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INTERNATIONAL JOURNAL OF FERTILITY AND STERILITY

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Editorial

The CROWN Initiative: Journal Editors Invite Researchers to Develop Core Outcomes in Women's Health

The Core Outcomes in Women's Health (CROWN) Initiative

Clinical trials, systematic reviews and guidelines compare beneficial and non-beneficial outcomes following interventions. Often, however, various studies on a particular topic do not address the same outcomes, making it difficult to draw clinically useful conclusions when a group of studies is looked at as a whole (1). This problem was recently thrown into sharp focus by a systematic review of interventions for preterm birth prevention, which found that among 103 randomised trials, no fewer than 72 different outcomes were reported (2). There is a growing recognition among clinical researchers that this variability undermines consistent synthesis of the evidence, and that what is needed is an agreed standardised collection of outcomes-a "core outcomes set"-for all trials in a specific clinical area (1). Recognising that the current inconsistency is a serious hindrance to progress in our specialty, the editors of over 50 journals related to women's health have come together to support The CROWN (CoRe Outcomes in WomeN's health) Initiative (Box 1).

Box 1: Aims of The CROWN Initiative

1. Form a consortium among all gynaecology-obstetrics and related journals to promote core outcome sets in all areas of our specialty.

2. Encourage researchers to develop core outcome sets using robust consensus methodology involving multiple stakeholders, including patients.

3. Strongly encourage the reporting of results for core outcome sets.

4. Organise robust peer-review and effective dissemination of manuscripts describing core outcome sets.

5. Facilitate embedding of core outcome sets in research practice, working closely with researchers, reviewers, funders and guideline makers.

www.crown-initiative.org

Development of consensus is required around a set of well-defined, relevant and feasible outcomes for all trials concerning particular obstetric and gynaecologic health conditions, such as preterm birth, incontinence, infertility and menstrual problems. With so many subspecialties involved, this is no easy task. Duplication of effort can be avoided by working with the Core Outcome Measures in Effectiveness Trials (COMET) Initiative, which is working towards core data sets for all medical specialties (3). Production of trustworthy core outcome sets will require engagement with patients, healthcare professionals, researchers, industry and regulators, and the employment of scientifically robust consensus methods (1). The data for these core outcome sets, once agreed upon, should be collected in trials and reported in publications as standard practice in the future.

Journal editors now invite researchers to take the lead in beginning this work. What will we do as editors to support them and their colleagues? First, we are drawing wide attention to The CROWN Initiative by publishing this editorial in the journals listed below. We shall ensure that the global research community, which includes our many reviewers, is aware of the need for core outcome sets. Submissions which describe development of core outcome sets, if deemed acceptable after peer review, will be effectively disseminated.

Our collaboration is not for enforcing harmony at the expense of innovation. To quote from the COMET home page (www.comet-initiative.org): "The existence or use of a core outcome set does not imply that outcomes in a particular trial should be restricted to those in the relevant core outcome set. Rather, there is an expectation that the core outcomes will be collected and reported, making it easier for the results of trials to be compared, contrasted and combined as appropriate; while researchers continue to explore other outcomes as well." We also expect that as new or superior ways of capturing outcomes emerge, core outcome sets

will themselves need updating.

Producing, disseminating and implementing core outcome sets will ensure that critical and important outcomes with good measurement properties are incorporated and reported. We believe this is the next important step in advancing the usefulness of research, in informing readers, including guideline and policy developers, who are involved in decision-making, and in improving evidence-based practice.

Acknowledgements

Note

Reproduced from *The Core Outcomes in Women's Health (CROWN) Initiative* with permission from the Royal College of Obstetricians and Gynaecologists and John Wiley & Sons Ltd.

Appendix

The CROWN Initiative includes the following journals, in alphabetical order (correct on 13th May 2014, up to date list available at www.crown-initiative.org):

1. Acta Obstetricia et Gynecologica Scandinavica
2. American Journal of Obstetrics & Gynecology
3. American Journal of Perinatology
4. Archives of Gynecology and Obstetrics
5. Australian and New Zealand Journal of Obstetrics and Gynaecology
6. Best Practice & Research: Clinical Obstetrics & Gynaecology
7. Birth: Issues in Perinatal Care
8. BJOG: An International Journal of Obstetrics and Gynaecology
9. BMC Pregnancy and Childbirth
10. BMC Women's Health
11. Climacteric
12. Clinical Obstetrics and Gynecology
13. Clinics in Perinatology
14. Cochrane Menstrual Disorders and Subfertility Group
15. Cochrane Pregnancy and Childbirth Group
16. Contraception
17. Current Opinion in Obstetrics and Gynecology
18. European Journal of Obstetrics & Gynecology and Reproductive Biology
19. Fertility and Sterility
20. Fetal Diagnosis and Therapy
21. Ginekologia Polska
22. Gynecological Surgery
23. Gynecologic Oncology
24. Gynecologic Oncology Reports
25. Human Fertility
26. Human Reproduction
27. Human Reproduction Update
28. Hypertension in Pregnancy
29. International Journal of Fertility and Sterility
30. International Breastfeeding Journal
31. International Journal of Gynecology & Obstetrics
32. International Urogynecology Journal
33. Journal of Family Planning and Reproductive Health Care
34. Journal of Gynecologic Oncology
35. Journal of Lower Genital Tract Disease
36. Journal of Midwifery & Women's Health
37. Journal of Obstetrics & Gynaecology
38. Journal of Obstetrics and Gynaecology Canada
39. Journal of Obstetric, Gynecologic & Neonatal Nursing
40. Journal of Perinatal & Neonatal Nursing
41. Journal of Perinatal Medicine
42. Maturitas
43. MCN The American Journal of Maternal Child Nursing
44. Menopause Review (Przegląd Menopauzalny)
45. Menopause: The Journal of The North American Menopause Society
46. Neurourology and Urodynamics
47. Obstetrics & Gynecology
48. Paediatric and Perinatal Epidemiology
49. Placenta
50. Prenatal Diagnosis
51. Reproductive Health
52. The Breast Journal
53. The European Journal of Contraception and Reproductive Health Care
54. The Obstetrician & Gynaecologist (TOG)
55. Twin Research and Human Genetics
56. Ultrasound in Obstetrics & Gynecology

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Review Article

The Luteal Phase after GnRHa Trigger-Understanding An Enigma

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Abstract

The luteal phase of all stimulated *in vitro* fertilization/intra-cytoplasmic sperm injection (IVF/ICSI) cycles is disrupted, which makes luteal phase support (LPS) mandatory. The cause of the disruption is thought to be the multifollicular development achieved during ovarian stimulation which results in supraphysiological concentrations of steroids secreted by a high number of corpora lutea during the early luteal phase. This will directly inhibit luteinizing hormone (LH) secretion by the pituitary via negative feedback at the level of the hypothalamic-pituitary axis, leading to a luteal phase defect. With the introduction of the gonadotropin-releasing hormone (GnRH) antagonist protocol, it became feasible to trigger final oocyte maturation and ovulation with a single bolus of GnRH agonist (GnRHa) as an alternative to human chorionic gonadotropin (hCG). GnRHa triggering presents several advantages, including the reduction in or even elimination of ovarian hyperstimulation syndrome. Despite the potential advantages of GnRHa triggering, previous randomized controlled trials reported a poor clinical outcome with high rates of early pregnancy losses, despite supplementation with a standard LPS in the form of progesterone and estradiol. Following these disappointing results, several studies now report a luteal phase rescue after modifications of the LPS, resulting in a reproductive outcome comparable to that seen after hCG triggering. We herein review luteal phase differences between the natural cycle, hCG trigger and GnRHa trigger and present the most recent data on handling the luteal phase after GnRHa triggering.

Keywords: HCG, GnRHa, Luteal Phase, IVF

Introduction

The luteal phase of the natural cycle

In the natural cycle ovulation is induced by a mid-cycle surge of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary elicited by a rise in the late follicular phase level of estradiol and progesterone. Ovulation marks the transition from follicular phase to luteal phase, characterized by the formation of a corpus luteum which releases steroid hormones, including progesterone and estradiol. Importantly, the steroid production is totally dependent on the pulsatile secretion of LH by the pituitary (1, 2).

Apart from securing the function of the corpus luteum, LH plays a crucial role during the luteal phase by up-regulating growth factors like vascular endothelial growth factor A (VEGFA) and fibroblast growth factor 2 (FGF2). In addition, cytokines are up-regulated and extragonadal LH-receptors are activated in the endometrium. All these factors are thought to enhance and support implantation and early neo-vascularization (1, 3).

The role of the previously described mid-cycle FSH surge during the natural cycle is not fully understood; however, FSH seems to promote oocyte nuclear maturation, i.e. resumption of meiosis and

cumulus expansion. Furthermore, FSH has been shown to induce LH receptor formation in the luteinizing granulosa cells, thus optimizing the function of the corpus luteum (1, 3).

Following implantation, the embryo gradually begins to secrete human chorionic gonadotropin (hCG) into circulation, structurally and biochemically similar to LH (3). The function of the corpus luteum requires a consistent hCG secretion until the luteo-placental shift around the 7th week of gestation, after which the placenta will be responsible for the continued secretion of steroids. The progesterone produced by the corpus luteum induces secretory transformation of the uterine glands, increases the endometrial vascularization and stabilizes the endometrial receptivity in preparation for embryo implantation. In addition, progesterone promotes uterine musculature quiescence thought to prevent uterine contractions, which could lead to expulsion of the embryo from the uterine cavity (2).

Apart from progesterone, the corpus luteum produces other steroid hormones, including estradiol. Estradiol has a modulatory effect on the secretory endometrial progesterone receptor concentration and may serve to replenish and maintain a sufficient level of endometrial receptors to secure an adequate response to progesterone (4).

The luteal phase of the stimulated IVF/ICSI cycle

Controlled ovarian stimulation (COS) is a key component of modern IVF treatment, as the availability of multiple oocytes for fertilization increases the chance of pregnancy (5).

In the stimulated *in vitro* fertilization/intra-cyto-plasmic sperm injection (IVF/ICSI) cycle, the luteal phase is physiologically abnormal. The most plausible reason for this is the multifollicular development achieved during the follicular phase, leading to a high number of corpora lutea. The collective steroid production of the corpora lutea will result in supraphysiological concentrations of progesterone and estradiol which directly inhibit the LH secretion by the pituitary via negative feedback actions at the level of the hypothalamic-pituitary-gonadal-axis (3). If un-supplemented, this will result in corpus luteum demise and early pregnancy loss (2). Therefore, to ensure the reproductive outcome of an assisted reproductive treatment (ART) cycle it is crucial to correct the luteal phase. This

can be achieved either by increasing the early luteal LH activity or by supplementing with steroid hormones (1) until the circulating hCG produced by the implanting embryo is sufficiently high to secure the function of the corpus luteum.

The luteal phase after hCG triggering

HCG has been the golden standard for ovulation induction for decades, functioning as a surrogate for the mid-cycle LH surge. HCG binds to and activates the same receptor as LH, the LH/hCG receptor (1), and, thus, by injecting a single bolus of hCG it is possible to trigger final oocyte maturation and ovulation.

After triggering of ovulation, it is necessary to maintain the function of the corpus luteum in order to secure a good reproductive outcome. HCG has a significantly longer half-life than that of endogenous LH and the bolus injected to trigger ovulation can support the corpus luteum for 7-10 days. After this period hCG is cleared from the circulation, and the corpus luteum is now totally dependent on the endogenous LH production and the hCG produced by the implanting embryo. However, during the early/mid luteal phase the hCG secretion from the embryo to the maternal serum is limited due to an absence of direct vascular communication (3). Moreover, the endogenous LH production is reduced by the supraphysiological concentrations of steroid hormone seen after COS. Thus, the result is a low LH activity leading to a decreased corpus luteum function during the early/mid luteal phase, necessitating luteal phase support (LPS) (1).

The best LPS strategy has yet to be defined, however progesterone administered either vaginally or intramuscularly is the first choice of treatment and is generally used for at least 15 days. Currently, the literature does not find any evidence for adding estradiol (2).

Regarding hCG triggering, the downside is that the trigger agent is very closely connected to the ovarian hyperstimulation syndrome (OHSS). This life threatening condition, characterized by massive enlargement of the ovaries and an increased vascular permeability, among others, is a iatrogenic complication following COS (5, 6). The main cause of OHSS is a combination of ovarian hyperstimulation with exogenous gonadotrophins and the use of hCG for final ovulation induction

(6). Thus, hCG causes a sustained luteotropic activity due to its prolonged circulating half-life (1, 7). Furthermore, even low doses of hCG during the luteal phase have been shown to affect the expression of vascular mediators, thereby increasing the vascular permeability (1).

The best strategy to prevent OHSS has previously been to indentify high-risk patients before ovarian stimulation, followed by the use of an appropriate COS protocol (8).

The luteal phase after GnRHa triggering

With the introduction of the GnRH antagonist protocol for the prevention of a premature LH surge, it became possible to trigger ovulation with GnRHa. The GnRH antagonist occupies the GnRH receptor without causing down-regulation, and by injecting a single bolus of GnRHa, the antagonist is displaced from the receptor. This activates the receptor, inducing a flare-up of gonadotrophins (LH and FSH), which effectively stimulate the final oocyte maturation and ovulation. However, important differences exist regarding the profile and duration of the LH surge after triggering with GnRHa compared to that of the natural cycle. In the natural cycle, the LH surge is characterized by three phases with a total duration of ~48 hours. After GnRHa triggering, the surge consist of two phases, only, with a duration of ~24-36 hours leading to a significantly reduced amount of LH released (1).

Apart from an LH surge, GnRHa triggering also induces an initial secretion of FSH resembling that of the natural cycle. This more natural surge of gonadotrophins after triggering with GnRHa may explain why some authors reported retrieval of an increased amount of mature oocytes compared to hCG triggering (1, 3).

The induced surge of gondotrophins results in an initial rise in the levels of progesterone and estradiol followed by a decrease during the next 24 hours prior to oocyte pick-up (OPU). Subsequently a second rise in the level of progesterone takes place as ovarian steroidogenesis shifts from follicular to luteal phase. In contrast, the estradiol level continues to fall (1).

After GnRHa trigger the circulating levels of progesterone and estradiol are significantly lower throughout the luteal phase as compared to those obtained after hCG triggering due to the shorter half-life of LH (~60 minutes) compared to that of hCG (>24 hours) (1).

The important clinical advantage of GnRHa triggering, however, is the reported significant reduction in or even elimination of OHSS (1, 3) caused by the shorter half-life of the endogenous LH surge compared with the continuous LH/hCG receptor stimulation after hCG triggering (1, 7).

As previously mentioned, the luteal phase after COS is defect due to supraphysiological steroid hormone concentrations inhibiting the LH secretion via negative feedback at the level of the hypothalamic-pituitary-gonadal-axis. As seen above the LH activity will be further compromised after GnRHa triggering due to the shorter duration of the endogenous induced LH surge and a potential weaker activation of the LH/hCG receptor. The result of this is a significant reduction in LH activity throughout the early/mid luteal phase leading to premature luteolysis and implantation failure (1).

In contrast, after hCG triggering, the luteal actions of LH will be covered by the bolus of hCG injected and then gradually by the hCG produced by the implanting embryo. Thus, supplementation with progesterone is sufficient to secure the reproductive outcome. However, after GnRHa triggering the lack of endogenous LH activity necessitates a modification of the standard luteal phase supplementation currently used after hCG triggering (1, 3).

Modified luteal phase support after GnRHa triggering

The initial randomized controlled trials (RCTs) reported a poor clinical outcome with an extremely high early pregnancy loss rate (EPL) when GnRHa was used to trigger final ovulation, despite the use of standard LPS with vaginal progesterone and oral estrogen (9-11). The most plausible reason for the poor results was a suboptimal LPS, and it was clear that the luteal phase after GnRHa triggering was significantly different from that seen after hCG triggering and a search for a more optimal LPS commenced.

A bolus of hCG

After the first disappointing results, trials were performed to explore the possibility of correcting

the luteal phase by injecting a small bolus of LH activity in the form of hCG. HCG in standard doses would increase the risk of OHSS, but by supplementing with a reduced dose, the treatment was thought to be safe, even for the OHSS high risk patient. Therefore, trials were conducted to explore the hypothesis that a small hCG bolus could rescue the luteal phase without increasing the risk of OHSS (5, 9, 12-15).

Humaidan et al. (9) conducted a RCT, randomizing 302 normoovulatory women undergoing IVF/ICSI to ovulation induction with either hCG or GnRHa. The GnRHa group was supplemented with 1500 IU hCG 35 hours after triggering besides a standard progesterone and estradiol support. The study reported delivery rates (DR) and early pregnancy loss rates (EPL) comparable to those of hCG trigger. In the group of women triggered with hCG, the OHSS incidence was 2% as compared to no cases after GnRHa triggering. The trial included normo-ovulatory women, only, and it was unknown whether the protocol would be safe for the OHSS high risk patient. However, the authors assumed it to be safe as more than one third of the patients in each group had more than 14 follicles $\geq$11 mm on the day of triggering-a level previously set to predict the occurrence of OHSS.

Following this study, the question to ask was: does GnRHa triggering followed by a bolus of 1500 IU hCG in a group of patients at risk of OHSS reduce the OHSS incidence compared with hCG trigger? This question was explored in a more recent study by Humaidan et al. (16), including 390 women undergoing IVF/ICSI. The study consisted of two RCTs, one study, randomizing patients at risk of OHSS and another, randomizing patients at low risk of OHSS. The ovarian response on the day of final oocyte maturation was used to create two risk groups with a cut-off level of >14 follicles $\geq$11 mm. The group at risk of OHSS had final oocyte maturation with either a bolus of GnRHa followed by a single bolus of 1500 IU hCG (n=60) or 5000 IU hCG (n=58). Similarly, women at low risk of OHSS were allocated to receive either a bolus of GnRHa followed by a total of two boluses of 1500 IU hCG, on the day of OPU and OPU+5 (n=125) or 5000 IU hCG (n=141). For luteal phase support all patients received a standard progesterone and estradiol supplementation.

No OHSS cases were seen in the group at risk of OHSS after GnRHa triggering despite supplementation with 1500 IU hCG, compared to an incidence of 3.4% in the group at risk of OHSS triggered with hCG. In contrast, two late-onset moderate OHSS cases were seen in the OHSS low-risk group triggered with GnRHa followed by two boluses of 1500 IU hCG, versus no cases of OHSS after hCG triggering. The authors concluded that future trials should focus on the minimal hCG activity needed for LPS in the low risk group to secure the reproductive outcome without increasing the risk of OHSS. The safety of the LPS protocol was previously tested among 12 hyper responders (15). Patients had a mean of 22 oocytes and all had embryo transfer (ET), which resulted in a live birth rate (LBR) of 50%. One moderate late-onset OHSS case occurred; however, the patient did not require hospitalization.

Using the same protocol, Radesic et al. (5) retrospectively explored its safety and efficiency in 71 OHSS high risk patients. The authors reported that the use of a GnRHa trigger in combination with 1500 IU hCG on the day of OPU resulted in a high ongoing pregnancy rate (OPR) of 52% without increasing the risk of severe OHSS. Despite the average patient producing a mean of 17 oocytes, only one patient (1.4%) was hospitalized due to OHSS in this high-risk group. All patients were supplemented with daily progesterone and estradiol.

These results were further corroborated by the conclusions of a recent international multicentre retrospective study. In this study Iliodromiti et al. (17) included 275 women at high risk of OHSS. The study reported an overall CPR of 42% and two cases of severe OHSS, only, (0.72%). The authors concluded that in women undergoing ovarian stimulation and who develop an excessive ovarian response, the use of a GnRH agonist trigger combined with a bolus of 1500 IU hCG at the time of oocyte retrieval provides an opportunity to proceed with fresh embryo transfer.

In another effort to find the optimal dose of hCG necessary during the luteal phase after GnRHa triggering, Castillo et al. (13) reported the outcomes of a retrospective study including 192 patients undergoing IVF/ ICSI from 2002-2006. Throughout the study period, the treatment proto-

col for luteal hCG administration changed, and the patients were grouped based on the dose received: group A (n=44) received 1000 IU, group B (n=115) received 500 IU and group (n=33) received 250 IU hCG i.m. A total of three fixed doses of hCG were administered starting on the day of OPU and every third day along with daily progesterone supplementation. Regarding the mean number of embryos transferred, there was a significant difference between the groups due to an actual trend of transferring fewer embryos. Despite these differences, the groups were comparable as regards EPL and clinical pregnancy rate (CPR). There was a clear trend of fewer cases of OHSS when using lower doses of hCG and the authors concluded that three doses of 1000 IU of hCG was unadvisable. The vast majority of the severe OHSS cases were late-onset (6/7), and 4/6 were related to multiple pregnancies. The results show a distinct relationship between the risk of OHSS and multiple pregnancies-single embryo transfer is therefore highly recommended in all patients at risk of OHSS development, regardless of trigger mode.

In another retrospective analysis, Shapiro et al. (12) reported a high ongoing pregnancy rate and no OHSS cases among 182 OHSS high-risk patients, using a so-called "dual-trigger". Patients received ovulation induction with a bolus of GnRHa as well as an average dose of 1428 IU of hCG, followed by LPS with progesterone and estradiol. Although retrospective, the results seem to indicate that dual-triggering can correct the luteal phase without causing OHSS among OHSS high risk patients.

Finally, Kol et al. (14) explored for the first time, the use of a LPS protocol without exogenous progesterone and estradiol after GnRHa triggering. The study included 15 normal responder patients with ≤12 follicles, who were supplemented with two boluses of 1500 IU of hCG, only, during the luteal phase. The boluses were administered on the day of OPU and OPU+4. The study reported an OPR of 47% and no OHSS development in any of the patients. These results seem promising as they might introduce the future exogenous progesterone free LPS for the normo-reponder IVF patient triggered with GnRHa.

In conclusion, supplementation with hCG rescues the luteal phase after ovulation induction with GnRHa, resulting in reproductive outcomes similar to that of hCG. However, it still needs to

be determined whether "dual-trigger", a single bolus of hCG or repeated low-doses of hCG is the best option. Regardless of the chosen protocol, it is crucial to individualize the luteal phase treatment with hCG according to the ovarian response to stimulation in an effort to reduce the risk of OHSS.

Recombinant LH

An alternative way of increasing the LH activity during the insufficient luteal phase after GnRHa triggering would be to administer repeated doses of recombinant LH.

This concept was explored in a proof-of-concept study performed by Papanikolaou et al. (18). The study included 35 normal responder patients randomized to receive ovulation triggering with either GnRHa or hCG. All patients received elective single embryo transfer (SET) after having undergone the same stimulation protocol. In the GnRHa group the luteal phase was supported with six alternate doses of 300 IU rLH, starting on the day of OPU and repeated every other day in addition to 600 mg daily of progesterone, administered vaginally. The study reported DR and EPL rates comparable to those of hCG trigger and no cases of OHSS were seen in either group.

The authors in this small group of normo-responder patients concluded that rLH effectively secures a good reproductive outcome after triggering with GnRHa without any OHSS development. The study was the first to assess the concept of applying repeated doses of rLH as LPS to overcome the luteal phase insufficiency after GnRHa triggering and the results seem promising. However, larger RCTs are necessary to draw conclusions about the safety and efficacy of this protocol. Furthermore, an obvious limiting factor for the use of rLH for LPS is the high cost of this preparation.

Intensive progesterone and estradiol support

As the standard LPS regimens turned out to be insufficient after GnRHa trigger (10, 11), US based research groups explored the use of a LPS protocol, consisting of progesterone and estradiol, only (6, 8, 12, 19).

