do not use abbreviations in title).

Running title is providing a maximum of 7 words (no more than 50 characters).

Abstract must include Background, Materials and Methods, Results, and Conclusion (no more than 300 words).

Keywords, three to five, must be supplied by the authors at the foot of the abstract chosen from the Medical Subject Heading (MeSH). Therefore; they must be specific and relevant to the paper.

The following components should be identified in the abstract:

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Ethical considerations: Please state that informed consent was obtained from all human adult participants and from the parents or legal guardians of minors and include the name of the appropriate institutional review board that approved the project. It is necessary to indicate in the text that the maintenance and care of experimental animals complies with National Institutes of Health guidelines for the humane use of laboratory animals, or those of your Institute or agency.

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Discussion: It should emphasize the present findings and the variations or similarities with other researches done by other researchers. The detailed results should not be repeated in the discussion again. It must emphasize the new and important aspects of the study.

Conclusion: It emphasizes the new and important aspects of the study. All conclusions are justified by the results of the study.

Acknowledgements: This part includes a statement thanking those who contributed substantially with work relevant to the study but does not have authorship criteria. It includes those who provided technical help, writing assistance and name of departments that provided only general support. You must mention financial support in the study. Otherwise; write this sentence "There is no financial support in this study".

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Abstract book:

Example: Nabavi SM. Stem cell therapy for multiple sclerosis. Cell J. 2013; 5 Suppl 1: Os-13.

Thesis:

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Example: Eftekhari Yazdi P. Comparison of fragment removal and co-culture with Vero cell monolayer's on development of human fragmented embryos. Presented for the Ph.D., Tehran. Tarbiyat Modarres University. 2004.

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Example: Jahanshahi A, Mirnajafi-Zadeh J, Javan M, Mohammad-Zadeh M, Rohani M. Effect of low-frequency stimulation on adenosineA1 and A2A receptors gene expression in dentate gyrus of perforant path kindled rats. Cell J. 2008; 10 (2): 87-92. Available from: http://www.celljournal.org. (20 Oct 2008).

Book:

Example: Anderson SC, Poulsen KB. Anderson's electronic atlas of hematology.[CD-ROM]. Philadelphia: Lippincott Williams & Wilkins; 2002.

Law:

Example: Embryo donation law. Iran Judicature, Official Gazette of the Islamic Republic of Iran. Available from: http://www.dastour.ir/Brows/?lid=245069.(20 Jul 2013).

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