The first report was by Engmann et al. (8) who randomized a total of 65 PCOS patients undergo-

ing IVF treatment. The patients were allocated to an ovarian stimulation protocol consisting of either GnRHa trigger after co-treatment with GnRH antagonist or hCG trigger after dual pituitary suppression, using a long GnRHa down-regulation protocol. The luteal phase supplementation in the GnRHa trigger group consisted of intensive support with progesterone and estradiol. Luteal serum levels were closely monitored and the administration of progesterone and estradiol was adjusted to maintain serum levels of >20 ng / ml and >200 pg/ml, respectively.

This protocol resulted in DR and EPL rates comparable to those of hCG triggering. Furthermore, the study reported a total elimination of OHSS after GnRHa triggering despite the fact that PCOS patients were included, many of which were at high-risk of developing OHSS after ovarian stimulation.

An important question that needs to be explored is whether this protocol applies to normo-gonadotrophic patients. LH levels are significantly higher in PCOS patients during the follicular and luteal phases, due to a higher frequency and amplitude of the LH pulse. Further, in PCOS patients the hypothalamus has a reduced sensitivity to negative feedback from the ovarian steroid hormone concentrations, in particular progesterone (1). This leaves PCOS patients with a significantly higher LH level during the luteal phase as compared to the normo-gonadotrophic patient.

In accordance with the results from Engmann et al. (8) and Shapiro et al. (12) reported good pregnancy outcomes and no cases of OHSS after the use of intensive LPS with progesterone and estradiol among 24 high responders. In their study, the luteal phase was also closely monitored to maintain levels of progesterone and estradiol of ≥15 ng/ml and ≥200 pg/ml, respectively.

Although the results seem promising in terms of the reproductive outcome and the total elimination of OHSS among OHSS high-risk patients, the study by Shapiro et al. (12) is obviously limited by its design and small study population and the results need to be confirmed in future larger RCTs.

Moreover, there are some potential biases in the study by Engmann et al. (8). Thus, a long GnRHa protocol was compared with a GnRH-antagonist/GnRHa protocol and the individualized LPS was only administered to the GnRHa group.

Importantly, the abovementioned encouraging results are contrasted by others. In a previous study performed by Babayof et al. (6) 28 PCO patients considered at high-risk of developing OHSS were randomized to receive either GnRHa or hCG triggering. Patients received intensive luteal support with 50 mg/day i.m. progesterone and the dose was doubled at serum levels of <12.5 ng/ml. Moreover, 4 mg/day of oral estradiol was given at serum levels <200 pmol/l. Despite the intensive LPS the reproductive outcome was disappointingly low with an OPR of 6% and an EPL of 80% in the group of patients receiving GnRHa for triggering.

These findings are further supported by Orvieto (19) who reported a low reproductive outcome in 67 OHSS high-risk patients despite the fact that they had a LPS protocol similar to the one suggested by Engmann et al. (8). Thus, there is clearly a need for RCTs to clarify the efficacy of the intensive progesterone and estradiol protocol among high-risk as well as low-risk patients.

Segmentation strategy

An alternative approach to encounter the luteal phase insufficiency seen after GnRHa triggering is to segmentate the IVF cycle, i.e. to stimulate in one cycle, trigger with GnRHa and transfer in subsequent frozen-thaw cycles. This seems to be a very safe approach for patients at risk of OHSS and recent trials suggest similar pregnancy rates between fresh and frozen-thawed embryos (20-22).

The concept was recently explored in a RCT by Shapiro et al. including 177 patients (20). A blastocyst transfer was performed in 103 patients. In the group randomized to fresh embryo transfer the final oocyte maturation was induced with either hCG alone or, using dual-triggering. The group receiving frozen-thawed embryos had a significantly higher reproductive outcome per transfer compared to the fresh transfer group. The authors concluded that the difference in outcomes probably was due to superior endometrial receptivity in the freeze all group.

To extend the indication, Shapiro et al. (21) conducted a similar study in high responder patients who were randomized to receive either fresh or frozen-thaw transfer. In both groups final oocyte maturation was induced with the use of a dual-trig-

ger. The CPR was 80 vs. 65%, in favour of frozen-thaw ET despite a superior embryo quality in the fresh group.

In summary, a freeze-all strategy further reduces the risk of OHSS and may be the best current option for patients with a very high risk of OHSS (22). However, there is a need for future trials to justify the use of oocyte/embryo cryopreservation as a routine approach. Importantly, this approach demands access to optimal cryopreservation programs (3).

Conclusion

Many recent publications indicate that the time has come for a paradigm shift in the triggering policy of ART. HCG has been the gold standard for ovulation induction, however, after the introduction of the GnRH antagonist protocol for the prevention of a premature LH rise, triggering of final oocyte maturation and ovulation with a single bolus of GnRHa is definitely an alternative.

GnRHa triggering possesses important advantages over hCG triggering, mainly in terms of a significant reduction in-if not total elimination of OHSS. Following the initial disappointing clinical reports several subsequent studies implemented a modified luteal support in terms of supplementation with either LH activity or luteal steroids. Using the modified LPS, the reproductive outcome increased remarkably and is now comparable to that seen after hCG triggering. Although the modified LPS has had a significant positive effect on the reproductive outcome after GnRHa triggering without increasing the risk of OHSS, the most optimal LPS still has to be investigated.

Until the optimal luteal supplementation protocol has been defined an alternative option in patients with an extreme ovarian response or with a significant comorbidity is a freeze all strategy and transfer in a subsequent natural or stimulated cycle.

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Original Article

Pregnancy Rate Following Luteal Phase Support in Iranian Women with Polycystic Ovarian Syndrome

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Abstract

Background: To assess the efficacy of luteal phase support (LPS) using intravaginal progesterone (P) on pregnancy rate in Iranian women with polycystic ovarian syndrome (PCOS) who used a combination for ovulation induction consisting of letrozole or clomiphene citrate (CC) and human menopausal gonadotropin (HMG).

Materials and Methods: This was a randomized clinical trial undertaken in a fertility clinic in Kashan, Isfahan Province, Iran. A total of 198 patients completed treatment and follow up. Base on chosen ovulation induction programs, they were divided into two following group: i. CC group (n=98) used a combination consisting of CC (100 mg×5 day) and HMG (150 IU×5 day) and ii. letrozole group (n=100) used a combination consisting of letrozole (5 mg×5 day) and HMG (150 IU×5 day). After human chorionic gonadotropin (hCG) administration (5000 IU), the patients (n=122) who randomly received intravaginal P (Cyclogest, 400 mg daily) were included in LPS group, while the rest (n=123) were included in non-P cycles group. The outcome was the comparison of chemical pregnancy rate between the groups.

Results: Our findings showed that LPS was associated with a 10% higher pregnancy rate than in non-P cycles, although this difference did not reach statistical significant (p=0.08). LPS improved pregnancy rate in both CC (4%) and letrozole (6%) groups. In addition, patients who used letrozole for ovulation induction along with intravaginal P showed higher pregnancy rates than CC group.

Conclusion: Administration of vaginal P for LPS may improve the pregnancy rate in women with PCOS using letrozole or CC in combination with HMG for ovulation induction (Registration Number: IRCT201206072967N4).

Keywords: Clomiphene, Letrozole, Progesterone, Luteal Phase, Polycystic Ovarian Syndrome

Introduction

The luteal phase has been defined as the period between ovulation and either the establishment of a pregnancy or the onset of menses two weeks later. Luteal phase defect (LPD) has been attributed mainly to inadequate production of progesterone (P) that is known as the major product of the corpus luteum, which is necessary for the establishment of pregnancy. As a result, P has been used as luteal phase support (LPS) in ovulation induction cycles for many years (1).

LPD has been reported in patients with polycystic ovarian syndrome (PCOS) that has been identified as most common endocrine disorder in women of reproductive age (2). This type of disorder causing abnormal follicular development and numerous antral follicles may be related to abnormal hypothalamic sensitivity to P. Furthermore, the granulosa cells of women with PCOS may have an inherent inability to secrete normal levels of P after luteinization if ovulation is achieved (3).

On the other hand, controlled ovarian hyperstimulation is generally used as treatment protocols for patients with PCOS. In controlled ovarian hyperstimulation cycles, multifollicular development and supraphysiologic steroid serum concentrations may negatively affect luteinizing hormone (LH) secretion. Disturbed LH secretion may induced LPD that leads to premature luteolysis, reduced LH concentration, low P level and shortened luteal phase (4).

Some studies have been shown that presence of LPS through administration of P has significantly affected the success of ovarian induction and intrauterine insemination (IUI) cycles (5, 6). Nevertheless, in the studies done by Ozornek et al. (7) and Kyrou et al. (8) they reported no benefit of LPS in patients who underwent stimulated IUI cycles. In another study has been concluded that P supplementations have low therapeutic value in LPD, beside taking clomiphene citrate (CC) for ovulation induction (9). Montville et al. strongly recommended luteal phase supplementations containing P in women with PCOS using letrozole for ovulation induction, while no positive effect of P on those stimulated with clomiphene citrate was detected (10).

Therefore, the previous studies have produced conflicting results, while the amount of data from well-controlled clinical trials is limited. Thus, further studies are required to describe the impact of treatment with P for LPS in stimulated cycles in PCOS before deciding to move forward with more invasive assisted reproductive technologies.

To best our knowledge, there had been no prospective trial investigating the need for P administration in the combination stimulation protocols in PCOS. In light of these observations, the aim of present study was to evaluate the effect of LPS with P on pregnancy rate in Iranian women with PCOS who were treated with either CC or letrozole in combination with human menopausal gonadotropin (HMG).

Materials and Methods

A randomized clinical trial with parallel design was employed to confirm the effect of LPS with P on pregnancy rate in patients with PCOS. This study was conducted in an infertility clinic affiliated with Shahid Beheshti Hospital in Kashan, Isfahan Province, central part of Iran, between Aprils and January 2011.

Patient population

Patients were eligible if they met following criteria: being 20-35 years of age; being married; not having non-classical adrenal hyperplasia, thyroid disorders and hyperprolactinemia; being Iranian; having effective speaking or listening skills; not having male factor for infertility; having normal uterine cavity and patency of fallopian tube as demonstrated by either hysterosalpingography (HSG) or diagnostic laparoscopy and hysteroscopy; and having Rotterdam diagnostic criteria. Based on random allocation sequence generated by one of researchers, enrolled participants (n=198) were divided into two main groups as follows: i. CC group (n=98) used a combination consisting of CC and HMG and ii. letrozole group (n=100) used a combination consisting of letrozole and HMG (Fig 1).
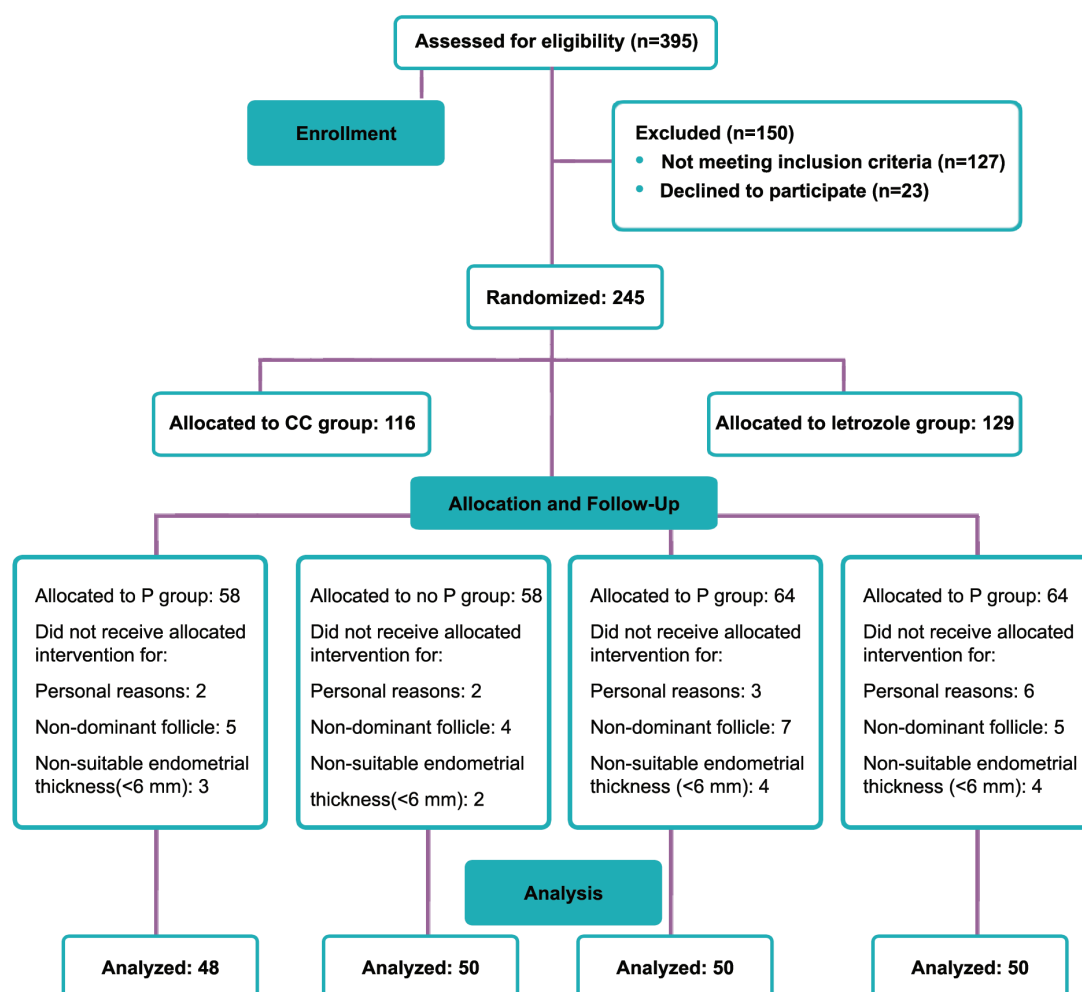
Fig 1: Patients flow chart.

Ovarian stimulation and luteal supplementation

On day 3 of the treatment cycle, baseline transvaginal ultrasounds scan (AU 350, Esaote, Milano, Italy) was performed. One physician carried out all sonograms and treatment protocols. The endometrial stripe was measured at its maximum anteroposterior thickness along the sagital axis of the uterine body. When there was no ovarian cyst on the scan, CC group received orally 100 mg clomiphene citrate (CC; Iran Hormone,Tehran, Iran) for 5 days starting on day 3 of the menstrual cycle, while letrozole group received 5 mg/day of letrozole (Femara; Novartis Pharma AG, Switzerland) from day 3 to day 7 of the menstrual cycle.

It is noted that HMG contains follicle-stimulating hormone (FSH) and LH. The dose and duration of HMG treatment was adjusted according to the patient's response, after monitoring the follicular development including the number of growing follicles. Therefore, in both groups, at least 5 ampoules (Merional, IBSA, Switzerland) in total dosage of 150 IU containing FSH were applied intramuscularly (IM) daily from day 5 to day 10. After day 10 of the menstrual cycle, all patients were evaluated every other day by a transvaginal ultrasound. When one or more dominant follicle(s) reached ≥18 mm, ovulation was triggered in form of an IM injection of 5000 IU human chorionic gonadotropin (hCG) (Choriomon, IBSA, Switzer-

land). Afterward, all patients (n=245) were randomly divided into two sub-groups. The patients (n=122) who used P suppositories (Cyclogest, 400 mg vaginally; Alpharma, England) were included in LPS group, while the rest (n=123) who did not use the supplement were included in non-P cycles group. LPS group used P suppositories daily starting on the day after hCG and was continued for 14 consecutive days.

Outcome measure was the sign of chemical pregnancy (positive β-hCG test i.e. >25 IU/mL). Pregnancy testing was performed by determining the quantitative serum βhCG level on day 14 after P administration.

Statistical analysis

For an expected pregnancy rate of 21% for patients with LPS and 12% for patients without LPS, a sample size of 50 patients per groups was required for a statistical power of 90% at a p level of 0.05. Socio-demographic characteristics of the groups were expressed as mean ± SD or case (percentage) elsewhere, while the collected data were compared using one-way analysis of variance (One-Way ANOVA) and Chi-squared tests. Comparison of chemical pregnancy between groups was performed by chi-squared test. Multivariable logistic regression was specified to evaluate association between pregnancy rate after LPS and variables of interest. The Statistical Package for Social Sciences (SPSS; SPSS Inc., Chicago, IL, USA) version 11.5 was used to assess the study data. P values were set as 0.05 for all analyses.

Ethical considerations

The Ethics Committee of the Kashan Medical University approved the study. The protocol was explained to the patients before they entered the study, while an informed consent was obtained from all.

Results

We included 198 participants in the present study, who had completed treatment and follow up. The socio-demographic characteristics between groups were compared according to age, duration of infertility, endometrial thickness and number of dominant follicle. Results showed that there were no significant differences between the groups except for the age (Table 1).

Table 1: Socio-demographic characteristics of participants in CC and letrozole groups with and without using P

Variable	CC (N=98)		Letrozole (N=100)	
	Using P	Not using P	Using P	Not using P
Age (Y) [a]	28.43 (4.43)	25 (3.50)	26.93 (4.72)	25.8 (3.30)
Duration of infertility (Month)	46.47 (38.01)	28.9 (32.5)	37.7 (35.27)	34.9 (29.9)
Total HMG (FSH received) ampoule	5.81 (0.56)	5.79 (0.71)	5.78 (0.59)	5.80 (0.57)
Endometrial thickness on hCG day (mm)	7.62 (0.88)	7.73 (1)	7.76 (1.09)	7.52 (0.92)
Number of follicle ≥18 mm on hCG day	1.77 (1.27)	1.78 (1.14)	1.78 (1.09)	1.86 (1.34)
Pregnancy rate	11 (27.5)	7 (17.5)	14 (35)	8 (20)

HMG; Human menopausal gonadotropin, FSH; Follicle-stimulating hormone, hCG; Human chorionic gonadotropin, CC; Clomiphene citrate, P; Progesterone and [a]; Values are mean (SD) or case (percentage). No significant differences between groups except for the age (p<0.001).

Progesterone supplementation was resulted in 10% higher pregnancy rate in LPS group than in non-P cycles group, although this difference did not reach statistical significance (p=0.08). LPS improved pregnancy rate in both CC (11 vs. 7, p=0.30) and letrozole (14 vs. 8, p=0.10) groups, although the difference is not significant. In addition, patients who used letrozole for ovulation induction had higher pregnancy rates when using intravaginal P support than CC group (14 vs. 11, p=0.40), although the difference is not significant.

To conduct thorough analysis on effect of P sup-

plementation on the pregnancy rate with consideration of other confounders, we applied logistic regression. The effect of the parameters (Table 1) on pregnancy achievement after P supplementation was examined using a robust logistic regression model. Variables entered the model were selected by means of univariate comparisons between two group of patients who did or who did not achieve a pregnancy if p<0.05 (presence of pregnancy following P supplementation). The association between P supplementation and achievement of pregnancy was marginally significant (OR: 0.741, 95% CI: 0.539–1.019, p=0.06, Table 2).

Table 2: Logistic regression used as a dependent variable for the achievement of pregnancy

Dependent variable: incidence of pregnancy	P value	SE	Odds ratio	95% CI	
				Lower	Upper
Independent variable					
Luteal support	0.06	0.162	0.741	0.539	1.019
Hosmer-Lemeshow test, p=0.98					

SE; Standard error and CI; Confidence interval.

Discussion

Currently, no information is available regarding the effect of LPS using P supplementation on pregnancy rate in stimulated cycles with combination therapies. This is the first study in which the effect of LPS with P on pregnancy rate was evaluated in Iranian women with PCOS who were treated with either CC or letrozole in combination with HMG for ovulation induction. Progesterone supplementation seemed to be of benefit in both the CC and letrozole treatment groups. The women with PCOS may inherently benefit from P supplementation in the luteal phase regardless of which medication is used for ovulation induction. P administration in PCOS patients has been demonstrated to decrease LH pulse frequency (11). In addition, the granulosa cells in women with PCOS may have intrinsic abnormalities in the response to both gonadotropin

action and steroidogenesis. Granulosa cells in patients with PCOS have been verified to have some changes in response to LH before ovulation (12).

While development of several follicles to attain ovulation and large amounts of P are the main cause of the ovarian stimulation, the treatment overrides the physiological feedback mechanisms. The luteal phase of these cycles is characterized by a momentary high level of one or both hormones, which suppress the levels of LH and FSH (13). It has been recommended that the low level of LH may lead to lack of luteotrophic support determined by low P levels or short luteal phase (14). This is in agreement with previous studies by Erdem et al., and Maher et al., who found LPS with vaginal P positively affects the success of stimulated IUI cycles (5, 6), but other studies reported no benefit of LPS with either P, gonadotropin-releas-

ing hormone (GnRH) agonist or hCG in patients who underwent ovulation induction (7, 8, 15).

In Balasch colleagues' study (9), twenty infertile patients being treated with CC and hCG for induction of ovulation with a defective luteal phase were assigned into two groups of treatment using P supplementation or control. Their findings showed that success rates were similar in both groups (20 and 30%, respectively). It has been concluded that progestational agents have low therapeutic value in luteal phase deficiency induced by CC. It should be noted that oral and intravaginal P has been used in Balasch's and present studies, respectively. P is usually well tolerated and the side effects encountered typically depend on the route of administration. But, the intravaginal method of P has gained popularity as a first option in luteal support treatment that is mainly due to patient comfort and practice efficiency (16). Local bioavailability in the uterus is greater after vaginal administration than with other routes (17) and this might be expected to result in an increased possibility of pregnancy.

It has been proposed that in IVF cycles, the use of GnRH analogs and gonadotropins cause multifollicular development, change of the hormonal environment, an increase in steroid serum concentration and an increase in risk of LPD (18). Nevertheless, in our study, the mean numbers of dominant follicles were 1.6. Some studies have noted that in cycles with mildly stimulated ovaries and less obvious follicular development (such as our study), there is no biological evidence to indicate that treatment with P in the luteal phase is necessary or improves pregnancy rates (19).

Tavanitou et al. (20) discovered that LH serum concentrations were significantly higher in patients administered CC. In other investigation have been shown that controlled ovarian stimulation with HMG in the follicular phase was an effective treatment for LPD associated with recurrent pregnancy loss (21). Understanding from induction ovulation with gonadotropins in hypophysectomized women had verified that it was essential to provide continued support in the form of hCG at least until the mid-late luteal phase (22). However, women undergoing ovarian stimulation are not totally hypogonadotrophic, so they need no support in luteal phase.

Moreover, the half-life of hCG is relatively long if at least 5000 IU (dosage of hCG in our study) are used for ovulation induction, so a biologically significant amount persists for at least 10 days until the embryo starts secreting hCG (23).

In addition, in present study, patients who used letrozole for ovulation induction had higher pregnancy rates when using P as compared to CC group. Studies on effect of P supplementation in patients with PCOS using either CC or letrozole are limited. In a study by Montville et al. (10), they have shown that women with PCOS who used letrozole for ovulation induction had higher pregnancy rate when using intravaginal P support than CC group. Nevertheless, in this study, we did not compare pregnancy rate between P and control groups regardless taking medication. Aromatase inhibitors such as letrozole are hypothesized to maintain normal hypothalamic pituitary feedback mechanisms, and in case of ovulation induction in women with PCOS, may act to increase follicular sensitivity to FSH through increasing intrafollicular androgen levels. Unlike CC, letrozole does not antagonize the estrogen receptor in the endometrium (24, 25). The lack of antagonism may contribute to increase pregnancy rate. In addition, the present study suggests that combination therapy of letrozole and luteal phase P improve pregnancy rate compared with letrozole alone. Letrozole may act to increase midluteal P levels after ovulation.

These observations would help to explain the benefits of CC, letrozole and HMG on the luteal phase that showed no significant relation in present study. Whether non-significant result is due to lack of LPD in mildly stimulated cycles (Table 1) or is due to the direct positive effect of CC and/or hMG or hCG on luteal phase is not clear.

Strengths of this study included matching properties such as duration of infertility, endometrial thickness and number of dominant follicles, indicating these confounders did not play a role in results. In addition, our trial included the same physician using the same clinical protocols for all patients. Most importantly, all patients followed the same lab protocols. We had attempted to adjust the results for parameters that were significantly different between the two study groups, but our conclusions are influenced by some limitation.

The obvious weakness is small sample size that did not provide an adequately powered analysis for the important confounders, so the tested outcome could be affected (pregnancy rate). Moreover, the lack of statistical significance of difference between groups in present study may be a result of not having the number of cycles required to reach appropriate statistical power. Perhaps the failure to observe a significant effect of P on pregnancy rate in the different studies may be explained in part by either small study sizes, inadequate statistical power to detect a significant difference, the use of different drugs for ovarian stimulation, as well as different types and dosages of P for LPS. Despite these limitations, our findings were the subject of thorough statistical analysis that added strength to our conclusions.

Undoubtedly there is a need for further prospective randomized studies, with larger samples and longer periods of follow-up, to confirm the real clinical benefit of luteal phase P administration (if any) before it is introduced into daily clinical practice.

Conclusion

Our results suggest that LPS with P may improve pregnancy rate in PCOS patients treated with either CC or letrozole in combination with HMG.

Acknowledgements

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Original Article

Effect of Piroxicam on ART Outcome: A Pilot Study

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Abstract

Background: One of the most important factors affecting success rates in assisted reproductive techniques (ART) besides the number of oocytes retrieved and high quality embryos derived from them is the technical aspects of embryo transfer. It seems that pretreatement with uterine relaxants can be helpful in preventing unpleasant cramps which can have an adverse effect on ART outcome. In this respect, some drugs such as prostaglandin inhibitors or sedatives have been evaluated but not confirmed yet remain controversial. This study was performed in order to assess the effect of administrating Piroxicam prior to embryo transfer on pregnancy rates in ART cycles.

Materials and Methods: This pilot study was performed from August 2010 through December 2011 on 50 infertile women in ART cycles. Recombinant follicle stimulating hormone (rFSH) with a long gonadotropin releasing hormone (GnRH) analogue protocol were used for controlled ovarian hyperstimulation. The subjects were randomly allocated into two groups of 25 patients after obtaining written consent. Group A received a 10 mg Piroxicam capsule 30 minutes before embryo transfer and group B was the control group with no treatment. Data were analyzed by Chi-square and analysis of variance (ANOVA).

Results: Pregnancy rate was 34% (n=17) totally, with 32% (n=8) in group A and 36% (n=9) in group B (p=0.75). Uterine cramps were experienced by 4 women (16%) in group B, while none were reported by women in group A (p=0.037).

Conclusion: It seems that Piroxicam administration 30 minutes prior to embryo transfer cannot increase pregnancy rates, but can prevent or reduce uterine cramps after the procedure.

Keywords: Embryo Transfer, Piroxicam, Pregnancy Rate, Assisted Reproductive Techniques

Introduction

Assisted reproductive techniques (ART) have contributed tremendously to the infertility treatment. As experience has accumulated in the past three decades, success rates have increased, making them applied worldwide (1). Different factors contribute to the success rates in the various stages of these procedures. One of the most important factors affecting success rates besides the number of oocytes retrieved, high quality embryos and uterine receptivity is the technical aspects of embryo transfer (2). Due to the vast research performed in recent years, the embryo transfer technique has improved in different aspects including

the type of catheters used and transfer of embryo under sonographic guidance. Also the effect of different factors such as administration of antibiotics and bed rest after transfer have been evaluated (3, 4). Regarding uterine factors, the absence of uterine contractions at the time of embryo transfer is reported to significantly affect endometrial receptivity (5, 6). Embryo transfer is an aggressive procedure that may induce endometrial inflammatory reaction and augmented myometrial contractility. In a non-pregnant uterus, uterine contraction patterns play an important role in human reproduction. It has been shown in different studies that suitable stimulation or prevention of uterine contractions after embryo transfer can increase the fertility rate (5, 7, 8).

In order to reduce uterine cramps, it is highly recommended to perform embryo transfer with the least trauma. In a study by Fanchin, they showed an increase in random fundocervical uterine contractions in cases of difficult embryo transfer (9). In another study, it was shown that applying a tenaculum to the cervix during a mock embryo transfer (ET) can increase uterine contractions (10).

Uterine contractions are induced by prostaglandin (PG) which is synthesized from arachidonic acid by cyclo-oxygenase (COX). It seems that pretreatment with uterine relaxants can be helpful in preventing unpleasant cramps. Theoretically non-steroidal anti-inflamatory drugs (NSAIDs) which block the action of COX can inhibit the production of PG and should have beneficial effect on pregnancy rates (11). In this respect some drugs such as prostaglandin inhibitors or sedatives have been evaluated but not confirmed, yet remain controversial. In a study by Bernabeu, in egg donation cycles, implantation rates did not show significant difference in oocyte recipients who had received indomethacin before transfer (12). In our previous study we compared the effect of indomethacin to hyoscine given before embryo transfer on ART outcome. It was shown that hyoscine administration 30 minutes prior to embryo transfer can significantly increase pregnancy rates by reducing uterine cramps (13).

Piroxicam is another NSAID which has been used before embryo transfer in various studies with different results. Its mechanism of action, although being similar to other NSAIDS, is not completely understood, but may be related to prevention of prostaglandin synthesis by a reversible inhibition of the cyclo-oxygenase enzyme (14).

Due to the controversies in different surveys, this study was performed to assess the effects of administrating Piroxicam prior to embryo transfer in ART.

Materials and Methods

This pilot study was performed in Vali-e-Asr Hospital from August 2010 through December 2011 after obtaining approval from the Ethical Committee of Tehran University of Medical Sciences in 230 patients who attended the infertility clinic. Inclusion criteria consisted of patients with the age group of 20-35 years old and with ART indication due to tubal factors, ovulation disorders or severe male factor. A long gonadotropin-releasing hormone (GnRH) analogue protocol for pituitary desensitization and recombinant follicle stimulating hormone (rFSH; Gonal-F, Merck Serono, Switzerland) were used for controlled ovarian hyperstimulation. Oocyte retrieval was performed 36-38 hours after human chorionic gonadotropin (HCG) administration which was given when at least two 18 mm follicles were detected. After microinjection, embryo formation and getting a written informed consent, fifty cases who had a good response (> 4 oocytes) during the controlled ovarian hyperstimulation (COH) for ART and who had embryos for transfer, were randomly divided into two groups. Group A received Piroxicam (10 mg, Tolid Daru, Iran) orally half an hour before embryo transfer and group B did not use any form of medication which is the conventional method used (control group). Embryo transfer was done using the Wallace catheter without sonographic control. The patients were asked about their feeling of lower abdominal pain which was considered as having or lacking cramps. Both groups rested for 30 minutes after embryo transfer. Systemic diseases and endometriosis were exclusion criteria. Demographic data, infertility history, endometrial thickness, number of oocytes and embryos transferred, presence of cramps after embryo transfer and ART outcome were recorded in a questionnaire and registered by SPSS version 16. Success rates were compared using chi-square and analysis of variance

(ANOVA).

Results

The demographic characteristics and infertility history in both groups showed no significant difference (Table 1). Mean endometrial thickness was 9.55 ± 2.06 mm and 9.68 ± 2.07 mm in groups A and B, respectively (p=0.82).

After embryo transfer, uterine muscle cramps were reported by 4 women (16%) in group B and none in group A (p=0.03). Seventeen pregnancies (34%) occurred in the 50 patients included in the trial with a pregnancy rate of 32% (n=8) and 36% (n=9) in groups A and B, respectively (Table 2).

Table 1: Demographic characteristics of the two groups under study

Group		Control (group B)	Piroxicam (group A)	Total	P value
Characteristics		**N=25**	**N=25**	**N=50**	
Age of women in years (Mean ± SD)		27.68 ± 4.58	28.649 ± 4.32	28.16 ± 4.45	NS**
Age of men in years (Mean ± SD)		32.86 ± 4.02	34.09 ± 4.09	33.48 ± 4.05	NS**
Type of infertility No. (%)	**Primary**	20 (80)	19 (67)	39 (78)	NS*
	Secondary	5 (20)	6 (24)	11 (22)	
Duration of infertility (Y)		6.18 ± 3.37	6.70 ± 3.94	6.44 ± 3.63	NS**
Cause of infertility No. (%)	**Male**	17 (68)	18 (72)	35 (70)	NS*
	Female	4 (16)	5 (20)	9 (18)	
	Both	4 (16)	2 (8)	6 (12)	

NS; Non-significant,; Chi-square test and **; ANOVA.*

Table 2: Comparison of outcomes of the two groups under study

Group outcome	Piroxicam	Control	P value
Abdominal muscle cramps No. (%)	0	4 (16)	0.03
β-hCG positive No. (%)*	8 (32)	9 (36)	NS

*NS; Non-significant and *; Chi-square test.*

Discussion

In ART cycles, different factors contribute to the success rates. Technical aspects of embryo transfer is one of the most important factors, besides the high quality embryos and uterine receptivity. Even with an atraumatic transfer, endometrial reaction can be induced and affect ART (2).

In recent years, attention has been paid to reduce or prevent uterine contractions or cramps using prostaglandin inhibitors or sedatives before transfer. Indomethacin and piroxicam are the two mostly cited prostaglandin inhibitors used before embryo transfer (12, 14). Regarding indomethacin, in a study by Bernabeu et al. (12), implantation rates did not show significant difference in oocyte recipients who had received indomethacin before transfer. In another study conducted by our group (2009) to compare the effect of indomethacin and hyoscine, we showed that indomethacin and hyoscine reduce uterine cramps (p=0.04) as compared to the control group. The results of the same study also showed that pregnancy rate is significantly higher in the hyoscine as compared to the indomethacin or control groups (p<0.04) (13). Hyoscine as an anti-muscarinic drug is supposed to reduce cervical spasm (15). Sirohiwal et al. (16) showed in a study that using hyoscine suppository can reduce cervical resistance during labor.

In different studies, Piroxicam is considered as a controversial topic. Moon et al. (17) studied the effect of administrating 10 mg beta-cyclodextrin piroxicam, one and two hours prior to embryo transfer. In this study, pregnancy rate was found to be higher (46.8%) than the control group (27.6%). Firouzabadi et al. (18) studied the effect of giving a single dose of piroxicam before embryo transfer on implantation and pregnancy rates in *in vitro* fertilization (IVF) cycles. The implantation and clinical pregnancy rates were significantly higher in the piroxicam treatment group compared with the control group. In contrast to the above mentioned studies, our results showed that although giving piroxicam before embryo transfer prevents uterine cramps, it has no significant effect on pregnancy rates in ART cycles. Similar to the present study, Asgharnia and Mehrafza (19) showed that piroxicam has no significant role in pregnancy rate. Also, Dal Prato et al. (14) showed that piroxicam administration before intracytoplasmic sperm injection has no additional effect on pregnancy outcome. Production of inflammatory cytokines is important for successful implantation. Prostaglandins promote decidualization of the endometrium and implantation by increasing vascular permeability.

In rodents and rabbits, implantation can be interrupted by injection of prostaglandin inhibitors (20, 21). Just before implantation occurs, there is an increase in endometrial vascular permeability which can be prevented by indomethacin. Also, an increase in prostaglandin levels in implantation sites has been shown during very early stage of implantation (22). The prostaglandins are supposed to be secreted either by the endomtrium or the embryo. It has been shown that blastocysts in human as well as sheep, cows, rabbits, and mice produce and secrete prostaglandins (23). Regarding the endometrium, although decidual synthesis of prostaglandins occurs, but its level is much lower as compared to proliferative and secretory endometrium which can be due to high progesterone levels during pregnancy. Nevertheless, an increase in prostaglandin E_2 (PE_2) synthesis at the implantation site is mainly due to the response of some signaling factors from blastocyst, such as the platelet-activating factor, and correlates with an increase in vascular permeability (22, 24). It is now well-accepted that decidua-derived PE_2 is one of the major regulators of trophoblastic invasion, activating other signaling proteins.

Since prostaglandins play a key role in implantation, due to the present evidence, it seems reasonable to omit NSAIDs administration as a means of reducing uterine contractions in embryo transfer procedure until further evidence can prove their benefits. In spite of our findings, since this study was performed on a limited number of patients and since other numerous factors are involved in this process, we recom-

mend that more precise studies be performed on a wider scale in order to obtain more accurate results.

Conclusion

It seems that piroxicam administration 30 minutes prior to embryo transfer can not significantly increase pregnancy rates, but can prevent or reduce uterine cramps after the procedure.

Acknowledgements

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Original Article

Comparison between Conventional Blind Embryo Transfer and Embryo Transfer Based on Previously Measured Uterine Length

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Abstract
Background: Embryo transfer (ET) is one of the most important steps in assisted reproductive technology (ART) cycles and affected by many factors namely the depth of embryo deposition in uterus. In this study, the outcomes of intracytoplasmic sperm injection (ICSI) cycles after blind embryo transfer and embryo transfer based on previously measured uterine length using vaginal ultrasound were compared.

Materials and Methods: This prospective randomised clinical trial included one hundred and forty non-donor fresh embryo transfers during January 2010 to June 2011. In group I, ET was performed using conventional (blind) method at 5-6cm from the external os, and in group II, ET was done at a depth of 1-1.5 cm from the uterine fundus based on previously measured uterine length using vaginal sonography. Appropriate statistical analysis was performed using Student's t test and Chi-square or Fisher's exact test. The software that we used was PASW statistics version 18. A p value <0.05 was considered statistically significant.

Results: Chemical pregnancy rate was 28.7% in group I and 42.1% in group II, while the difference was not statistically significant (p=0.105). Clinical pregnancy, ongoing pregnancy and implantation rates for group I were 21.2%, 17.7%, and 12.8%, while for group II were 33.9%, 33.9%, and 22.1, respectively. In group I and group II, abortion rates were 34.7% and 0%, respectively, indicating a statistically significant difference (p<0.005). No ectopic pregnancy occurred in two groups.

Conclusion: The use of uterine length measurement during treatment cycle in order to place embryos at depth of 1-1.5cm from fundus significantly increases clinical and ongoing pregnancy and implantation rates, while leads to a decrease in abortion rate (Registration Number: IRCT2014032512494N1).

Keywords: Ultrasound, Embryo Transfer, Uterine

Introduction

Embryo transfer (ET) is one of the most important steps in assisted reproductive technology (ART) cycles. The goal of ET is to deliver good quality embryos in early stages of development (zygote to blastocyst) to a uterus with suitable endometrium. It has been demonstrated that even with the transfer of high quality embryos, the success rate of the ART program is low and only 15-20% of the transferred embryos will implant (1). It has been also estimated that up to 85% of the embryos transferred fail to implant (2).

There are multiple factors affecting the success of embryo transfer such as: embryo quality, uterine contractions, use of tenaculum, easy

or difficult transfer, volume of transfer media, transfer technique, and depth of uterine transfer, while some of these factors are more important than others. One of the factors impacting the ART cycles' outcome is the embryo transfer technique (3). Some clinicians believe that the impact of the transfer technique on the *in vitro* fertilization (IVF) results is as important as embryo quality, while any difficulty in ET may influence the implantation rate, significantly (4). One of the important aspects of the transfer technique is the depth of embryo deposition. If the deposition site is too deep, the chances of catheter touching the fundus and damaging the endometrium are increased. It has been demonstrated that touching the endometrium can stimulate junctional zone contractions (5) which may increase the chance of ectopic pregnancy.

In a study by Woolcutt et al. regarding "blind transfer", they have reported high rate of touching fundus or tubal ostia (6), and some other studies have indicated increased risk of ectopic pregnancy in cases of transfer close to the uterine fundus (7, 8). Therefore, the depth of the embryo deposition in the uterine cavity may influence the implantation success rates (9). Chun et al. showed a direct relation between implantation and pregnancy success rates with the length of the uterine cavity (10). The best site for ET to achieve higher pregnancy rates seems to be at a distance >10 mm and <20 mm from the fundus (11). There are several methods for ET. In the blind transfer method (with or without clinical touch), the embryo is planted 5-6 cm from the external os (12, 13), in the second method, the ultrasonography-guided embryo transfer is applied (2, 14), and in the third method, the embryo is transferred based on previously measured uterine length by a sound (metal or plastic) (15, 16).

There has been some controversy over the advantage of performing ultrasonography during ET. The positive impact of this procedure on the pregnancy rate has been reported in several studies (16, 17). In contrast, some other studies have reported no advantage in using ultrasonographic guidance (18-20). Since in many IVF centres in Iran, ET is performed blindly; therefore, in this study, we aimed to compare the outcome of conventional (blind) ET method with the ET method based on previously uterine length measurement using vaginal sonography.

Materials and Methods

This was a prospective randomized clinical trial performed during January 2010 to June 2011. One hundred and forty women undergoing intracytoplas-

mic sperm injection (ICSI) during this period in Infertility and Reproductive Health Research Center (IRHRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran, were enrolled in the study. Donor cycles and frozen ET were excluded. The study was approved by the Research Committee of the Infertility and Reproductive Research Centre of Shahid Beheshti University. A signed written consent was obtained from all participants.

The type of stimulatory cycle [agonist (Superfact; Hoechst, Frankfurt, Germany) or antagonist (Cetrotide, EMDSerono, Inc., Germany)] was selected based on the age of woman and other factors. Ovarian stimulation was done using recombinant follicle-stimulating hormone (r-FSH; Gonal-F; Serono Laboratories Ltd., Geneva, Switzerland) or purified FSH (Merional or Fostimon; IBSA, Switzerland), in single or combination formula.

When the leading follicles reached 17-18mm in diameter, human chorionic gonadotropin (hCG) 10,000 units (IBSA, Switzerland) was administered. The uterine length was measured, on the day of hCG administration, by recording the distance from the external os to the end of uterine cavity using vaginal sonography. Oocyte retrieval was performed 34-36 hours later, while the day of ET was determined by convenience. The patients were randomly divided into two groups. In group I, ET was performed using conventional (blind) method based on the sense and experience of the physician. In group II, the ET was performed based on previously measured uterine length using vaginal ultrasound and the embryo deposition was done at the depth of 1-1.5 cm from the top of uterine cavity. Furthermore, all ETs on even days were enrolled in group I, whereas all ETs on odd days were enrolled in group II.

Procedure

All women were placed in the lithotomy position (with an empty bladder) and a sterile metal speculum was placed to expose the cervix. The cervical mucus was cleared using ringer solution, then the external os washed with media (Ham's F-10 liquid, Sigma, Germany). In all cases, a Cook catheter (COOK Medical, USA) was used. First the outer catheter and then the inner catheter that was loaded with the embryos was placed. In group I, embryos were blindly deposited at the middle portion of the uterine cavity, approximately at 5-6 cm distance from the external os and based on physician experience.

In group II, embryos were deposited at 1-1.5 cm

from the uterine fundus based on the previously measured uterine length without touching fundus and without ultrasound use during the procedure.

After slow withdrawal of the catheter and speculum, all women rested for an hour.

The difficulty of the ET was determined by the physician. When the catheter easily passing through the cervical canal was denoted easy, whereas any resistance to the insertion of the catheter, requiring a tenaculum, or future time-consuming manipulations were denoted difficult ET.

For supporting the luteal phase, all patients received Cyclogest vaginal suppository (400 mg BID) (Actover, Alpharma, England). Fourteen days after ovum retrieval, beta-hCG (β-hCG) level was measured. Ultrasound was performed three weeks later to determine clinical pregnancy and at 10-12 weeks of gestation, to determine ongoing pregnancy.

Statistical analysis

Appropriate statistical analysis was performed using Student's t test and Chi-square or Fisher's exact test. The software that we used was PASW statistics version18. The power analysis of study was 80% and a p value <0.05 was considered statistically significant.

Results

A total of 140 fresh and non-donor embryo transfers were performed. The conventional blind method was performed on eighty cases of group I and the previously measured uterine length method using vaginal ultrasound was performed on 60 cases of group II. The baseline and clinical characteristics of patients including age, duration and type of infertility, and etiology of infertility were compared between two groups (Table 1). No statistically significant differences were found between the two groups in other variables such as type of stimulation, type of gonadotropin used, number of retrieved oocytes, number of transferred embryos, endometrial thickness, uterine length, and easy or difficult transfer (Table 2), except for the day of transfer that in group II at 72 hours was later than group I at 48 hours after ovum pick up.

According to our results, although the chemical pregnancy rate was higher in group II (42.1 vs. 28.7%), the difference was not statistically significant (p=0.105). The clinical pregnancy, ongoing pregnancy and implantation rates were higher in group II (33.9 vs. 17.7% and 22.1 vs. 12.8%, respectively), indicating that the difference is statistically significant.

Abortion rate was higher in group I (34.7 vs. 0%). Except for the demise of one embryo in a twin pregnancy, no other abortion in the first trimester was recorded in group II. No ectopic pregnancy was detected in both groups (Table 3).

Table 1: The comparison of baseline and clinical characteristics between two groups

Patient	Frequency (%)		P value
	Group I N=80	Group II N=60	
Age[a] (Y)	31.68 ± 0.69	31.4 ± 82	0.790[b] (NS)
Type of infertility			0.122[c] (NS)
Primary	61 (76.2%)	52 (86.7%)	
Secondary	19 (23.8%)	8 (13.3%)	
Cause of infertility			0.950[d] (NS)
Female factors	24 (30.8%)	16 (26.7%)	
Unovulation	10 (12.5%)	9 (15%)	
Tubal factor	25 (31.2%)	17 (28.3%)	
Male factors	37 (47.4%)	31 (51.7%)	
Female and male	11 (14.1%)	9 (15%)	
Unexplained	6 (7.7%)	4 (6.6%)	

Signification at the 5 precent level.
[a]; Mean ± standard deviation, [b]; T test, [c]; Chi-square analysis, [d]; Fisher exact test and NS; Non-significant.

Table 2: The comparison of the other studied variables between two groups

	Frequency (%)		
	Group I	**Group II**	**P value**
	N=80	**N=60**	
Treatment cycle			0.283[a] (NS)
Agonist	49 (61.3%)	42 (70%)	
Antagonist	31 (38.7%)	18 (30%)	
Drug			0.151[a] (NS)
Single	29 (36.3%)	29 (48.3%)	
Combination	51 (63.7%)	31 (51.7%)	
Transfer type			0.130[b] (NS)
Easy	72 (90%)	58 (96.7%)	
Hard	8 (10%)	2 (3.3%)	
Uterine length[c]	74.94 ± 1.33	74.97 ± 1.21	0.989 (NS)
Stimulation days[d]	8.43 ± 0.20	8.40 ± 0.24	0.923[c] (NS)
No. of oocytes[c]	8.62 ± 0.57	9.73 ± 0.80	0.252 (NS)
No. of embryos[d]	5.14 ± 0.40	5.50 ± 0.45	0.555[c] (NS)
No. of ET[d]	2.42 ± 0.08	2.45 ± 0.10	0.848[c] (NS)
End thickness	8.58 ± 0.24	8.72 ± 0.31	0.73 (NS)
Transfer time[d] (Hour)	63.60 ± 2.18	69.20 ± 1.98	0.060[c] (NS)

Signification at the 5 precent level.
NS; Non-significant, ET; Embryo transfer, [a]; Chi-square analysis, [b]; Fisher exact test, [c]; T test, and [d]; Mean ± standard deviation.

Table 3: The comparison of pregnancy results between two groups

	Frequency (%)		
	Group I	**Group II**	**P value**
	N=80	**N=60**	
Chemical pregnancy	23 (28.7%)	24 (42.1%)	0.105[a] (NS)
Clinical pregnancy	17 (21.2%)	19 (33.9%)	0.0135[a]*
Ongoing pregnancy	14 (17.7%)	19 (33.9%)	0.0131[a]*
Abortion rate	8 (34.7%)	0 (0%)	0.005[a]*
Implantation rate[b] (%)	12.86 ± 2.71	22.12 ± 4.57	0.086[c]*

*NS; Non-significant, ET; Embryo transfer, [a]; Chi-square analysis, [b]; Mean ± standard deviation, [c]; T test and *; significant(s).*

Discussion

Despite many advances in the practice of ART cycles, the implantation and clinical pregnancy success rates are low even for patients with many oocytes and good quality embryos (1, 2). ET technique and the depth of embryo deposition in uterus are important factors which could affect the results (3, 9, 10). The optimal depth of embryo deposition has been suggested to be 1-2 cm from fundus (11, 21, 22), but determination of this depth have been carried out by different methods that lead to controversial results.

In many IVF centres in Iran, the conventional (blind) method is used. Although ultrasonic-guided ET have been suggested to improve the outcomes in some studies (16, 17), some other studies have not confirmed this finding (18, 19). In a study by Lambers et al., the outcome of ART cycles using previously measured uterine length method did not differ from cycles using ultrasound-guided ET method (20). Full bladder during abdominal ultrasonography is difficult and painful for many patients and is time-consuming for the physicians, so it is better and easier to perform ET procedure by empty bladder. Our study indicated that embryo transfer based on previously measured uterine length method results in significantly higher pregnancy and implantation rates as compared with blind method.

Increased clinical and ongoing pregnancy and implantation rates in this method may be due to determination of exact and suitable depth of uterus for embryos placement that indicates the importance of the fundal site of uterus for better implantation. The length of uterus is a factor affecting the outcome of ART, whereas this measurement is different among women and changes during the drug stimulation and cycle to cycle (10, 23). Therefore, the blind method seems not to be a suitable method since ET is done in a certain depth of uterus for all patients.

A decrease in abortion rate in our study is another point to support the importance of better site determination in ET success.

One of the different points between two groups in our study was the time of ET, suggesting that in group II, the day 3 ET showed better result as compared to the day 2 ET, but was not significant-ly different. This difference may affect the results, but some studies have showed that day 2 ET and day 3 ET have had similar reproductive outcomes (24, 25).

Conclusion

It seems that the detection of uterine length by ultrasound during the treatment cycle and performance of ET at the depth of 1-1.5 cm from fundus may improve the outcome of ART cycles as compared to a blind approach, while leads to a decreases in abortion rate.

Acknowledgements

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Original Article

Age, Body Mass Index, and Number of Previous Trials: Are They Prognosticators of Intra-Uterine-Insemination for Infertility Treatment?

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Abstract———
Background: To examine whether pregnancy rate (PR) of intrauterine insemination (IUI) is related to certain demographic factors, such as age and body mass index (BMI), along with number of IUI cycles performed, a set of infertile Saudi women.

Materials and Methods: During this prospective study (a 24-month period), 301 Saudi women with infertility underwent IUI in our infertility clinic. We investigated whether PR is correlated with patient age and BMI, and the number of IUI trials, in order to determine if they could be used as prognosticators of pregnancy success.

Results: The highest PR was 14.89% for ages 19-25 and the lowest PR was 4.16% for ages 41-45, indicating no statistically significant difference among PR in all age groups (p value of 0.225). Also, in terms of BMI, the highest PR was 13.04% for BMI $\geq$35 and the lowest was 7.84% for BMI of <25 to 18.5, indicating no significant difference among different BMI groups (p value of 0.788). One-cycle treatment, as expected, was more successful (PR=12.84%) than 2-cycle treatment (PR=5.75%), however, 3-5-cycles treatment still showed encouraging results (PR=17.24%); but the difference did not reach statistical significance (p value=0.167).

Conclusion: PR after IUI treatment remained approximately 10% from 19 to 40 years of age and declined after 40. Although no significant difference was observed among different age groups, earlier treatment is still recommended. There was a positive but not statistically significant correlation between PR and patient's BMI indicating that BMI is not a determining factor. There was also no correlation between PR and number of IUI trials. Patients can thus try as many times as they want before moving on to *in vitro* fertilization (IVF) treatment.

Keywords: Intrauterine Insemination, Age, Body Mass Index, Pregnancy Rate

Introduction

The advantages of intrauterine-insemination (IUI) with and without mild ovarian stimulation were early recognized and IUI was applied for treating couples with borderline male, cervical, immuno-logical, or unexplained infertility factors (1). Additionally, IUI has been shown to be a much less expensive and less invasive procedure in comparison with the *in vitro* fertilization-embryo transfer (IVF-ET) (2).

Following IUI, pregnancy rates vary widely due to multiple factors including heterogeneity and variability of studied patients and parameters (3-7). These variations could also be due to population differences and circumstantial variability under which each study was conducted (1).

The objective of the present study is to audit the clinical outcomes of IUI as a mild infertility treatment in a set of female patients in Saudi Arabia and to examine whether clinical outcomes were related to patient demographic factors such as age and BMI, as well as, the number of IUI treatments, in order to be used as success prediction factors.

Materials and Methods

During a 24-month period, between January 2010 and December 2011 inclusive, 301 Saudi women with infertility underwent extensive investigation consecutively. That included pertinent infertility-related history, general physical and pelvic examination, and assessment of the reproductive organs by appropriate imaging and endoscopy. Baseline serum levels of follicular stimulating hormone (FSH), luteinizing hormone (LH), thyroid stimulating hormone, and prolactin were always analyzed. In addition, assessment of the male partner included at least two semen analyses, with at least one analysis being performed before and after sperm wash to determine the total motile sperm count and modes of sperm progression. Further evaluation of the male partner included analysis of serum levels of FSH and testosterone, when indicated. Eligible women for IUI-treatment were then stimulated with different kinds of ovulation induction hormones. Most patients were stimulated with highly purified gonadotropins (hMG), such as Merional, 75 i.u./day or Puregon, 50 i.u./day (both from IBSA, Switzerland). Only cases that did not show any sign of response with-

in the first ten days of treatment were excluded. The number of treatment days differed based on various factors, like age, body mass index (BMI), and cause and history of infertility. A number of patients were stimulated with clomiphene citrate, 100 mg/day (Merk, Germany) for 5-6 days. All patients were monitored with ultrasound for ovulation induction and follicle maturation. When a maximum of two leading follicles reached at least 17 mm in diameter, patients were injected with 5,000 IU of human chorionic gonadotropin hormone (HCG) 36 hours prior to IUI.

On the day of IUI, fresh semen of the husband was prepared for insemination. The final sperm specimen was mixed in 0.5 ml of HEPES-buffered media (Quinn's Advantage Medium, with HEPES, SAGE (Pasadena, USA), supplemented with serum (Quinn's Advantage Serum Protein Substitute SPS, SAGE (Pasadena, USA). All pre- and post-preparation semen parameters were recorded.

Pregnancy was determined by βHCG values obtained on the sixteenth day after IUI, and then confirmed by ultrasound four weeks later.

Statistical analysis

Data were analyzed using the SPSS statistical software package, version 19 (SPSS Inc., Chicago, USA). Chi-squared test was used to compare pregnancy rates with respect to all variables. P-value less than 0.05 were considered statistically significant.

Ethical considerations

The Institutional review board of the College of Medicine at King Saud University approved this study with approval number E-12-642. Informed written consents were also obtained from all human adult participants in the study.

Results

Women were stratified by age as shown in table 1. The median age of the entire group was 31, with a range of 19-45. The highest pregnancy rate was 14.89% in age group 19-25, as compared with the lowest PR of 4.16% in age group 41-45. This difference was not statistically significant (p=0.225). One pregnancy occurred, at age 44, among 24 women over 41 years.

BMI was stratified according to the World Health

Organization (WHO) definition (8, 9). The relationship between BMI and pregnancy rates among all groups is shown in table 2. The median BMI of the group was 30.14. The highest PR was 13.04% with BMI ≥35, while the lowest PR was 7.84% with BMI from 18.5 to less than 25. This difference was also not statistically different (p=0.788).

The relationship between the number of IUI cycles performed and pregnancy rates is shown in table 3. The differences between one, two, and three to five cycles did not reach statistical significance (p=0.167), however, there was a trend that suggested the first treatment cycle to be the most successful.

Table 1: Relationship between age and pregnancy rate of infertile women treated with intrauterine insemination

	Age (Y)	Number of patients and (%)	Number of pregnancies and (PR%)	P value/χ^2
I.	19-25	47 (15.51%)	7 (14.89%)	
II.	26-30	106 (34.98%)	6 (5.66%)	
III.	31-35	81 (26.73%)	11 (13.58%)	0.225
IV.	36-40	43 (14.19%)	5 (11.63%)	χ^2=5.673
V.	41-45	24 (7.92%)	1 (4.16%)	
		Total: 301	**Overall PR: ~10%**	

PR; pregnancy rate.

Table 2: Relationship between body mass index (BMI), and pregnancy rate of infertile women treated with intrauterine-insemination

	BMI (Kg/m²)	Number of patients and (%)	Number of pregnancies and (PR)	P value/χ^2
I.	Normal (18.5 - <25)	51 (16.94%)	4 (7.84%)	
II.	Over-Weight (25 - <30)	97 (32.23%)	9 (9.28%)	0.788
III.	Obese (30 - <35)	84 (27.91%)	8 (9.52%)	χ^2=1.054
IV.	Highly Obese (≥35)	69 (22.92%)	9 (13.04%)	

PR; Pregnancy rate and BMI; Body mass index.

Table 3: Relationship between Number of intra-uterine insemination cycles performed and pregnancy rate in saudi infertile women

	Number of IUI Cycles	Number of patients and (%)	Number of pregnancies and (PR)	P value/χ^2
I.	One	148 (49.17%)	19 (12.84%)	0.167
II.	Two	87 (29.04%)	5 (5.75%)	χ^2=3.576
III.	3-5	66 (21.93%)	5 (17.24%)	

PR; Pregnancy rate and IUI; Intra-uterine insemination.

Discussion

We audited results from a single infertility clinic in the Kingdom of Saudi Arabia, to determine whether women's age, and BMI, as well as, the number of IUI treatments performed could be used as prognostic indicators of pregnancy success.

Data in table1 shows relatively good outcome up to the age of 40, while some studies reported good outcomes only up to 30 years of age, (10-12) and reporting favorable outcomes up to age 35 (13-15). Although there was a decline by approximately 50% in PR in the 26-30 age group, the difference was not statistically significant. There may be a logical explanation related to local socio-demographic factors. Women of 26-30 years of age comprise the most active reproductive group as they are either recently or just married and are actively trying to conceive. Therefore, those who fail to spontaneously conceive may have more complicated infertility issues, and IUI may not be the best treatment for them. With the exception of the 26-30 age group, the results were in line with those published by others (11).

The lowest pregnancy rate (4.16%) was in the over-40 group (one success out of 24 cycles). These findings are in agreement with those of others (10). One study (16) reported PR of only 2% for 40 years old or older patients. Therefore, many authors advised women of that age not to undergo IUI more than once. Moreover, one study (14) reported no pregnancy after age 40, while another (17) reported no pregnancy after age 44. Consequently, it was advised that infertile women of 43 or older seek directly other alternatives (18).

We found that normal BMI was found in only 16.8% of women while 27.8% were highly obese. These statistics are very disturbing from a public health point of view as this group of patients is a random sample of Saudi women. It has been proven that overweight/obesity has become a world epidemic, (19) that is associated with multiple health issues including pregnancy related complications (20). Obesity is related to ovulation dysfunction that leads to limited fertilization, and menstrual disorders, which may lead to impaired implantation (21-23). Ovulation interruptions are known to be more related to polycystic ovarian syndrome which is more common in obese women. The incidence of intrauterine lesions such as myomas and/

or polyps is also more frequent in obese women (24, 25). Other complications during and after birth (19, 20) will not be discussed as they do not pertain to this study.

We found, however, no statistically significant difference in PR between different BMI groups, including the highly obese group (BMI ≥35). A study (26) reported that ovulation induction with an aromatase inhibitor (letrozole 2.5 mg for 5 days) and IUI treatment, showed no significant difference for different BMI's. Another study (27) reported that obese women produce fewer follicles and require higher doses of medication; the success rate however was comparable to that of women with normal weight.

On the other hand, some studies (23, 24, 28) reported that overweight and obese sub-fertile women had a reduced probability of successful fertility treatment and their pregnancies were associated with more complications and higher costs. Few other studies (29-31) have also reported that weight loss helped the ovulation process in some obese women and enhanced their infertility treatment outcome.

About half (49.17%) of the patients underwent IUI only once, with PR of 12.84%. Then the PR declined to 5.75% with 29.04% of the patients who tried IUI twice. The PR then climbed up again to 17.24% with those who underwent 3-5 IUI cycles (21.93% of the patients), although with no significant difference among all three groups. A study (32) compared PR from one to six IUI cycles, and found that the PR of the first cycle is significantly higher than the rest of the IUI cycles. A similar study (14) found that the PR rate increased up to the third trial and decreased thereafter, contrary to a study (13) that found IUI pregnancy rates were significantly higher after the third cycle. Another study (33) found no superiority of a specific cycle which is more consisted with our findings. Campana et al. (17) found significantly higher cumulative pregnancy rates with up to six IUI's compared with those with up to three IUI. Botchan et al. (34) reported pregnancies at the eighteenth IUI cycle (one pregnancy out of eighteen patients) and above, (five pregnancies out of ninety-five cases). So, it may be advisable that couples who have concerns about their time or finances, be counseled to proceed with IVF-ET if their first IUI trial

Conclusion

We audited a group of 301 infertile Saudi women treated with IUI and found that the pregnancy rate remained approximately 10% for age groups between 19 and 40. While after 40 it declined noticeably, which means that direct IVF treatment would be in their favor. There was a positive but not statistically significant correlation of pregnancy rates with BMI; (from 8% PR, for normal BMI, to 13%, for highly obese), i.e. female high BMI is not a discouraging factor for IUI treatment. There was also no clear correlation between PR and the number of IUI trial; and it is therefore the patient's choice when to move to IVF treatment instead.

Acknowledgements

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Original Article

Polycystic Ovary Syndrome in University Students: Occurrence and Associated Factors

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Abstract

Background: The aim of this study was to assess the occurrence of polycystic ovary syndrome (PCOS) and its association with body composition among students in University of Sharjah (UOS).

Materials and Methods: This cross-sectional study included a total sample size of 50 female students registering in undergraduate programs at the University of Sharjah using convenience sampling technique. A pretested interview schedule was administered to elicit information pertaining to personal background and medical history related to PCOS. A diagnostic ultrasound scan was performed for determining PCOS along with a body composition analysis using bioelectrical impedance analysis (BIA) technology.

Results: Twenty percent (10 out of 50 participants) were diagnosed with PCOS, of whom only 4 individuals were previously diagnosed with PCOS and aware of their conditions, while the reports showed 16% with oligomenorrhea, 4% with polymenorrhea, and none with amenorrhea. A positive family history was indicated as reported by 22% of the total participants. Significant difference between the body weights of participants having PCOS (66.7 kg) and those without it (58.8 kg) were noted (p=0.043, t=2.084). On the other hand, the body composition related variables including waist-hip ratio (WHR), fat-free mass (FFM), percent body fat (PBF) and visceral fat area (VFA) were relatively higher in participants having PCOS than those without it. However, there was no statistical significance of differences. Comparatively, the participants with PCOS had lower bone mineral density (BMD) than those without it, whereas the difference was statistically non-significant.

Conclusion: The occurrence of PCOS in the present study is consistent with the global prevalence. Comparatively, the body composition of PCOS females is different from the normal females. Further studies are required in the Middle East region on larger sample sizes and broader aspects of health including lifestyle and dietary components to understand these differences.

Keywords: PCOS, Body Composition, Menstrual, Ultrasound

Introduction

Polycystic ovary syndrome (PCOS) is a disorder in the function of an endocrine gland that affects the ovaries (1), involves hyperandrogenism and diminishes reproductive function (2). The disease affects around 1 in 10 women, making it the most common endocrine disorder amongst women of reproductive age (3). Some of the clinical manifestations of this disorder are irregular menstruation, infertility, weight gain, hirsutism, and acne. Also, the biochemical diagnostic features of PCOS include anovulation, insulin resistance, and hyperandrogenaemia (4). Thus, PCOS would likely increase a woman's chance of having diabetes mel-

litus, hypertension and inflammation. It has been demonstrated that women with PCOS may have higher risks of cardiovascular, sleep apnea and infertility (5-8). Diagnosis of PCOS is usually based on typical signs and symptoms, physical appearance, biochemical evidence of hyperandrogenism and ovarian dysfunction (9). An ultrasound examination of the uterus/ovaries is the most reliable technique used due to morphological diagnosis of polycystic ovaries (10).

Considering the magnitude and consequences of PCOS compounded by the social apprehensions related to the nature of problem, it is important to assess its occurrence in the young adults. University female students constitute a homogenous group of population whose outreach is feasible and they are the future mothers of the society. Ironically, university students may appear healthy and not realize that they have PCOS until problems in conceiving are encountered during marriage. Lack of information about association of PCOS with other health parameters and unawareness of its diagnostic criteria may have a major impact on the presence of this disease among university females. Therefore, the objectives of the present study were to assess the occurrence of PCOS and to study its association with body composition among female students at University of Sharjah, United Arab Emirates.

Materials and Methods

A cross-sectional study was conducted at University of Sharjah in United Arab Emirates between January 2012 and June 2012. All female students registering in undergraduate programs at the University of Sharjah were included in the present study. However, those students who were pregnant at the time of the survey period were excluded. Accordingly, fifty female students were selected using the convenience sampling technique (11). Objectives of the study and assessment needed were explained, and an informed consent was then obtained from all the participants. Furthermore, the study was approved by the Research Committee of Department of Clinical Nutrition and Dietetics, College of Health Sciences, University of Sharjah.

A pretested interview schedule was administered to collect information from the subjects. Herein, the students provided demographic information that included personal information (age, college and marital status); medical history related to PCOS; status of

menstrual cycle like normal (bleeding at intervals between 22 to 40 days intervals), oligomenorrhea (bleeding at intervals of greater than 40 days) and polymenorrhea (bleeding at intervals of less than 22 days); use of hormonal pills; family history of PCOS; perception of body weight; and attempt to lose weight. Body composition data were also collected systematically at the initial clinic visit.

The required measurements were taken as follows: i. body composition of the participants was determined using the bioelectrical impedance technology (Biospace Co. Ltd., Seoul, Korea); ii. body mass index (BMI) was calculated in kg/m^2 and defined according to World Health Organization (WHO) (12); iii. waist-hip ratio (WHR) was determined by the waist circumference divided by the hip circumference; iv. fat free mass (FFM) was determined by fat free mass including weight of skin, bones, ligaments, tendons, organs and water content; v. percent body fat (PBF), defined according to Li et al. (13) was calculated by the amount of fat in the body composition; vi. visceral fat area (VFA; the area in cm^2 of organ fat or intra-abdominal fat) is located inside the peritoneal cavity, packed in between internal organs and torso, as opposed to subcutaneous fat found underneath the skin and intramuscular fat, which is interspersed in skeletal muscle (14); vii. bone mineral density (BMD; gram per square centimeter) is the bone mass after developmental period is completed (15). BMD was also measured using the Body Composition Analyzer.

Polycystic ovary was defined as the presence of at least 1 ovary at >10 cm^3 in volume and/or at least 1 ovary with ≥12 follicles that measured 2-9 mm in diameter. Ovarian assessments were made using an ultrasound instrument (Siemens, Erlangen, Germany). The procedure of the instrument manufacturer was followed.

Statistical analysis

Data obtained were statistically analyzed using Statistical Package for the Social Science (SPSS: SPSS Inc., Chicago, IL, USA) software version 17. Descriptive data were reported as means ± SD. Demographic and medical history variables were expressed in frequencies and percentages. Significance of difference in the variables between participants with or without PCOS was determined using student's t test. A p value of less than 0.05 was considered to be statistically significant.

Results

Demographic characteristics of the participants are given in table 1. Among participants, 72% were from the medical and health sciences colleges and 28% were from other different colleges. The age of the participants ranged from 17 to 23 years with the mean age of 19.4 years. Only one out of 50 participants was married. Her gynecology history revealed that she had para 1- one live child.

Table 1: Demographic characteristics of participants (n=50)

Variable	Participants %
Colleges	
Medical and health colleges	72 (36)
Other colleges	28 (14)
Age (Y)	
(17-19)	58 (29)
(20-23)	42 (21)
Marital status	
Unmarried	98 (49)
Married	2 (1)

The distribution of participants according to medical history related to PCOS is presented in table 2.

Medical history revealed that the status of menstrual cycle was normal in majority (80%) of the participants, while the finding showed that 16% had oligomenorrhea, 4% had polymenorrhea, and none had amenorrhea. Out of these, 8% had been dealing with PCOS for more than two years, 6% for over a year, and another 4% for less than 6 months. Glucose intolerance was reported in 2%, while 14% described other associated problem, specified as anemia. Twelve percent of participants took hormone pills for regularizing their menstrual cycles. While 8% of participants were on the treatment for less than 6 months, 4% were on treatment between 6 months to a year.

Ironically, only 8% of participants were previ-

ously diagnosed with PCOS, 76% had not been diagnosed earlier, and 16 % were unaware of any previous diagnosis of PCOS. About 22% showed to have the positive family history, 76% had no family history, and 2% were unaware of their family history regarding occurrence of PCOS. Amongst the individuals with positive family history, 8% reported in their mothers and sisters, 4% in cousins and 6% in their aunts. Thirty percent of participants reported to have difficulties in maintaining normal weight. When enquired about their perception of body weight, two-third of them confessed that they perceived their body weight as "normal", 14% as "underweight", 18% as "overweight" and 4% as "obese".

During the last one year, weight loss was attempted by almost half of the participants (n=24). Out of these, 16 % sought out professional support for losing weight during this period. The ultrasound scan results confirmed the diagnosis of PCOS in 10 out of 50 participants (20%).

The means and standard deviations of body composition variables of the participants are represented in table 3. In addition, significance of difference between the group with PCOS and that without PCOS is presented for each variable.

The weight of the participants ranged from 39 kg to 98 kg, with a mean weight of 60 ± 11 kg. Participants with PCOS (66.7 kg) were found to be significantly heavier than those without it (58.8 kg) (p=0.043, t=2.08).

Mean BMI of the participants was 22.9 ± 3.5 ranging from 16.5 to 31.3. Almost three-fourths of the total students were categorized as "normal", while BMI of 26% was above normal. Participants with PCOS had higher BMI than those without it; however, no significant difference was found.

As evident from the table 3, the mean values of WHR, PBF, FFM, and VFA were found to be higher in participants with PCOS in contrast to those without PCOS. However, statistically significant difference could not be established at p<0.05.

BMD of the participants, on an average, was 2.3 ± 0.28 g/cm^2 and it ranged from 1.8 to 3.1 ± 0.28 g/cm^2. There was no significant difference between the BMD of those with PCOS and those without it at p<0.05.

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Table 2: Medical history of participants related to PCOS (n=50)

Variable	Participants %
Status of menstrual cycle	
Normal	80 (40)
Oligomenorrhea	16 (8)
Polymenorrhea	4 (2)
Relevant diseases	
None	84 (42)
Glucose intolerance	2 (1)
Other	14 (7)
Use of hormonal pills	
No	88 (44)
Yes	12 (6)
Previous diagnosis of PCOS	
No	76 (38)
Yes	8 (4)
Doesn't know	16 (8)
Family history of PCOS	
No	76 (38)
Yes	22 (11)
Doesn't know	2 (1)
Perception of own body weight	
Normal	64 (32)
Underweight	14 (7)
Overweight	18 (9)
Obese	4 (2)
Weight loss attempt	
No	52 (26)
Yes	48 (24)

PCOS; Polycystic ovary syndrome.

Table 3: Means and standard deviations of body composition variables in participants with PCOS and those without PCOS

Variables	With PCOS	Without PCOS	t	P value
Weight (kg)	66.7 ± 14.4	58.8 ± 9.7	2.08	0.043*
Body mass index (kg/m^2)	24.1 ± 3.9	22.6 ± 3.43	1.21	0.230
Waist hip ratio	0. 87 ± 0.06	0.84 ± 0.04	1.82	0.076
Percent body fat (%)	36.8 ± 8.7	33.7 ± 6.8	1.20	0.235
Fat free mass (kg)	48.1 ± 12.4	43.1 ± 10.6	1.28	0.207
Visceral fat area (cm^2)	77.6 ± 26.3	64.9 ± 28.0	1.29	0.202
Bone mineral density (g/cm^2)	2.31 ± 0.21	2.33 ± 0.30	0.173	0.863

; Significant at p<0.05 and PCOS; Polycystic ovary syndrome.

Discussion

Polycystic ovary syndrome is the most common endocrine disturbance that affects women. The aim of this study was to assess the occurrence of PCOS and its association with body composition among students in University of Sharjah. Our study reported that oligomenorrhea occurred in 16% of female students. Avvad et al. (16) reported that the menstrual irregularity in the early post-menarchal years may be an early sign of PCOS. Kitzinger and Wilmot (17) supported the presence of either irregular, absent or disrupted periods in women. van Hooff et al. (18) suggested that about 50% of the oligomenorrhic adolescents will develop PCOS as adults. A positive family history has been indicated in PCOS (19). Comparatively higher figures have been reported in earlier studies, 35% in mothers and 40% in sisters (20). Body dissatisfaction is observed to a greater extent in females suffering from PCOS. Himelein and Thatcher (21) as well as Trent et al. (22) confirmed that the common symptoms in PCOS (menstrual irregularities, obesity, male-pattern facial and body hair, acne, and other skin problems) contributed to poor body image and self-esteem and correlated with low quality-of-life scores. Moran et al. (23) concluded that there are potential barriers to successful weight management in young women who do not suffer from PCOS and additional barriers in women having PCOS.

The prevalence of PCOS (20%), based on our ultrasound findings, is consistent with those of other studies reporting prevalence of PCOS (8-33%) in women of reproductive age (24, 25). One-fourths of the total subjects in the current study were found to have BMI above normal; however, there was no significant difference between the BMI of subjects with or without PCOS. Yucel et al. (26) also revealed similar findings. Eleftheriadou et al. (27) reported a slightly higher percentage of overweight adolescents with PCOS than those without it. The histogram of BMI distribution in participants was found to be skewed towards the left, though it was not statistically significant. Similar to the current findings, no significant differences were reported between females with PCOS and controls in terms of WHR (28), PBF (29) as well as BMD (30). Barber et al. stated that it was global obesity (weight in the current study) rather than the abnormal regional fat distribution (VFA and WHR values in the current study) that characterized the PCOS women (30). However, WHR value of PCOS women was reported significantly higher than that of control subjects (29).

Conclusion

The prevalence of PCOS in the present study is consistent with the global occurrence. Comparatively, the body composition of PCOS females is different from the normal females in

terms of favoring more body weight, body fat, WHR and BMI. On the other hand, BMD is lesser in PCOS females than their normal counterparts. However, the further studies are needed in the Middle East region on larger sample sizes and broader aspects of health including the lifestyle and dietary components to understand the differences in weight in females suffering from PCOS.

Acknowledgements

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Original Article

Age at Menopause and Its Main Predictors among Iranian Women

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Abstract————

Background: Since time of menopause is influenced by a variety of racial, environmental, and physiological factors, determining age at natural menopause and its main indicators seems to be necessary. The present study attempted to determine average age at menopause and its main predictors among Iranian women.

Materials and Methods: This descriptive-analytic study was carried out on 400 postmenopausal women aged 43 to 65 years attending the health centers in Hamadan, Hamadan Province, Iran, during 2013. Due to potential effects of oral contraceptive pills (OCP) on age of menopause, we considered two groups of women with and without OCP use using cluster sampling method. Data were collected through individual interviews at the health centers.

Results: The findings showed significant univariate relationships between age at menopause with some baseline variables including mother's age at menopause ($p<0.001$), mother and spouse with high educational level ($p<0.001$), passive cigarette smoking ($p<0.001$), weekly physical activity ($p<0.001$), and high family income ($p<001$). Adversely, smoking was associated with early menopause.

Conclusion: The postmenopausal women doing intense weekly physical activity, having mothers with late menopause, having higher monthly income, and experiencing later-age pregnancy are likely to reach menopause later than their contemporaries, while smokers have an early menopause.

Keywords: Iran, Menopause, Women

Introduction

Age at menopause is naturally influenced by a variety of racial, environmental, and physiological factors (1). Even, recent genome-wide association studies have been successful in revealing genetic determinants of age at natural menopause (2-4). In fact, some women reach menopause at an early age with unknown reason, which could be the result of an inherited issue or a one-time genetic mutation. Also, age at menopause may be potentially influenced by chronological age, mother's age at menopause, menstrual cycle characteristics, and markers of ovarian reserve (5, 6). Besides, some environmental and iatrogenic factors including cigarette smoking, chemotherapy, ovarian surgery, or damage the reproductive or endocrine system have been identified to accelerate or delay menopausal age through inducing defects in hormonal pathways such as ovarian dysfunction (7, 8).

Postmenopausal women are reported more than 15% of the population in developed countries, but this rate is 5-8% in less developed regions of the

world. By 2030, the world population of menopausal and postmenopausal women is assumed to increase to 1.2 billion, with 47 million new cases each year. As women are spending more than 1/3 of their life in the postmenopausal period and the number of postmenopausal women is increasing, physical and mental health in this period are of greater importance. Identifying the factors associated with late or early menopause is necessary because age at menopause has been associated with risk for the onset of several chronic diseases such as cardiovascular diseases, breast and endometrial cancers and osteoporosis (9).

The major role of ethnicity in determining age at menopause as well as its main predictors has been assessed in different societies. It is noted that Hispanic and African-American women reach menopause earlier, while Chinese and Japanese women reach menopause later than the average Caucasian women experiencing menopause at about age 51.5 (10). However, no comprehensive information is available in age at menopause and its main determinants among Iranian women. The present study attempted to determine average age at menopause and its main predictors among Iranian women.

Materials and Methods

This descriptive-analytic study was carried out on 400 postmenopausal women aged 43 to 65 years old attending the health centers in Hamadan, Hamadan Province, West of Iran, for general counseling or treatment of menopausal symptoms, during 2013. The study was performed according to the Helsinki declaration protocol. The objectives of the study were explained to all participants and informed consent was then obtained from them. The study was approved by the Ethical Committee of Hamadan University of Medical Sciences. Inclusion criteria were as follows: natural cessation of menses ≥12 months, not being surgically menopause and not receiving hormone therapy. Due to potential effects of oral contraceptive pills (OCP) on age of menopause, we considered two groups of women with and without OCP use using cluster sampling method. The participants were interviewed to record baseline information regarding menarche age (year), marital status (married), age of first and last pregnancy (year), number of parity,

quality of menstrual cycle (regularity), duration of breast feeding (year), history of cigarette smoking, level of physical activity (hour/week), as well as socioeconomic status.

Results were reported as mean ± standard deviation (SD) for the quantitative variables and as absolute frequencies and percentages for categorical variables. Categorical variables were compared using chi-square test or Fisher's exact test when more than 20% of cells with expected count of less than 5 were observed. Continuous variables were also compared using t test. Predictors showing a statistically significant relation with menopausal age in univariate analysis were taken for multivariate logistic regression analysis to investigate their independence as predictors. Odds ratio (OR) and 95% confidence intervals (CI) were calculated. P values of 0.05 or less were considered statistically significant. All the statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS; SPSS Inc., Chicago, IL, USA) version 15.0.

Results

Findings showed that the mean age of women was 57.4 ± 5.1 years, of which 1.3% were less than 45 years, 9.0% were aged 46 to 50 years, 29% were aged 51 to 55 years, and 60.8% were aged 56 years or more. The mean menarche age was 13.6 ± 1.3 years, the mean age at marriage was 15.8 ± 3.6 years, and the mean age at first pregnancy was 18.4 ± 3.5. The mean age of natural menopause was 49.6 ± 4.02 years and its median was 50.0 years. Among them, 17/5% were ≤45 years old, 45% were 50-46 years old, and 37/5% were ≥51 years old.

The baseline characteristics of participants in the two groups are summarized in table 1. Except for history of infertility that was more frequent in OCP non-users as well as weekly physical activity that was intense in OCP users, both groups were similar in terms of other baseline variables. The estimated mean age of menopause for the entire sample was 49.67 ± 3.83 years, ranged 39 to 60 years, while for OCP users was 49.60 ± 4.33 years and for OCP non-users was 49.61 ± 3.52 years with no significant discrepancy. Regarding association between age at menopause and other characteristics, multivariate logistic regression analysis shows positive association between age at menopause with me-

narche age (Beta=0.125, p=0.012) and last pregnancy age (Beta=0.258, p<0.001). Also, using t test or one-way ANOVA analyses, we could show significant univariate relationships between age at menopause and some baseline variables including mother's age at menopause (p<0.001), mother and spouse with high educational level (p<0.001), passive cigarette smoking (p<0.001), weekly physical activity (p<0.001), and high family income (p<0.01) (Table 2). Furthermore, our data showed that age at menopause is predictable by last pregnancy age using multivariable linear regression model. Adversely, smoking was associated with early menopause.

Table 1: Baseline characteristics in OCP user and non-user women

Characteristics	OCP user (n=243)	OCP non-user (n=157)	P value
Women age (Y)	57.24 ± 4.96	57.69 ± 5.35	0.404
Age at menopause (Y)	49.60 ± 4.33	49.61 ± 3.52	0.979
Age at menarche (Y)	13.60 ± 1.25	13.66 ± 1.30	0.616
Age at first pregnancy (Y)	18.23 ± 3.36	18.71 ± 3.61	0.182
Age at last pregnancy (Y)	33.28 ± 5.62	33.16 ± 5.66	0.845
Age at marriage (Y)	15.75 ± 3.36	15.95 ± 3.95	0.562
Mothers' age at menopause (<45 year)	38 (15.6)	29 (18.5)	0.459
Parity	3.60 ± 0.61	3.47 ± 0.70	0.611
Irregular menstrual cycles	59 (24.3)	39 (24.8)	0.899
Breast feeding >1 year	211 (86.8)	132 (84.1)	0.228
History of infertility	2 (0.8)	12 (7.6)	<0.001*
Cesarean section delivery	7 (2.9)	10 (6.4)	0.052
Married status (Yes)	190 (78.2)	117 (74.5)	0.397
Mother with high educational level (College)	21 (8.6)	22 (14.0)	0.152
Spouse with high educational level (College)	48 (19.8)	35 (22.3)	0.689
Mother job status as employed	4 (1.6)	7 (4.5)	0.074
Smoking	3 (1.2)	3 (1.9)	0.587
Passive smoking	105 (43.2)	55 (35.0)	0.103
Weekly physical activity >20 hours/week	100 (41.2)	52 (33.1)	0.005*
High family income (>1000000 Tomans)	25 (10.3)	9 (5.7)	0.139
Number of family members	7.35 ± 1.88	7.21 ± 2.50	0.550

; P<0.05 and OCP; Oral contraceptive pills.

Table 2: Multivariable linear regression model for identifying the determinants of the age at menopause

Characteristics	P value	Beta	SE
Age at menarche (Y)	0.190	0.148	0.113
Age at last pregnancy (Y)	0.018*	0.065	0.027
Mothers' age at menopause (Y)	<0.001*	3.643	0.397
Mother with high educational level (college)	0.263	0.320	0.285
Spouse with high educational level (college)	0.821	0.055	0.242
Passive smoking	<0.001*	-1.817	0.313
Weekly physical activity >20 hours/week	<0.001*	0.780	0.202
High family income (>1000000 Tomans)	0.002*	0.806	0.256

*; P<0.05.

Discussion

The mean age at menopause was 49.6 ± 4.02 years according to previous studies (11). Our findings showed that the postmenopausal women doing intense weekly physical activity, having mother with late menopausal age, having higher monthly income, and experiencing later-age pregnancy are likely to reach menopause later than their contemporaries, while smokers have early menopause.

Age at menopause varies across different nations, while is shown a raise in all nations. But, this trend seems paradoxical because existence of several determinants. In this regard, different range of menopausal age and various related determinants have been reported from different countries. In a study by Dratva et al. (12), the median of menopausal age was 54 years and the major determinants of early menopause were current smoking, high body mass index (BMI), and low physical activity, while the determinant for late menopause was multiparity. In this context, the use of OC caused different effects on timing of menopause. In another study by Parazzini et al. (13), the mean age at spontaneous menopause was 51.2 years, and they also reported late menopause age was significantly associated with lower educational level, high BMI, a later age at menarche, lifelong irregular menstrual cycles, and higher parity, whereas smokers showed lower age at menopause. Nagel et al. (14) showed that increasing age at first full term pregnancy and a longer time interval until having regular menses are associated with later onset of natural menopause. Also, as compared to never smokers, current smokers were younger at menopause. High carbohydrate diet and high intake of vegetable, fiber and cereal products are inversely related to the age at natural menopause. Women with higher intake of fat, protein and meat had a delayed onset of natural menopause. Meschia et al. (15) reported the mean age at menopause is 50.9 years. Also, they showed that women smoking had a lower mean age of menopause than non-smokers. Moreover, a low body mass index (BMI) and an early age at menarche were related to early menopause in the crude analysis. In Bromberger's observation, median age of menopause was 51.5 years for the whole sample. Median age at menopause were earlier for women who were African-American, smokers, and underweight, as well as who experienced irregular menstrual cycles (16). In our survey, the mean age at menopause was 49.67 and its median was 50.0 years that is consistent with the finding of other studies conducted in Iran that were included different sources of patients (17-21). A study has showed that 1% of women reach menopause by age 40, 10% by age 46, and 90% by age 55.0, approximately (22). Smokers reached menopause about 1.5 years earlier than nonsmokers due to the effect of nicotine on estrogen production and

catabolism (23). Our study also suggested several new variables to consider in predicting age at menopause. Our results confirmed that there is association between intense weekly physical activity and age at menopause. Menopausal age is accelerated in women who exercise excessively. Also, those with higher socioeconomic level experience later menopause. Furthermore, we showed an association between a mother's and a daughter's age at menopause, as shown previously. In fact, when a mother experiences menopause early, her daughter will have poor ovarian reserve between ages 35 and 49 (24-26). This commonality may be due to some genetics variations. Genome-wide association studies (GWAS) have been successful in showing genetic determinants of age at menarche and age at natural menopause. More than 30 novel genetic loci have been identified in GWAS for age at menarche and 17 for age at natural menopause (27). Also, newly genetic hypothesis is supported by twin studies that attribute 63% of the concordance to shared genetic material. Pedigree analyses have uncovered a potential dominant pattern of inheritance of early menopause and premature ovarian failure through maternal or paternal relatives (28, 29). In total, identifying markers predicting age at menopause would be of significant value to women and clinicians. Time to menopause could influence treatment choice (medical or surgical) for women with disorders of menstruation. However, no single test can predict age at menopause, sum of these determinants should be considered to optimize predicting age at menopause. Limitation of this study should be discussed. The women analyzed were part of a large study whose main goal was to describe the characteristics of women attending health centers located in a city of Iran. Thus, they cannot be considered as a representative population of the Iranian women. However, the general characteristics of this population are similar with those of Iranian women.

Conclusion

In this study, physical activity, education, mother's age at menopause, last pregnancy age, income and smoking are found to be related with age at natural menopause. Our findings showed that the postmenopausal women doing intense weekly physical activity, having mother with late menopausal age, having higher monthly income, and experiencing later-age pregnancy are likely to reach menopause later than their contemporaries, while smokers have early menopause. This result would help in the planning of health services in menopause units as a new, important issue in developing countries like Iran. Additionally, these services should be available for all women at menopause age.

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Original Article

Sexual Functioning among Married Iranian Women with Polycystic Ovary Syndrome

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Abstract

Background: This study aimed to assess sexual functioning among women with polycystic ovary syndrome (PCOS) in Iran.

Materials and Methods: A cross-sectional study was conducted to ascertain factors related to sexual functioning in 300 PCOS patients attending to the private practice centers in Kashan, Isfahan Province, Iran, from May to October 2012. The Female Sexual Function Index (FSFI) was used to measure sexual functioning. Moreover, the socio-demographic details and clinical information of PCOS including obesity, hirsutism, acne, menstrual cycle disturbances, infertility and endocrine profile were recorded for each patient.

Results: Overall the prevalence of female sexual dysfunction (FSD) was 16.6%. In particular patients indicated poorer sexual functioning for the desire (48.3%) and the arousal (44.7%) subscales. Multiple logistic regression analysis suggested patients with lower educational level (OR: 2.94; 95% CI: 1.46-5.92) and irregular menstrual status (OR: 4.61; 95% CI: 1.93-11) were more likely to report sexual dysfunction.

Conclusion: The findings suggest that desire and arousal were the most prevalent sexual disorders reported in this patient population. In addition, findings suggested that women with limited or no formal education and a history of menstrual irregularities were the most likely to report female sexual dysfunction. Further investigations are needed to examine female sexual functioning among women with PCOS, to educate their health care providers, and to develop therapeutic interventions.

Keywords: Sexual Dysfunction, Polycystic Ovary Syndrome, Women

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. It is estimated that 5 to 10% of women suffer from the disease (1). The symptoms typically associated with PCOS are irregular menstruation, hirsutism, obesity, infertility, anovulation and acne, leading to a significant reduction in female quality of life (QOL), marital maladjustment and impaired sexual functioning (2, 3).

Sexuality is an important and complex domain in QOL studies. Prevalence of female sexual dysfunction (FSD) may vary according to cultural, racial and health status. Impaired sexual functioning in women with PCOS has been often neglected

or studied incidentally. Characteristics associated with PCOS may adversely affect sexual health. Women struggling with PCO, have reported feeling less attractive and having lower sexual satisfaction when compared to women without PCOS (4). Contrary to hypothesis stating that elevated androgen levels in PCOS increase female libido, women with PCOS have reported decreased sexual satisfaction and feeling less attractive (5-7). In one study findings suggested an elevated body mass index (BMI) did not affect sexual function or intercourse frequency, but a higher BMI resulted in a decrease in sexual satisfaction (8). Hahn et al. (2) reported that hirsutism decreased women sexual function more than obesity.

Studies examining the sexuality of patients with PCOS focused on the psychosexuality or subject's sexual orientation (9-11). Since there are multiple factors that can impair the sexual function of these patients, it is essential to evaluate the importance of this problem and the main factors contributing to this disorder. In the Iranian population, there has been no study related to sexual functioning among women with PCOS yet. This study was designed to investigate whether clinical and hormonal characteristics in women with PCOS influenced their sexual functioning. The intent is to facilitate an understanding of the relationships between these variables and to guide interventions that might improve the sexual function of patients struggling-with symptoms of PCOS.

Materials and Methods

Design and data collection

This was a cross-sectional study of women with PCOS who attended two private gynecology clinics in Kashan, Isfahan Province, Iran, from May to October 2012. The Ethics Committee of the Tarbiat Modares University approved the study. Patients with confirmed diagnosis of PCOS were invited to participate in the study. After explaining the study objectives, a written informed consent was obtained from all participants and they were then requested to complete the study questionnaires. Inclusion criteria were as follows: 15-40 years of age, married, Iranian, as well as having two of the following Rotterdam diagnostic criteria: i. polycystic ovaries being detected by ultrasound scan (presence of 12 follicles or more in

one or both ovaries and/or increased ovarian volume >10 ml), ii. clinical signs of hyperandrogenism (hirsutism score based on hirsutism score greater than 7 or obvious acne) and/or an elevated plasma testosterone (testosterone >2. 0 nmol/l) (12-13), and iii. having an interval between menstrual periods >35 days and /or amenorrhea, defined as the absence of vaginal bleeding for at least 6 months (i.e.199 days) (14). Exclusion criteria were as follows: diagnoses of non-classical adrenal hyperplasia; thyroid dysfunction or hyperprolactinemia; communication concerns, specifically the inability to speak or listen attentively; previous or current psychiatric diagnosis or using psychiatric medications including antidepressants; and taking any prescription medication (except allergy medications and occasional pain medications) for at least three months before entering the study.

Measures

Sexual function

Female sexual function was evaluated using a detailed 19-item questionnaire [the Female Sexual Function Index (FSFI)] described by Rosen et al. (15). This standardized questionnaire evaluates six domains of female sexual functioning during a four-week period that is identified as desire, arousal, lubrication, orgasm, satisfaction and pain during sexual intercourse. The domain of female sexual arousal disorder is assessed in terms of frequency, level, confidence and satisfaction with eight questions. It is further divided into two separate domains of lubrication (four items) and arousal (four items). These items assess both the peripheral (lubrication) as well as the central (subjective sexual arousal and desire) components. Other domains assessed include pain (three items), orgasm (three items) and satisfaction (three items). A scoring algorithm is applied to each domain and a composite score is obtained. Scores ranged for items 3-14 and 17-19 are 0-5, and for items 1, 2, 15 and 16 are 1-5. By adding the scores of the individual items comprising the domain and by multiplying the sum by the domain factor, individual domain scores are then obtained. Maximum scores for factors are as follows: 0.6 for desire, 0.3 for arousal and lubrication, and 0.4 for orgasm, satisfaction and pain. A total score is obtained by add-

ing the six domain scores. The full-scale score range is from 2.0 to 36.0, with higher scores associated with a lower degree of sexual dysfunction. Women who scored <3.9 for all six domains are identified as sexual dysfunction. In this study, we used the cut-off points of the Persian version translated by Mohammadi et al. Therefore, a score <3.3 in the desire domain, score <3.4 in arousal and orgasm, score <3.8 in satisfaction and pain, score <3.7 in lubrication, and total score <28 were considered as female sexual dysfunction (FSD). Validity and reliability of Persian version of the questionnaire has been well documented (16). In order to carry out the test-retest reliability, a total of 30 patients, randomly selected from the original group, completed again the FSFI two weeks later, in the same manner as the first one. The test-retest reliability of the scale was estimated by intraclass correlation coefficient (ICC). The ICC was satisfactory (0.80, p<0.05).

Clinical symptoms of PCOS

1. Menstrual history: patients were asked to choose the best option indicating their menstruation interval during the preceding 12 months of the following category: <21 days, 21-34 days, 35-60 days, >199 days, and being variable.

2. Reproductive history: women were asked to categorize their reproductive history based on the following criteria: i. having been pregnant: all births, no losses; ii. having been pregnant: some births, some losses; iii. having been pregnant: no births, all losses; and iv. never being pregnant.

3. BMI: weight and height were calculated by the following formula for all participants, weight/height squared (kg/m^2).

4. Body hair: clinical assessment of hirsutism was determined using the Ferriman-Gallwey scoring system (F-G score). Nine body sites (the upper lip, chin, chest, upper back, lower back, upper abdomen, lower abdomen, arm, and thigh) were graded from 0 (no terminal hair) to 4 (severe hirsutism). Scores can range from 0 to 36. A score of 7 or above was considered positive for hirsutism (17).

5. Acne: acne was determined using the Global Acne Grading System (GAGS). The GAGS considers six locations on the face and chest/upper back. The borders on the face are defined by the hairline, jawline, and ears. The score of each location is a factor presenting affected surface area as

well as distribution and density of pilosebaceous units. The chest and upper back are also included because their involvement is critical in order to assess the severity of acne and to decide on treatment option. The score of each location is separately determined in a 0-4 point scale that means the sum of scores belonging to a location (18).

Socio-demographic status

The study used years of formal education as a measure of socioeconomic status that was categorized into five levels: no education, first level (1 to 5 years), second level (6-9 years), third level (10-12 years) and fourth level (more than 12 years). Different studies from Iran showed that education could be a good proxy measure for socioeconomic status of Iranians (19).

Laboratory measures

An overnight 8-hour fasting venous blood sample was obtained from each subject on the second or third day of their spontaneous or progesterone-induced menstrual cycles. Serum total testosterone (TT), sex hormone-binding globulin (SHBG), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were concomitantly assessed in all participants by ELISA (DRG Instruments GmbH, Marburg, Germany). TT and SHBG were used to calculate the free androgen index (FAI) as TT (nmol/l)/ SHBG (nmol/l) ×100, suggesting to be a useful indicator of abnormal androgen status (20).

Data analysis

Data are presented as number (%), unless otherwise indicated. To explore the association between the socio-demographic and PCOS characteristics with sexual function (Table 1), the method of multivariate logistic regression analysis was applied. Odds ratios (ORs) and 95% confidence intervals (CI) were calculated. Independent variables included the PCOS characteristics that were dichotomized, converted and coded as dummy variables. For example, menstrual cycle was converted to 1 that indicates amenorrhea, oligomenorrhea, polymenorrhea, or being variable, or to 0 that indicates the remaining, while the variables remaining in the model were reported. A value of p<0.05 was accepted as significant. Statistical analysis was performed using Statistical Package for the Social Sciences 15.0 (SPSS Inc., Chicago, IL, USA).

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Demographic data	
Age (Y)*	26.56 ± 4.44
**Education **	
The first level	32 (10.7)
The second level	50 (16.7)
The third level	126 (42)
The fourth level	92 (30.7)
Duration of marriage*	10.02 ± 4.20
Parity*	0.51 ± 0.77
Clinical	
Hirsutism score *	6.7 ± 5.73
Acne score *	10.54 ± 7.26
Interval between menstruation (days)	
<21	8 (2.7)
21-34	109 (36.3)
35-60	19 (6.3)
>199	31 (10.3)
Variable	133 (44.3)
**Reproductive history **	
Never being pregnant	193 (64.3)
Having been pregnant: all births, no losses	32 (10.7)
Having been pregnant: some births, some losses	17 (5.7)
Having been pregnant: no births, all losses	58 (19.3)
BMI (kg/m²)	
<25	130 (43.3)
25-30	120 (40)
>30	50 (16.7)
Endocrine	
LH (IU/l)*	8.28 ± 6.16
FSH (IU/l)*	6.09 ± 4.42
Testosterone (nmol/L)*	1.24 ± 0.23
SHBG (nmol/L)*	55.57 ± 43.87
FAI *	10.21 ± 34.45

*PCOS; Polycystic ovary syndrome, BMI; Body mass index, LH; Luteinizing hormone, FSH; Follicle-stimulating hormone, SHBG; Sex hormone-binding globulin, FAI; Free androgen index, *; Mean ± SD and **; N (%).*

Results

Socio-demographic characterize and clinical symptoms

In all, 300 women with PCOS were included in the study during the six-month enrollment. The mean (SD) age of patients was 26.5 (4.44) years. The majority of women had education beyond high school (72.7%, n=218). More than two-thirds of patients had never been pregnant and had not successfully carried a pregnancy to term (n=251), of whom most reported having abnormal menstruation (n=191). Our inclusion criteria were FG score more than >7 and testosterone level >2. However, our findings showed that the mean values of FG score and testosterone were 6.7 and 1.24, respectively. According to Rotterdam criteria, having two of the diagnostic criteria is enough. In other word, if a patient complains of irregular menstrual cycles and her sonography results also indicates polycystic ovary, it is considered as a common case of PCOS. For this reason, the mean scores of hyperandrogenism are lower than inclusion criteria. Socio-economic and clinical characteristic of the patients are presented in table 1.

FSFI subscale's scoress

Fig 1 presents a summary of the mean scores of the six subscales, indicating FSFI, while the overall prevalence of FSD is 16.66% (n=50/300). The items are arranged from highest to lowest scores as follows: i. desire (48.3%, n=145/300), ii. arousal (44.7%, n=134/300), iii. pain (39%, n=183/300), iv. lubrication (21.3%, n=64/300), v. orgasm (15%, n=45/300), and finally vi. satisfaction (13%, n=39/300).

Factors contributing to sexual dysfunction according to logistic regression

Multiple logistic regression analysis suggested a positive association between FSD and menstrual disturbance (OR: 4.61; 95% CI: 1.93-11). In other word, women with menstrual irregularities reported higher levels of sexual dysfunction when compared to PCOS women with regular menstruation cycles. Moreover, FSD was significantly higher in the presence of low level of education (OR: 2.94; 95% CI: 1.46-5.92, Table 2).

Error bars: 95% CI
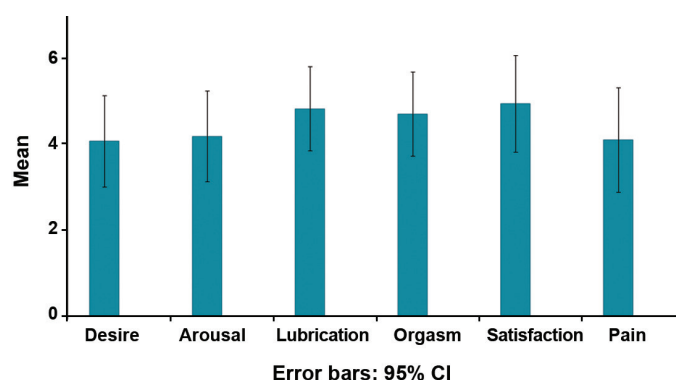
Fig 1: Mean FSFI scores of subscales in PCOS patients.

Table 2: Logistic regression analysis including socio-demographic and clinical symptoms predicting FSFI score among PCOS patients

Independent variables†	OR (95% CI)	SE	P value
Education	2.94 (1.46-5.92)	0.35	0.002
Menstrual	4.61 (1.93-11)	0.44	0.001

†; Age, F/G and acne scores, duration of marriage, parity, endocrine profile and BMI were included in the regression analysis as continuous variables and other variables were used as dummy variables. Only significant results are presented. FSFI; Female sexual function index, PCOS; Polycystic ovary syndrome, CI; Confidence interval and OR; Odds ratio.

Discussion

Since PCOS often manifests itself through marriage and having sexual activity, its psychosexual implications are found to cause profound emotional distress in affected women (21). The concept of sexual problems has not been discussed in Iranian PCOS patients. The objective of this study was to further examine the impact of PCOS on female sexual function in an Iranian population sample and to identify potential demographic and patient-related risk factors for FSD.

Given the effect of PCOS on women's physical health and emotional well-being, it is undoubted that a substantial proportion of patients reported sexual impairment and problems. In our study, the overall prevalence of FSD was 16.66%. The most significantly influenced domains in these participants were in line with the results of Aslan et al. (22) about desire and arousal. Sexual arousal as a separate component of the sexual response cycle was first recognized by Kaplan (23), while arousal problems are often considered to be attributed to inhibited desire that may occur independently (24). The changes that occur in a woman's physical appearance as a result of PCOS, particularly hirsutism, acne and obesity, along with menstrual irregularity and infertility, have been found to be a leading cause of psychological morbidity (9, 25-31). Moreover, psychological inhibition may result in inadequate vaginal lubrication and cause coital pain. Almost 39% of the women reported having pain during intercourse, while sexual aversion, inconvenient relationship effects, and development of additional sexual dysfunction were also reported. The pain may prevent intromission, while considering to prevalence of infertility in this population, the importance of this issue is obvious.

In this study, 15% of the studied sample reported orgasm disorder. Depression, poor body image and low self-esteem were frequently seen in PCOS patients (32-36), indicating as a main cause of orgasm disorders in these women. In our study, 13% of women experienced a lack of satisfaction with their sexual relationship. In a study by Hahn et al. they assessed the quality of life (QOL), psychological well-being and sexual satisfaction of 120 patients with the diagnosed polycystic ovary syndrome and showed a subjective deterioration of general well-being as well as an increase of psy-

chological disturbances and sexual problems in women with PCOS (2). In another study by Elsenbruch et al. (9) they observed that the manifestations of PCOS, such as infertility, hirsutism, acne, and obesity, lead to reduction in QOL and to serious limitation of sexual satisfaction.

In the present study, we further determined factors that may influence sexual function in PCOS women. Of the socio-demographic data analyzed, education level showed strong correlation with the likelihood of FSD. Lower educational levels are positively associated with the presence of sexual dysfunctions, as also shown by similar findings of studies conducted in Turkish, Africa, and USA (37-41).

In the current study, women with menstrual irregularities reported higher levels of sexual dysfunction when compared to PCOS women with regular menstruation cycles. A negative effect of menstrual problems on the quality of life of patients has been previously discussed by other authors as well (28). Menstrual irregularities can have important social consequences, especially for Muslim women. For example, it is forbidden for a menstruating woman to perform many religious activates, like prayer; therefore, prolonged bleeding disrupts household patterns in such a way that family and community members may become aware of a woman's situation if her period persists for more than the expected number of days (42, 43). Menstrual irregularities may also have adverse consequences for women's intimate relations and for other aspects of their reproductive and general health. For example, in Islam, man is forbidden to have intercourse with his wife during her menses as in Judaism and Zoroastrianism.

Surprisingly, we did not find any association between FAI levels and FSFI domain scores. Our results are consistent with the findings by Davis et al. (44), indicating no association between low sexual domain scores and low free testosterone serum levels. Thus the hyperandrogenism characteristic of PCOS does not predict satisfactory sexual functioning in our sample, even though endogenous testosterone is known to play an important role in this regard in normal women (45, 46). In contrast, a previous study in non PCOS women showed that a positive correlation between higher testosterone level and ability to achieve an orgasm in women of reproductive age (47). It is possible that a positive

association between androgen levels and satisfying sexual functioning is masked by the effects of the PCOS phenotype on self-esteem, which is crucial to sexual functioning. It is well known that the hyperandrogenic phenotype deleteriously affects the emotional condition of patients with PCOS. Such symptoms may be associated with reduced sexual and body image satisfaction (30, 48). In addition, a negative self-image, a higher BMI, depression, sexual dysfunction, reduced lubrication, and lower sexual excitement have been reported in women with higher testosterone levels (2).

As FSD is known as a common health problem in PCOS women, some controversy exists concerning the prevalence of FSD, while unique national, religious and cultural variations may contribute to risk factors of FSD. However, a thorough evaluation between different studies is affected by the lack of a uniform validated FSD questionnaire, setting, definition of FSD characteristics of the study population and the method of evaluation. A main methodological problem is use of the internationally accepted FSD questionnaires. At present, the FSFI is the most commonly used FSD questionnaire that has acceptable reliability and validity (16). Strengths of the current study were excellent response rate and verification of findings by gynecology physicians. We evaluated sexual function in married Iranian women with PCOS using a standardized questionnaire and a collection of questions designed for studying characteristics of PCOS, specifically. Despite the importance of the present findings, this study has some limitations. Firstly, we were not able to include a matched control group because of difficulties in screening and diagnosis of PCOS, as discussed above.

The current results, therefore, are applicable to identify the differences within the PCOS population. Secondly, the data were collected from a married Iranian patient sample; therefore, the findings should not be extrapolated to the general population and need to be studied in larger sample size. Thirdly, we were not able to determine the direction of causality between our variables. Moreover, all included patients in this study were married for cultural reasons (sex and infertility) in Iran. Additional prospective researches are needed to investigate the link between infertility and FSD and to determine the relationship between other known risk factors and sexual function.

Conclusion

This is a pioneer study in Iran investigating sexual problems in women with PCOS reporting sexual dysfunction, accounted as one-fifth of total participants. Desire and arousal disorders were the most common sexual dysfunction reported by Iranian women with PCOS. Our finding revealed that subjects with limited or no formal education and a history of menstrual irregularities reported greater sexual dysfunction using the FSD scale. In order to determine the causes of FSD, the topic needs further exploration involving intervention at regular health care visits. Clinician should consider religious and cultural background of their patients, especially in view of the factors influencing FSD.

Acknowledgements

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Original Article

The Study of Sexual Satisfaction in Iranian Women Applying for Divorce

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Abstract————

Background: Marital instability is affected by many factors. In Iran, socio-cultural and political limitations are obstacles for sexuality-related studies; therefore, insufficient information is available in this area. In the present research, we investigated the relationship between marital instability and sexual satisfaction among Iranian women.

Materials and Methods: A case-control study was carried out to investigate women applying for divorce in comparison with our controls during 2011 to 2012 in Isfahan, Iran. Data gathering was done using a questionnaire including two parts: socio-demographic information and factors influencing sexual satisfaction. Larson Inventory of Sexual Satisfaction for determining sexual satisfaction was used to determine sexual satisfaction.

Results: Divorce rate is significantly related to sexual satisfaction (p=0.009). There were also significant relationships between sexual satisfaction and the following variables: age, economic status, amount of income, duration of marriage, number of children, housing, alcohol/drug abuse by spouse, being beaten by spouse, compulsory marriage, second marriage of spouse, and being happy with current partner.

Conclusion: Sexual satisfaction plays an important role in marital stability of Iranian women. Thus, development of practical strategies in order to provide cultural intervention is needed to improve Iranian couples' awareness of their sexual relationship. Indeed, trainings in communication skills through sexual encounters are essential.

Keywords: Sexual, Divorce, Women

Introduction

Sexuality is an important part of the whole person, while is considered as integral component of health and general well-being in order to have better quality of life (1, 2). Sexuality affects individual's social life by influencing his/her behavior toward himself/herself, his/her sexual partner, and all other people (1, 3). Satisfaction of these sexual desires has a determinant role in order to form the human personality, while separation of these desires from any human behavior is inevitable (3).

Sexual satisfaction as a pleasurable feeling resulting from individual behaviors and interpersonal interactions is defined as judgment and analysis of one's own sexual behavior that is unlike some sources considering sexual satisfaction as a means of "orgasm" (4).

Sexual satisfaction is one of the necessities for a strong and sustainable marital relationship, and is correlated with mental health, general happiness, professional achievements and successful social interactions (5).

Sexual satisfaction is affected by different factors like job stress, couples' struggles, education level, cultural influences, economic problems, moral

and sexual consistency, and physical and mental diseases (3).

Sexual dissatisfaction leads to negative mental and spiritual effects, like disappointment, depression, insecurity, unhappiness, as well as spiritual, mental and personality imbalance. These complications result in diminished ability and creative power, serious conflicts, as well as negative emotions as annoyance, jealousy, competition to suppress each other, lack of self-confidence and being ignored (3, 6). Several studies have revealed that sexual dissatisfaction is the primary cause of nearly 80% of marital conflicts, of which 61.4% would later end in divorce (4, 5). However, evaluating and speaking about these problems is usually neglected because of feeling modest, shy, afraid, anxious, embarrassed, inefficient and sinful. These wrong thoughts develop in a way that some women consider "initiating sexual relationship" as losing their chastity and personality. They do not consider the joy of sex as their own right and suffer from having sex (3). These unhealthy relationships between couples are aggravated through tensions and conflicts and will widen the gap between couples, leading to unstable family foundation and increased risk of divorce (6-8).

Moreover, in a number of societies like Iran, marital conflicts and divorce are considered as one of the social inconveniences, which create severe mental tensions. Besides, divorce is not a simple solution for the women, while the husbands who are disrupted mentally and psychologically resist divorcing their wives (4).

Sexuality issues remain a taboo in Iran. Furthermore, according to the records of judicial institutions, the divorce has been increasing at an alarming rate since 2001. Moreover, some studies in this regard showed that sexual dissatisfaction have psychopathology impact on couples and are experienced by many of them who get divorced (4, 9-12). Therefore, performing this study was necessary in order to evaluate prevalence and level of sexual satisfaction in women applying for divorce and to compare it with a control group.

Materials and Methods

This case-control study included 65 randomly selected women applying for divorce in Isfahan Center of Legal Medicine Organization, Isfahan, Isfahan Province, Iran, during 2011 to 2012. Furthermore, the control group was comprised of 65 randomly selected females from normal population. Age and length of marriage were two factors used for case-matching. This study was approved by Ethic Committee of Legal Medicine Research Centre, Tehran, Iran. All participants also gave an informed written consent. Inclusion criteria were as follow: fertile females older than 18 years old, ability to give informed consent, ability to provide enough information, and being healthy. Exclusion criteria were as follow: chronic medial disease affecting sexual satisfaction, unable to provide enough information, unable to sign informed consent, infertility problem, and females younger than 18 years old.

A female physician helped all participants to fill out a questionnaire designed for this purpose that included two parts: socio-demographic information and factors influencing sexual satisfaction. In order to clarify variables leading to divorce, Larson Inventory of Sexual Satisfaction (ISS) was used to determine sexual satisfaction.

In Iranian population, ISS was previously used as a tool for evaluation of sexual satisfaction with good validity and reliability. The standard questionnaire has 32 items with 16 negative and 16 positive items. Iranian version of ISS with good validity and reliability consists of 25 items (see appendix A) and includes a 5-option Likert scale as follows: never, rarely, sometimes, often, and always (in a 0-4 score range). Based on the scores obtained, each group was placed into four sub-groups: completely satisfied (101-128), relatively satisfied (76-100), slightly satisfied (50-75), and dissatisfied (<50) (12, 13).

Data were gathered without recording names or any other identifying information to keep patient information confidential. Then, data were analyzed using independent t test (for comparing quantitative variables between groups), Chi-square test (for comparing qualitative variables between groups), Fisher's exact test (for comparing quantitative variables when the anticipated number was <5), and Pearson correlation coefficient (for determining relationship between quantitative variables). All analyses were conducted using the Statistical Package for the Social Sciences (SPSS; SPSS Inc., Chicago, IL, USA) version 16.0 and a p value <0.05 was considered statistically significant.

Results

Comparison and statistical evaluation of socio-demographic information and factors influencing sexual satisfaction are detailed in table 1.

Table 1: Comparison and statistical evaluation of socio-demographic information and factors influencing sexual satisfaction

Parameters	Groups Case	Control	P value	
Age (Y)	29.6 ± 6.51	35.31 ± 10.7	0.000	T student
Age of spouse (Y)	35.02 ± 7.41	40.74 ± 12.01	0.001	T student
Age difference between spouses (Y)	5.42 ± 4.31	5.46 ± 3.6	0.948	T student
Education level N (%)				
Illiterate	0	0	0.129	Fisher exact test
First to fifth grade	2 (3.1%)	7 (10.8%)		
Sixth to eighth grade	7 (10.8%)	12 (18.5%)		
High school diploma	43 (66.2%)	29 (44.6%)		
Associate's degree	2 (3.1%)	1 (1.5%)		
Bachelor's degree	10 (15.4%)	13 (20%)		
Post graduate degrees	1 (1.5%)	3 (4.6%)		
Education level of spouse N(%)			0.086	Mann-Whitney
Illiterate	0	0		
First to fifth grade	10 (15.4%)	4 (6.2%)		
Sixth to eighth grade	17 (26.2%)	13 (20%)		
High school diploma	31 (47.7%)	28 (43.1%)		
Associate's degree	1 (1.5%)	2 (3.1%)		
Bachelor's degree	4 (6.2%)	13 (20%)		
Post graduate degrees	2 (3.1%)	5 (7.7%)		
Employment status N (%)			0.420	Fisher's exact test
Housewife	44 (67.7%)	47 (72.3%)		
Student	5 (7.7%)	5 (7.7%)		
Worker	1 (1.5%)	0		
Employee	7 (10.8%)	10 (15.4%)		
Unemployed	8 (12.3%)	3 (4.6%)		
Employment status of spouse N(%)			0.193	Fisher's exact test
Housewife	9 (13.8%)	3 (4.6%)		
Student	1 (1.5%)	0		
Worker	11 (16.9%)	14 (21.5%)		
Employee	12 (18.5%)	19 (29.2%)		
Unemployed	32 (49.2%)	29 (44.6%)		
Economic status N(%)			0.004	Mann -Whitney
Good	9 (13.8%)	17 (26.2%)		
Intermediate	37 (56.9%)	43 (66.2%)		
Poor	19 (29.2%)	5 (7.7%)		

Parameters	Groups		P value	
	Case	Control		
Amount of income N(%)			0.011	Mann -Whitney
High	2 (3.1%)	6 (9.2%)		
Sufficient	35 (53.8%)	46 (70.8%)		
Insufficient	28 (43.1%)	13 (20%)		
Length of acquaintance prior to marriage (Y)	9.63 ± 30.16	8.96 ± 32.20	0.906	T student
Age at time of marriage (Y)	20.94 ± 4.59	21.56 ± 6.11	0.516	T student
Age of spouse at time of marriage (Y)	26.35 ± 5.96	27.02 ± 6.12	0.532	T student
Duration of marriage (Y)	8.66 ± 6.45	13.75 ± 13.23	0.006	T student
Number of children N(%)			0.010	Mann -Whitney
Zero	25 (38.5%)	14 (21.5%)		
One	21 (32.3%)	14 (21.5%)		
Two	15 (23.1%)	20 (30.8%)		
Three	2 (3.1%)	9 (13.8%)		
More	2 (3.1%)	8 (12.3%)		
Residence N (%)			1.000	Fisher exact test
City	62 (95.4%)	61 (93.8%)		
Village	3 (4.6%)	4 (6.2%)		
Housing N (%)			0.000	Fisher exact test
On lease	36 (55.4%)	15 (23.1%)		
Owned	13 (20%)	40 (61.5%)		
Her parent's house	8 (12.3%)	3 (4.6%)		
Her spouse's parent's house	8 (12.3%)	6 (9.2%)		
Others	0	1 (1.5%)		
Having a special bedroom N(%)			0.192	Chi-square test
Yes	40 (61.5%)	47 (72.3%)		
No	25 (38.5%)	18 (27.7%)		
Living with other(s) N (%)			0.120	Fisher exact test
Yes: her parents	13 (20%)	4 (6.3%)		
Her spouse's parents	6 (9.2%)	7 (10.9%)		
Others	2 (3.1%)	1 (1.6%)		
No	44 (67.7%)	52 (81.3%)		

	Groups			
Parameters	Case	Control	P value	
Any types of addiction N (%)			1.000	Fisher exact test
Yes	0	1 (1.5%)		
No	65 (100%)	64 (98.5%)		
Drug abuse by spouse N (%)			0.000	Chi-square test
Yes	24 (36.9%)	1 (1.5%)		
No	41 (63.1%)	64 (98.5%)		
Alcohol abuse by spouse N (%)			0.000	Fisher exact test
Yes	21 (32.3%)	0		
No	44 (67.7%)	65 (100%)		
Being beaten by spouse N (%)			0.000	Fisher exact test
Yes	31 (47.7%)	0		
No	34 (52.3%)	65 (100%)		
Past medical history N (%)			0.233	Chi-square test
Positive	8 (12.3%)	13 (20%)		
Negative	57 (87.7%)	52 (80%)		
PH of spouse N (%)			0.393	Chi-square test
Positive	16 (24.6%)	12 (18.5%)		
Negative	49 (75.4%)	53 (81.5%)		
Sexual problems of spouse N (%)			0.027	Chi-square test
Positive	9 (13.8%)	2 (3.1%)		
Negative	56 (86.2)	63 (96.9%)		
Compulsory marriage N (%)			0.008	Chi-square test
Yes	13 (20%)	3 (4.6%)		
No	52 (80%)	62 (95.4%)		
Second marriage of spouse N (%)			0.000	Fisher exact test
Yes	13 (20%)	0		
No	52 (80%)	65 (100%)		
Being happy with current partner N (%)			0.000	Chi-square test
Yes	29 (44.6%)	5 (7.7%)		
No	36 (55.4%)	60 (92.3%)		
Sexual satisfaction score*	55.48 ± 10.14	61.03 ± 13.47	0.009	T student

Data are means ± SD.

As shown, the mean total scores were 55.48 ± 10.14 and 61.03 ± 13.47 in case and control groups, respectively, indicating a significant difference between two groups.

Assessing sexual satisfaction in detail revealed that our cases had experienced lower levels of satisfaction than controls. Notably, the majority percentages in each group were seen in the slightly satisfied level that is opposite to the completely satisfied level from the sixty-five people evaluated in our case group (Table 2). In the case group, 40 (61.5%) and 25 (38.5%) individuals indicated "sexuality worsening" and "without any changes in sexual pattern", respectively, whereas in the control group, 14 (21.5%) and 39 (60%) individuals indicated "sexuality worsening" and "without any changes in sexual pattern", respectively, suggesting significant differences between two groups (p=0.000).

By comparing sexual satisfaction scores with other mentioned factors, we found that having a special bedroom (p=0.037) and having past medical history (PMH) (p=0.011) have significant effects on sexual dissatisfaction.

Significant differences were seen between groups in the following items: "My spouse enjoys having sex with me" (p=0.039), "I have an attractive and exciting sex life" (p=0.002), "My spouse is a good partner for our sexual activities" (p=0.000), "Sexual activity manifolds kind feelings between us" (p=0.001), "My spouse avoids having sex with me" (p=0.005), "Sexual activity with my spouse results in at least one orgasm for me" (p=0.040), "My spouse is very sentimental about my sexual needs and inclinations" (p=0.014), "My spouse cannot make me feel sexually fulfilled after sex" (p=0.004), "I am satisfied with my spouse's special manner of making love" (p=0.000), "My spouse and I try to find the way to have pleasurable sexual activities" (p=0.008), "I have not been interested in participating sexual activities with my spouse in the preceding months" (p=0.016), "I am satisfied with my sex life" (p=0.000), and "I know myself as a successful person in having sex with my spouse" (p=0.035).

Table 2: Frequency distribution of sexual satisfaction within groups

Level of sexual satisfaction	Groups Frequency (percent)		P value
	Control	Case	
Completely satisfied	0	0	0
Relatively satisfied	7 (10.8%)	2 (3.1%)	
Slightly satisfied	48 (73.8%)	43 (66.2%)	
Dissatisfied	10 (15.4%)	20 (30.8%)	0.041

Discussion

Our data demonstrated a decline in divorce rate by longer marital life. Such a decline can be explained that as the years pass, martial satisfaction is to some extent and woman thinks less about sexual activities. As an individual grows older, he/she obtains various experiences and skills for confronting or coping with problems, so individual's expectations change toward marital satisfaction, This may help couples in overcoming their issues regarding sexual desire, sexual performance, and frequency of sexual activity, while undo the negative effects of confounding items in order to have sexual satisfaction (3).

This is not in accordance with the findings of Shahsiah et al. (13), in which they highlighted the role of sexual satisfaction. They explained that at the beginning of a married life, sexual motivations hide marital concerns, like spousal disputes, blaming each other, economic problem, housing, raising children, etc. However, as time passes, problems have accumulated to the point where marriage starts falling apart.

We found a negative significant relationship between the number of children and divorce rate. Raising children in a family requires wife and husband to spend more time and energy as the result of their additional supportive role as mother and father; therefore, they are subjected to motivations that lead to dangerous behavior and that result in collapse of their marital commitment (3).

Nassimi and Mahdavi showed that there was a positive significant relationship between education and number of children with sexual satisfaction and there was a negative significant relationship between age and sexual satisfaction (14).

Nevertheless, these findings are in disagreement with those of Green et al. and Glazier et al. (15, 16), who explained woman with higher educational level and with monetary independence feels to be useful, whereas the likelihood of divorce and displeasure relating to life will be increased.

Groot et al. (17) suggested that socio-cultural similarities (e.g. the spouse's age diversity) cause more secure families due to similarities in their life styles and mutual understanding among partner, especially in sexual issues. Their results showed that those with an age difference less than 10 years were more fulfilled with both their marital and sexual life. On the contrary, Litzinger and Gordon (18) found that there is no significant relationship between sexual satisfaction and age difference among spouses.

A consistent or contradictory relationships are affected by factors like education level and age difference between spouses, whereas few studies evaluating the factors like younger age at time of marriage, low education, premarital pregnancy, short premarital acquaintance, personality maladjustment, and low socioeconomic conditions indicated no significant relationships.

Our results showed that divorce rate was significantly more in drug addict spouse. Increasing marital instability due to irresponsible behaviors of individual abusing drug or alcohol in family and society affect marital relationships, indicating relationship between addiction and sexual satisfaction is not significant(4), as shown in this study.

And finally, we tried to evaluate the pivotal role of sexual satisfaction in marital stability of Iranians using a questionnaire; however, participants were likely to respond with considerable bias or to answer in a socially desirable manner. Also, the participants' hesitation to share their private marital relationships should be considered.

Furthermore, we pointed out undesired level of satisfaction seen in our participants as a sample of women living in city of Isfahan Through comprehensive questionnaire and female physician helped us to overcome some of these obstacles. It is impossible to assess the extent to which participants answered truthfully, and the extent to which they prevaricated.

Conclusion

Our findings suggest that sexual satisfaction plays a pivotal role in marital stability of Iranians. Therefore, development of practical strategies in order to provide cultural intervention is needed to improve Iranian couples' awareness of their sexual relationship, as well as training in communication skills through their sexual encounters are essential. Since in this study, sexual dissatisfaction was revealed to be an underlying problem leading to divorce, sex education of couples before marriage seems to be of importance. We recommend conducting other prospective studies after education

of couples to evaluate the relationship between sexual satisfaction and divorce rate in Iranian population.

Acknowledgements

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Original Article

A Survey on Oocyte Donation: Turkish Fertile and Infertile Women's Opinions

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Abstract

Background: There are various treatment options for infertility, and new techniques are also being developed as it is an important healthcare problem affecting approximately 15-20% of married couples. The purpose of this study was to evaluate the level of information of fertile and infertile Turkish women on oocyte donation in order to understand their awareness of the legal, ethical, social and religious issues regarding this technique and to compare these two groups in terms of these variables.

Materials and Methods: This cross-sectional study included infertile women being treated at the assisted reproductive technologies (ART) program of a university hospital and women who had presented at the gynecology outpatients department of the same university for routine check-ups and who had no previous history of infertility. After consulting with specialists in the field and searching the related literature, a data collection form having 22 questions for infertile women and 18 questions for fertile women was prepared.

Results: The women were asked whether they would use the oocytes of another woman if necessary. The results showed that 67.6% of the fertile women said they would never want to use this method, while 63.9% of the infertile women stated they may accept to use this method under certain conditions (two distinct answers appeared in the answers, some women stated they would prefer donated oocytes from close relatives, while others stated they would prefer oocytes from total strangers), such as from a close relative or from someone they do not know at all.

Conclusion: Infertile women mentioned that they could use illegal routes if necessary to have a child at much higher rates than stated by fertile women. This shows that desire to have a child is a strong source of motivation in Turkey.

Keywords: Third-Party, Assisted Reproduction, Infertility, Oocyte Donation

Introduction

Childbearing is an important goal for marriage and seen as a vital means of stability and satisfaction in married life in many Islamic societies. Thus, being unable to fulfill this pri¬mary goal, an infertile couple is highly likely to be regarded as a failure (1-3). Within this context, infertility is considered as an important public problem which may affect the spouses' relationships or even threaten their marriage. In addition, the social environment aggravates the situation even further by bringing the couple face to face with society's expectations (1).

Currently, there are various treatment options for infertility, and new techniques are also being developed as it is an important healthcare problem affecting approximately 15-20% of married

couples (4-9). There are a number of different assisted reproduction modalities, some of which involve a third party, such as gamete or embryo donation or surrogate motherhood. Women with ovarian failure were considered irreversibly sterile until approximately 20 years ago, but advances in assisted reproductive technologies (ART) have changed that view. Oocyte donation today offers women with premature ovarian failure or a rapidly diminished ovarian reserve (DOR), a very realistic chance of pregnancy. Current oocyte donation is commonly achieved by *in vitro* fertilization (IVF) using the oocytes retrieved from healthy young donors after controlled ovarian hyperstimulation and the sperm of the recipient's partner, with the resulting embryos then transferred to the uterus of the recipient. A successful pregnancy established in a recipient woman using donated oocytes was first reported in 1983. Since then, oocyte donation has become a logical extension of assisted reproduction technology (10, 11).

The practice of oocyte donation involves ethical, social, religious, psychological and medical issues. Scant attention has been given to the medical risks of oocyte donation. The risks of oocyte donation mean that special scrutiny should be paid to the treatment of oocyte donors during the donation process. There are several side effects and long term risks that may be associated with being an oocyte donor, which include pain, infection, bleeding as a result of the oocyte retrieval process, premenstrual syndrome like symptoms, ovarian hyperstimulation syndrome (OHSS), and a controversial risk of ovarian cancer from the medications the donor uses. The risks of oocyte donation necessitate the inspection of the treatment of oocyte donors during the donation process (12, 13).

Treatment by oocyte donation, as one of the most contentious issues surrounding assisted reproduction, elicits active debate within many societies with regard to its moral, ethical and religious aspects (8, 14-19). Oocyte donation could be more complicated in Islamic societies where some may even believe that third party reproduction is not permissible under Islamic rules or forbidden by the legislation law. Many countries have passed necessary legal regulations in accordance with their own values and beliefs and started oocyte donation. The rights of the donor, the recipient couple and the child have been determined by law in the countries where the procedure is permitted (15, 16, 20-25). Islam is the dominant religion in Turkey and almost 99% of the population is Muslim (1). According to the law enforcement act on assisted reproduction treatment, only sperm and oocytes obtained from married couples can be legally used in Turkey. Sperm donation, embryo sharing and surrogate motherhood are all forbidden as well as oocyte donation. There are few studies reflecting the opinion of Turkish people toward oocyte donation (17, 26, 27).

At present, there are approximately 120 fertility clinics operating in Turkey. Some of the fertility clinics are sited in public or university hospitals, but the majority of them are established in private hospitals or operate as independent centers. In March 2010, a new version of the regulations, the "Legislation Concerning Assisted Reproduction Treatment Practices and Centers", was introduced in Turkey. In the revised legislation, a number of new restrictions have been declared including limitations regarding the licensing of private IVF centers, specifications on gamete and embryo storage and restrictions on the number of embryos that can be transferred to a patient [only one for women aged under 35 in their first and second cycle of IVF, and a maximum of two embryos for women in their third or subsequent cycles or over 35 years of age (28)].

The use of donor eggs, sperm, or embryos is more of a social or cultural problem than a medical one (29-30). Legal arrangements regarding oocyte donation are, therefore, subject to the cultural beliefs and opinions of the majority of the community about the procedure, individuals are free to use a legal procedure according to their own values. However, the fact that it is illegal can prevent those who want to use it and can push them towards using illegal routes (17, 26, 31). Law makers and legislative bodies define laws in a way that reflects the opinion of the majority. We believe that the opinions of the infertile couples are the most important in this matter. Infertile couples strive to find solutions for both infertility and associated negative effects, and therefore try many treatment options. At this point, we feel that infertile women's opinions on oocyte donation must be taken into account.

The purpose of study was to evaluate the level of

information of fertile and infertile Turkish women on oocyte donation in order to understand their awareness of the legal, ethical, social and religious issues regarding this technique and to compare these two groups in terms of these variables.

The study intended to provide answers to the following questions:

1. What are the opinions of fertile and infertile women regarding oocyte donation and the legal arrangement in Turkey?
2. Would fertile and infertile women accept being an oocyte donor or recipient?
3. Are fertile and infertile women aware of the legal, ethical, religious and social aspects of oocyte donation?

Materials and Methods

This cross-sectional study was carried out between October 2008 and January 2009 in Ankara, Turkey. Women applying to Gulhane Military Medical Academy gynecology and infertility clinics who were willing to participate and who met the inclusion criteria were included. The inclusion criteria for fertile women were: having conceived spontaneously and having no other gynecological problems, while for infertile women, being under treatment in ART outpatient clinic and having no other additional gynecological problems.

A data collection form was developed by researchers after evaluation of the relevant literature (5, 26, 31). The validity of the content was examined and approved by experienced infertility professionals (the chief of the outpatient ART clinic, the nurse of the outpatient ART clinic, an academic staff who has worked on psychosocial aspects of infertility, and a researcher who is under infertility treatment herself) to confirm the study's general appropriateness and applicability. The questionnaire consisted of 22 questions for infertile women and 18 questions for fertile women identifying the women's socio-demographic characteristics (age, level of education, and occupation), history of infertility, and knowledge and opinions about oocyte donation.

In the questionnaire, women were first asked if they had previously heard about oocyte donation, and those who had heard about it were asked to define the procedure. This was done to verify the actual knowledge level of women about the sub-

ject. Later, all were informed about the oocyte donation and women then answered the rest of the questionnaire.

The prepared questionnaire was first administered to 10 fertile and 10 infertile women as a pilot study to ascertain whether the items could be easily understood. Since no problems were detected/reported by women in the pilot stage, the questionnaire was used as is.

The women were provided information on the study in small groups at the waiting hall and those who consented to participate were taken to another room to fill in the data collection forms with face-to-face interviews. A total of 97 infertile women that attended the ART program of Gulhane Military Medical Academy, *In Vitro* Fertilization Center and 105 fertile women with no previous history of infertility were included within the scope of the study.

Ethical consideration

A detailed report about the study, including the purpose, possible benefits, methods and data collection means, is presented to the Gulhane Military Medical Academy Ethical Committee. Our study was then approved by this ethical committee. All participants were informed and their oral and written consents were taken. After their consent, all participants were interviewed by the researcher for about 20 minutes each, and filled the data collection forms.

Statistical analysis

The data were analyzed using the "Statistical Package for Social Sciences" (SPSS) version 15.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics, such as frequency, percentages, means and standard deviations were used to describe the sample and main variables. The appropriateness of the variables (age, duration of marriage and monthly income) was checked by a single sample Kolmogorov-Smirnov test and found to have normal distribution. Chi square test and indepent-samples t test were used to compare infertile and fertile women. Values of p less than 0.05 were considered statistically significant.

Results

The socio-demographic characteristics of the

women are presented in table 1. In table 2, it can be seen that the subject of oocyte donation had previously been heard by 54.6% of the infertile and 41.9% of the fertile women. However, only 40.2% of the infertile women and 18.1% of the fertile women could correctly define "oocyte donation". There was a statistically significant difference between the two groups (p=0.000, χ^2=12.04).

The fact that oocyte donation is illegal in Turkey was known by 70.1% of the infertile women and 50.5% of the fertile women. There was a statistically significant difference between the infertile and fertile women regarding their knowledge on illegality of oocyte donation in Turkey (p=0.003, χ^2=8.08, Table 2). Both fertile and infertile women felt that current legal arrangement for oocyte donation in Turkey was appropriate at a rate of 71.4 and 57.7%, respectively.

The percentage of women who did not want oocyte donation to be legal in Turkey under any circumstances were 50.5 and 44.3% for the infertile and fertile women, respectively (Table 2).

The women were asked whether they would use the oocytes of another woman if necessary to have a child. About 67.6% of the fertile women said they would never want to use this method, while 63.9% of the infertile women stated that they may want to use this method under certain conditions (such as from a close relative or from someone they do not know at all). The difference between the fertile and infertile women was significant (p=0.000, χ^2=20.10, Table 2).

The women were then asked whether they would donate oocytes for someone else if necessary. About 58.1% of the fertile women said they would never want to make a donation, while 55.7% of the infertile women said they may donate their oocytes under certain conditions. The difference between the fertile and infertile women was significant (p=0.03, χ^2=3.82, Table 2).

Table 1: The sociodemographic characteristics of the women

	Fertile women N=105 $\overline{X} \pm SD$		Infertile women N=97 $\overline{X} \pm SD$		P	t
Women's age (Y)*	32.41 ± 6.19		30.62 ± 3.88		0.016	2.43
Duration of marriage (Y)*	9.25 ± 6.29		6.44 ± 3.37		0.000	3.91
Monthly income (Turkish Lira)*	2768.47 ± 1141.80		1982.29 ± 687.07		0.000	5.87
	N	%	N	%	P	χ^2
Educational status**						
Primary education	4	3.8	13	13.4		
High school	41	39.0	57	58.8	0.000	19.60
University- or higher	60	57.1	27	27.8		
Employment status**						
Working	75	71.4	35	36.1	0.000	25.39
Not working	30	28.6	62	63.9		

*; Independ-samples t test and **; Chi Square test were used.*

Table 2: Fertile and infertile women's knowledge about oocyte donation and its practicabilty in Turkey

	Fertile women N=105		Infertile women N=97			
	N	%	N	%	P	χ²
Knowledge about oocyte donation						
Heard	44	41.9	53	54.6	0.047	3.27
Not heard	61	58.1	44	45.4		
Defining "oocyte donation"						
Defines correctly	19	18.1	39	40.2	0.000	12.04
Can not define	86	81.9	58	59.8		
Knowledge on illegality of oocyte donation in Turkey						
Thinks oocyte donation is legal	-	-	-	-		
Thinks oocyte donation is illegal	53	50.5	68	70.1	0.003	8.08
No opinion	52	49.5	29	29.9		
Opinions related to current rules and legislation that prohibit oocyte donation						
Oocyte donation must be kept illegal	75	71.4	56	57.7	0.29	4.15
Rules and legislation on oocyte donation must be revised	30	28.6	41	42.3		
Considering oocyte donation's legality in the future						
Never	53	50.5	43	44.3	0.23	0.76
Yes	52	49.5	54	55.7		
Acceptance of utilizing another woman's oocytes to have a child if necessary						
Never	71	67.6	35	36.1	0.000	20.10
Under some circumstances (Oocytes of a close relative or from a person whom she does not know)	34	32.4	62	63.9		
Desire to be an oocyte donor for someone else if necessary						
Never	61	58.1	43	44.3	0.035	3.82
Under some circumstances (For a close relative or a person whom she does not know)	44	41.9	54	55.7		

Chi Square test was used.

In table 3, it can be observed that, both fertile and infertile women stated that oocyte donation may be accompanied by legal, ethical, social and religious problems. The most common (64.8% of the fertile and 41.2% of the infertile women) concern regarding these problems was the emergence of the donor in the following years for financial or emotional demands from the family or the child. The other concerns were the fear of a consanguineous marriage later in life since the biological mother is not known (51.4% of the fertile and 39.2% of the infertile women), the genetic features of the biological mother remaining unknown for both the couple and the child to be born (43.8% of the fertile and 35.1% of the infertile women), and the possibility of elderly couples having children with this method (33% of the fertile and 12.4% of the infertile women).

The percentage of women who stated they would choose adoption in case they could never have a child at all were 81.0% of the fertile and 60.8% of the infertile women in our study. However, 20.6% of the infertile women indicated that they would choose oocyte donation as a second option, even if they knew that it was illegal. The difference between the fertile and infertile women was significant (p=0.00; χ^2=19.26, Table 4).

Table 3: Fertile and infertile women's opinions on the advantages and possible problems that oocyte donation may bring

	Fertile women		Infertile women			
	N=105		N=97			
	N	%	N	%	P	χ^2
This technique will provide infertile couples to have child and give them ease against their physical and psychological problems	60	57.1	47	48.5	0.13	1.58
The emergence of the donor later in life would lead to financial or emotional demands from the family or the child	68	64.8	40	41.2	0.001	11.21
I think that couples using this technique will not feel themselves as real mothers and fathers	21	20.0	24	24.7	0.26	0.65
The genetical features of the child to be born will not resemble his/her mother's features, thus this would be a problem	46	43.8	34	35.1	0.130	0.61
Elder women should not have child through this method	33	31.4	12	12.4	0.001	10.57
The children born via this method will never know their exact genetic origins, hence this would result in consanguineous marriages in the future	54	51.4	38	39.2	0.054	3.052
This practice is not appropriate to my religious beliefs	15	14.3	18	18.6	0.26	0.67
This practice is an absolute contradiction with the Turkish family structure, hence it should not be implemented	26	24.8	32	33.0	0.12	1.66

Chi square test was used.

Table 4: Comparison of fertile and infertile women's future plans regarding the use of oocyte donation in case they do not have any other choice

	Fertile women N=105		Infertile women N=97			
	N	%	N	%	P	χ^2
Prefer to live without a child	13	12.4	12	12.4		
Adopt a child	85	81.0	59	60.8		
Go to a country where occyte donation is legal	5	4.8	6	6.2	0.000	19.26
Try to utilize oocyte donation even it is illegal	2	1.9	20	20.6		

Chi square test was used.

Discussion

This study evaluated the knowledge level and opinions of both fertile and infertile Turkish women on oocyte donation. There have been published studies investigating public opinions about oocyte donation (17), IVF staff attitudes regarding oocyte donation (32), views of infertile women on surrogacy and oocyte donation (27), and gamete donation (26) in Turkey. Approximately half of the fertile and infertile women in the study had heard of oocyte donation. However, only a very small percentage of the fertile women and approximately half of the infertile women could correctly define oocyte donation. This indicates that infertile women seek various treatment options to eliminate infertility and its effects; therefore, they are more informed about the subject. Similar to our results, Khalili et al. (32) reported that half of the Iranian community knew the meaning of oocyte donation. Isikoglu et al. (17) have reported that 29.74% of women knew about oocyte donation in their similar study from Turkey. The high rate of having heard of oocyte donation in our study may be due to the increased awareness of the community in the three years between the two studies.

Both the fertile and infertile women in our study were aware that oocyte donation is illegal in Turkey. They were all approving the illegality of oocyte donation. However, 6 of every 10 infertile women reported that they could donate their oocytes for another woman under certain conditions (the donor is a close relative, never knowing the donor, etc.), and more than half said they could take oocytes from another woman if necessary. The attitude of community towards oocyte donation in different societies is still a controversial issue (32). Of previous researches in Turkey, one had reported that 23.3% of infertile women have stated they could accept oocytes from another woman, while 33.8% have stated they could donate oocytes (26). Another study on fertile women had reported that 82.76% of women have a positive attitude to oocyte donation (17). A study from Sweden (15) had found that one sixth of women felt they could donate oocytes for a woman they did not know, while another study (33) had reported 66% of the subjects stating they could donate oocytes for their siblings. A study from Iran (32) had reported that there were not so much difference between Christian and Muslim communities towards their reaction to oocyte

donation and the majority of Iranian public supported oocyte donation as an alternative way of overcoming infertility.

These results indicate that the percentage of Turkish infertile women with a positive attitude towards oocyte donation is constantly increasing and the method is today deemed to be more acceptable both in Turkey and in other countries.

Fertile women have a more unfavorable approach both to oocyte donation and acceptance compared to infertile women. Fertile women wanted oocyte donation to be kept illegal in the future and stated they would not use it even if necessary. However, although infertile women wanted it to be kept illegal in the future, they felt they could use it if necessary. This result is important as it indicates that infertile women feel a conflict between oocyte donation and the desire to have a child. Fertile women have a more negative attitude towards oocyte donation probably because they are not faced with infertility (16).

Approximately half of fertile and infertile women stated that oocyte donation would enable infertile couples to have children, and therefore provide physical and psychological comfort for them. Svanberg et al. (15), Purewal and Vanden Akker (34) have also reported that oocyte donation is a useful method for childless couples. However, both the fertile and infertile women in our study felt that legalization of oocyte donation could lead to ethical, legal, social and religious problems. This result could be related to ethical, sociocultural and religious characteristics of the Turkish society.

The percentage of women who believed oocyte donation would harm religious values or the family structure was quite low in our study. Isikoglu et al. (17) have similarly reported that less than half the participants stated their beliefs prevented oocyte donation.

Infertile women in the current study mentioned they could use illegal routes if necessary to have a child at much higher rates than fertile women. This shows that desire to have a child is a strong motivation in Turkey. To conclude, it is demonstrated that there are infertile couples who try to find and willing to use third-party assisted reproduction techniques, although illegal in this country. It is possible (and known) that some couples travel abroad to certain countries where oocyte donation is legal to make use of the method.

However, employing these techniques without vast information could harm both the couple- the family and the child born as a result. The infertility nurse also has a responsibility to inform the infertile couple about all procedures, whether legal or illegal. The nurse needs to know the characteristics of the group, he/she, is communicating with, so that information can be provided properly. These characteristics would encompass the cultural values that could influence the final decision.

This study has been conducted in an infertility outpatient center in the capital city of Turkey, Ankara. Therefore, as a limitation, the results deriven should not be generalized.

The religious beliefs of the subjects could have influenced the answers given to the questionnaire. We would suggest "larger and possibly multi centered researches" on the topic, including subjects from various religious and cultural societies. Since the majority of the population believes Islam in our country, and since other religious societies are rather concentrated in small groups in various cities, it was not possible for us as a small group of researchers to reach a larger pool of data from different religions. Our results, therefore, reflects the opinions of a Turkish population who all believe Islam.

Finally only fertile and infertile women were included in this study. But the treatment process and the choice of therapeutic options necessitates the husbands' opinion in the decision making. Therefore, it would be interesting to search husbands knowledge and approach to oocyte donation in future studies. Missing the male counterpart's opinions may, therefore, be considered another limitation of this study.

Conclusion

This study shows that approximately half of respondents had heard of oocyte donation; however, only a very small percentage of fertile women and approximately half of infertile women could correctly define it. The majority of both the fertile and infertile women were

aware that oocyte donation is illegal in Turkey. Infertile women have a more favorable approach and support oocyte donation as an alternative route for childless couples, compared to fertile women. Infertile women also mentioned that they could use illegal routes if necessary to have a child at much higher rates than stated by fertile women. This shows that the desire to have a child is a strong source of motivation in Turkey. Health care professionals need to be aware of the emotional and psychosocial impact of being childless in Turkish society.

The explanation of the current legal status in Turkey and the advantages and disadvantages of donation regarding the couple and the child to be born should be included in this presentation. The healthcare staff should provide the necessary guidance after checking the motivation of the couple.

Acknowledgements

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Original Article

The Attitude of South Korean People Regarding Usage of The Internet Perinatal Consultation

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Abstract

Background: To the general public, the Internet is an acceptable method of obtaining information. It also plays an important role in guiding patients and solving their problems. We investigated the clinical characteristics of an Internet website to provide guidelines and tips for consultation.

Materials and Methods: In this retrospective observational study, we analyzed the use of a free public Internet perinatal consultation website provided by the Ministry of Health and Welfare of Korea. We evaluated 2,254 Internet perinatal consultations and assessments of prenatal and obstetrics from August 2006 to December 2009. We evaluated the patients' questions based on Williams' textbook categories and their clinical characteristics.

Results: The mean age of patients seeking consultation was 33.9 ± 13.2 years, and parity was 1.2 ± 0.5. The most commonly asked questions were about drug safety during pregnancy (20.4%), and questions regarding prenatal care (18.7%) and labor pain (15.4%) were the second and third most commonly asked questions, respectively.

Conclusion: The Internet can provide good information to patients. Thus, guidelines regarding pregnancy-related questions and answers should be created. Obstetricians could use our data to identify question tendencies.

Keywords: Delivery, Internet, Pregnancy, Perinatal care

Introduction

Women who wish to become pregnant usually seek educational methods such as books and magazines for safe fertility and delivery. The Internet is currently a common and acceptable method by which patients seek answers to their questions. Based on 33 websites about Internet usage by pregnant women, 96.6% of women have Internet access at home (1). Pregnant women are often very nervous and curious about changes that occur to their body. However, some cannot easily get in touch with a doctor for counseling. Thus, women desire an access method not only for education, but also to easily and quickly obtain answers.

In the present study, we investigated the clinical characteristics of questions on an Internet website to provide guidelines and tips for consultation.

Materials and Methods

In this retrospective observational study, we analyzed the use of a free public Internet perinatal consultation website provided by the Ministry of Health and Welfare of Korea. This website provides information on pregnancy, delivery, child care, and infant health programs and provides a multilingual system for immigrants as well.

The website also offers perinatal consultations and assessments of gynecologic problems free of

charge. On average, there were 25,192 logins per day. The service was accessible via a Korean web domain. Fourteen obstetricians conducted the consultations using a private personal computer. Pediatric doctors also consulted with patients about pediatric problems. The consultant had a duty to reply to each patient's questions. Women asked questions on the site, and an automatic, immediate, repetitive alarm sounded on the consultant's telephone until a reply was updated on the website. The consultant was required to complete the reply while on duty. Duty days covered all 365 days of the year. All replies were completed within 24 hours.

We evaluated 2,254 consultations from August 2006 to December 2009. A total of 122 consultations occurred during the initiation year, whereas 802 consultations were performed in 2009. Two research nurses and one obstetrician evaluated each consultation. We evaluated the questions based on Williams' textbook categories and patients' clinical characteristics.

Ethical considerations

Approval for this study was given by the Human Ethics Committee at SCH (Soonchunhyang University) Medical Center (Bucheon, Korea). We mention that no personal data is published and the privacy of the users was respected.

Statistical analysis

Results are presented as number and percentage values. Variables were expressed using the number and the percentage. Statistical data were generated with the SPSS version 12 for Windows (SPSS Inc., Chicago IL, USA).

Results

The mean age of patients seeking consultation was 33.9 ± 13.2 years, and parity was 1.2 ± 0.5. Most of the consultations were with women (2,125, 94.3%) (Table 1), and the greatest proportion of consultations (73.6%) were with women aged 25 to 35 years (Table 2). A total of 1,997 (88.6%) married women received consultations (Table 3). The residential distribution was as follows: Seoul and the central area, 55.1%; other provinces, 39.4%; and foreign countries, 2.2% (Table 4). Approximately 60.7% of the patients

who received a consultation did not want their consultations to be publicly shared (Table 5). The consultations according to trimester were first trimester, 24.6%; second, 19.2%; third, 12.8%; and postpartum, 17.7%. A total of 25.2% of the consultations were performed during the preconception period (Table 6). All of the Internet consultations were performed in the Korean language. We divided the questions based on Williams' textbook categories (2). The most commonly asked question was about drug safety during pregnancy (20.4%), and questions regarding prenatal care (18.7%) and labor pain (15.4%) were the second and third most commonly asked questions, respectively. Approximately 11.3% of the questions concerned postpartum care. Questions about laboratory interpretations during pregnancy, such as the quad test and breastfeeding methods or complications constituted 9.2% and 8.6% of questions, respectively. Labor and delivery questions, such as questions about induction or cesarean section, comprised 6.3% of the questions. Questions regarding hypertension and diabetes were raised at prevalence rates of 4.3% and 6.3%, respectively (Fig 1).

Table 1: Sex distribution of clients

Sex	Number	Percentage
Male	104	4.6
Female	2125	94.3
Missed data	25	1.1
Total	2254	100

Table 2: Age distribution of clients

Age	Number	Percentage
<25	113	5.0
25-35	1660	73.6
36-45	396	17.6
>45	68	3.0
Missed data	17	0.8
Total	2254	100

Table 3: Marital status of clients

Marital status	Number	Percentage
Married	1997	88.6
Unmarried	186	8.3
Missed data	71	3.1
Total	2254	100

Table 4: Residential distribution of clients

Area	Number	Percentage
Seoul and Gyeonggi	1241	55.1
Other provinces	889	39.4
Foreign country	50	2.2
Missed data	74	3.3
Total	2254	100

Table 5: Agreement to publicly share information

Agreement	Number	Percentage
Yes	870	38.6
No	1368	60.7
Missed data	16	0.7
Total	2254	100

Table 6: Gestational period of clients by trimester

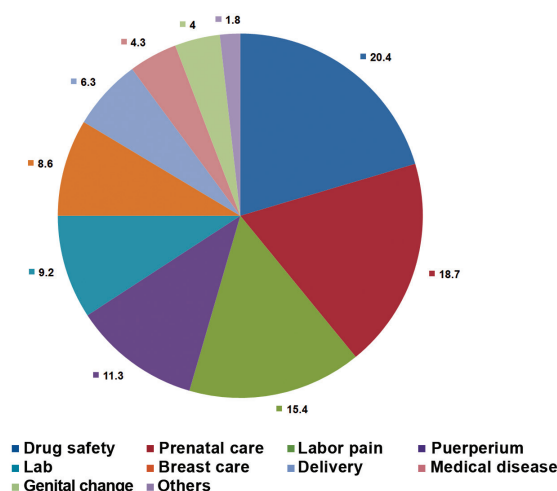| G.A.* by trimester	Number	Percentage
Pregestation	567	25.2
First	554	24.6
Second	432	19.2
Third	288	12.8
Postpartum	398	17.7
Missed data	17	0.8
Total	2254	100

; Gestational age.

Fig 1: Percentage of asked questions by subject.

Discussion

In Korea, there are almost 80 known websites about pregnancy, delivery, and child care (3). Our dataset is the largest in Korea and is representative of Korea, as the evaluated website is supported by the Ministry of Health and Welfare of Korea. Therefore, our data are representative of the characteristics of pregnancy-related questions.

The Internet is useful for pregnant women and plays a role in decision-making. In a Web survey of Internet use in 24 countries, most women (97%) used the Internet for pregnancy-related questions (1). Mankata described hospital and physician referral as the most prevalent topic (15%) for Internet consultation (4). However, our data revealed that Korean pregnant women were most curious about drug safety during pregnancy (20.4%).

The preconception period was also a common topic of consultation based on our data. Mankata reported that 5% of those asking questions were husbands, similar to our study (4.7%). Women in other countries were interested in and had many questions regarding pregnancy, but the topics in which they were interested were not the same. Pregnancy-associated questions are common on the Internet, which is a very cost-effective way to obtain information (4). However, a review article about Internet consultations revealed that most studies on this topic have provided little evidence and have been poorly designed (5).

Conclusion

The Internet can provide good information, but that information is sometimes confusing and may be inaccurate. If the website evaluated in this study had an automatic system for differentiating questions based on keywords, trimester, previous related questions, and so forth, the questions could easily be categorized and the evaluation time could be reduced. Thus, such a system should be created for pregnancy-related questions and answers. Culture and health care systems might influence the nature of the most frequent questions. We inferred that Korean women are very anxious about the health of the baby, so they were very eager to obtain information about drug safety and preconception care.

Acknowledgements

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Original Article

A Sectional Study: The Relationship between Perceived Social Support and Depression in Turkish Infertile Women

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Abstract

Background: Studies conducted on infertile women in the literature investigated some features such as depression, anxiety, loneliness, and social support. However, there has been no study examining the relationship between levels of perceived social support and depression in infertile women. Considering this deficiency, the study was conducted to determine the relationship between perceived social support and depression in infertile women. The purpose of this study is to determine the relationship between perceived social support and depression in infertile women.

Materials and Methods: This descriptive and sectional study was conducted between 16 April and 31 October 2012 in *in vitro* fertilisation (IVF) Centre of Fırat University Research Hospital. Sampling formula was used in cases when the number of elements in the population was not known to calculate minimum sample size required to be included in the study. A total of 238 women who applied to the relevant centre between the specified dates constituted the sample group of the study. A Questionnaire Form, Beck Depression Inventory (BDI) and the Multidimensional Scale of Perceived Social Support (MSPSS) were used to collect the data. A pilot study was carried out on nine infertile women. As a result of the pilot study, we formed the final version of the Questionnaire Form. The data of these nine women were not involved in the research. The data obtained from the study was assessed using Statistical Package for the Social Sciences (SPSS; SPSS Inc., Chicago, IL, USA) version 15.0. Percentage distribution, mean, t test, one-way analysis of variance (One-Way ANOVA), and Pearson correlation analysis were used to evaluate the data.

Results: The women's total mean score on the BDI was 12.55 ± 8.07. Scores obtained by women on the MSPSS was 15.75 ± 8.53 for the subscale of friend, 21.52 ± 8.20 for the subscale of family, and 15.62 ± 8.45 for the subscale of significant others. The women's total MSPSS score was 52.89 ± 21.75.

Conclusion: A significant, negative relationship was found between total BDI score with subscale and total mean scores of MSPSS (r= -0.596, p<0.01). Symptoms of depression decreased as the women's perceived social support increased.

Keywords: Depression, Infertility, Social Support, Sectional Study, Nursing/Midwifery

Introduction

The family is the smallest union in society that is based on marriage and blood relation and involves relationships between wife, husband, children and siblings (1). The family is responsible for continuing the human race and raising generations appropriate for society's expectations (2). Having a child is an expected and desired outcome of the conjugal community. As in all societies, marriage is associated with having a child in Turkish society. Therefore, almost all of the married couples or those who intend to get married plan on having a child (3-5). Not all couples, however, are easily able to have a child and may suffer from infertility.

The World Health Organization (WHO) defines "infertility" as the failure of getting pregnant in spite of the couple having unprotected regular sexual intercourse for at least one year (6, 7). The worldwide infertility rate is between 8 and 12% and varies between 10 and 20% in Turkey (6, 8); however, this rate has increased in recent years. This increase is associated with numerous factors such as the change in traditional roles or late marriage and couples' plans for children. There have also been increases in assisted reproductive techniques, greater access and use of infertility centres, as well as inclusion of infertility diagnosis and treatment in health insurance. From a social perspective, there has been increased accessibility of contraceptive methods; increased social acceptability of infertility; changes in use of alcohol, smoking, and substance abuse; changes in dietary habits, and increased stress and sexually transmitted diseases (7-13).

The effects of infertility on individuals' emotions are complicated and these effects vary based on the duration of infertility, individuals' capacities for adaptation, reasons and prognosis of infertility, and emotional and social supports (14-16). Infertility not just a situation about the function of reproduction it also appears as a potential crisis causing social and psychological exposure (17-20). It is estimated that almost 86.8% of infertile women have anxiety and 40.8% have depression. Infertility is a complex life crisis that adversely affects the couples' social lives, emotional conditions, marriage relations, sex lives, future plans, self-esteem and body images. Infertility should be considered as a bio-psychosocial crisis requiring psychological counselling, which is an integral part of a multidimensional solution (21). Some studies have reported a decrease in the level of depression, anxiety, mental distress, marital violence, and increased rate of pregnancy following psychosocial interventions (14, 22-26). Likewise, infertility treatment is expensive in terms of economy, stressful in terms of emotions and a physically painful process, all of which require adjustment within the couple. Infertile couples might subsequently develop guilt, a sense of worthlessness and depression (2, 19, 20).

Social support, which is a source of coping, is of great importance for the infertile woman to help preserve her physical and mental health. Social support is a valuable coping method that contributes to love, affection, confidence, self-expression, self-knowledge and sense of belonging. Even if it cannot eliminate the stressful situation, it enables individuals to be more optimistic by decreasing their levels of anxiety. It helps individuals in coping with challenging situations and generating new solutions, and decreasing their desperation (4, 27, 28).

Infertility is a condition adversely affecting the woman in terms of biologic, psychological and social aspects. Thus, midwives/nurses who are assigned to support couples in the infertility diagnosis and treatment process have great responsibilities. Infertility nursing is a process that starts in the polyclinic and extends to the operating room; it prioritises psychological and social conditions of couples and includes care during all kinds of medical and surgical treatments (29). During the process of infertility, the general purpose of care is to evaluate the physical, psychological, and social conditions of couples; to determine problems and needs in this field; and thus to provide convenient consultancy and training services (30). The consultancy to be provided to infertile women will positively affect their social support, success of the treatment, and women's health in the solution of problems.

Based on these important benefits, this study was conducted to determine the relationship between perceived social support and depression

in infertile women.

Materials and Methods

This descriptive and sectional study was conducted in the IVF Centre of a university hospital in the city centre of Elazig (Turkey) between 16 April 2012 and 31 October 2012. The centre where study data were collected was selected because it is the largest unit in the province of Elazıg and renders services to women from surrounding cities and with diverse socio-cultural features.

The population of the study consisted of women who applied to the IVF Centre of Firat University Research Hospital for infertility treatment. The study sample group comprised 238 women who applied to the relevant centre for infertility treatment between the specified dates.

The number of infertile women who accessed the relevant centre for *in vitro* fertilisation (IVF) treatment each year is not known, since the required statistical records have been not regularly kept. Therefore, a sampling formula was used in this situation where the number of elements in the population was not known in order to calculate the minimum sample size required for inclusion in the study. This formula is as follows (31, 32):

$n = t^2 * p * q / d^2$
n=Necessary sample size
p=Standard of deviation
q=1- Standard of deviation
t=Z score
d=Margin of error (Confidence interval)
$n = (1.96)^2 * 0.15 * 0.85/(0.05)^2 = 195.9216$

The number of participants calculated as representing the population was at least 196 individuals. Those women who met the sample criteria and accepted to participate in the study within the period when the researcher was present in the centre were included in the study until the minimum number (196) was attained. This number was reached within 4.5 months. Considering that data loss may occur during the data collection process, the sample size was extended to a total of 238 individuals. The inclusion criteria of the sample group were that women were literate, had been diagnosed with infertility and in the process of having treatment, and they had

no serious medical history that threatened life and did not receive treatment because of this reason. The exclusion criteria of the sample group were being not literate, and having a serious medical history.

Collection of the data

The study data were collected between 16 April 2012 and 31 August 2012. During the data collection process, the following forms were used: "Questionnaire Form" prepared by researchers, "Beck Depression Inventory" (BDI) for evaluation of depression, and the "Multidimensional Scale of Perceived Social Support" (MSPSS) for determination of the perceived social support. The questionnaire and scales were administered by researchers during face-to-face interviews with women who applied to the IVF Centre. Each woman was interviewed in a separate room in the related centre to enable them to answer the questions comfortably. The questionnaire and scales took about 10-15 minutes in total to complete.

Data collection instruments

Questionnaire form

The questionnaire form, which was prepared by researchers based on a literature review, has 26 questions (3, 4, 33-36). The form includes questions that determined the women's socio-demographic, health, menstruation and infertility characteristics.

Beck Depression Inventory (BDI)

The 21-item BDI was developed by Beck et al. in 1961 for the purpose of evaluating the physical, emotional, and cognitive symptoms observed during depression. The purpose of the scale is to objectively determine the symptom levels of depression, rather than diagnosing the depression (37, 38). Each item on the scale is scored between 0-3. A higher total score shows the severity of depression symptoms. The validity and reliability study of the Turkish version of the scale was conducted by Hisli in 1988. It indicated that when the BDI score is 17 and above, this enabled the differentiation of depression to be diagnosed with an accuracy of 90% (38). While the criterion-dependent valid-

ity was r: 0.75, reliability of split-half method was r: 0.74 and it was reported to be usable in Turkey (38). In this study, the Cronbach's alpha coefficient for internal consistency was 0.82.

Multidimensional Scale of Perceived Social Support (MSPSS)

The 12-item MSPSS developed by Zimet et al. in 1988 to subjectively assess the social support was used in this study (39).

The validity and reliability study of the scale was conducted by Eker and Arkar in 1995 (40). The MSPSS contains 12, 7-point, Likert-scaled items which comprise three subscales; family, friends and significant other. Each subscale contains 4 items. In the scale, items 3, 4, 8, and 11 measure the family support; items 6, 7, 9, and 12 measure the friend support; and items 1, 2, 5, and 10 measure the support of significant others. The higher the score obtained from the scale signifies a higher level of perceived social support (39-41). In this study, the Cronbach's alpha internal consistency coefficients were 0.95 for the subscale of family support, 0.94 for the subscale of the friend support, and 0.91 for the subscale of significant other support. The total Cronbach's alpha internal consistency coefficient of the scale was 0.94.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS; SPSS Inc., Chicago, IL, USA) version 15.0 was used to assess the study data. Percentage distributions, mean, t test, one-way analysis of variance (One-Way ANOVA), and Pearson correlation analysis were used to analyse the data.

Ethical considerations

Before commencing the study, approval was obtained from the Ethics Committee of Ataturk University Faculty of Health Sciences and then written permission was received from the hospital where the study would be conducted. Before starting the data collection process, in order to protect the rights of women included in the study, they were informed about the purpose of the study and that the data would be kept confidential. Any questions from the women were answered and they were given relevant information after the completion of the questionnaire.

Results of the study could be generalised to infertile women in the study group.

Results

Table 1 and table 2 illustrate descriptive characteristics of women included in the study. Table 2 illustrates the distribution of women's infertility features and the women's opinions on infertility.

Table 3 illustrates the distribution of lowest and highest scores obtained from the BDI and MSPSS, along with the women's mean scores. The women's total mean score on the BDI was 12.55 ± 8.07. Scores obtained by women on the MSPSS was 15.75 ± 8.53 for the subscale of friend, 21.52 ± 8.20 for the subscale of family, and 15.62 ± 8.45 for the subscale of significant others. The women's total MSPSS score was 52.89 ± 21.75.

Table 4 illustrates the relationship between BDI with subscale mean scores and total mean scores of the MSPSS. A statistically negative significant relationship is determined between the score of the subscale "friend" in MSPSS and the total score of BDI at the level of $p<0.01$. There is a statistically negative significant relationship between the score of the subscale "family" in the MSPSS and the total score of BDI at the level of $p<0.01$. A statistically negative significant relationship was found between the score of the subscale "significant other" in the MSPSS and the total score of the BDI at the level of $p<0.01$.

According to these results, a statistically negative significant relationship was determined between the scales (r: -0.596, $p<0.01$). In other words, it was observed that as the women's perceived social support increases, the symptoms of depression decrease.

Table 5 illustrates some of the women's sociodemographic characteristics and the comparison of mean scores of the scales of the BDI and MSPSS. No statistically significant difference was determined between the women's remaining features, except for the mean scores of the BDI according to the women's educational and income status. The difference between women's working condition, marriage duration and elapsed time following the diagnosis of infertility and their total mean scores of MSPSS was found to be statistically significant ($p<0.05$).

Table 1: Distribution of women's socio-demographic and medical characteristics

Characteristics (N=238)	Mean	Standard deviation
Age	31.9	6.2
Age of marriage (Y)	23.7	6.1
Menstruation duration	5.9	1.6
Age of husband (Y)	35.2	5.9
Duration of marriage	8.2	5.8
Educational status	Number	%
Low education level	202	84.9
High education level	36	15.1
Working condition of women		
Employee (officer + worker)	32	13.4
Unemployed	206	86.6
Educational status of husband		
Low education level	167	70.2
High education level	71	29.8
Occupation of husband		
Officer	67	28.2
Worker	34	14.3
Self-employed	137	57.5
Residence where they have lived for the longest period		
Village-district	80	33.6
Province	158	66.4
Residence in the city centre where the treatment is conducted		
Yes	148	62.2
No	90	37.8

Characteristics (N=238)	Mean	Standard deviation
Family Type		
Nuclear family	213	89.5
Extended family	25	10.5
Income status		
High	58	24.4
Low	180	75.6
Social security		
Available	205	86.1
N/A	33	13.9
Mode of marriage		
Arranged marriage	135	56.7
Love marriage	103	43.3
Previous depression treatment		
Yes	20	8.4
No	218	91.6
Depression treatment after diagnosis of the infertility		
Yes	19	8.0
No	219	92.0
Menstrual regularity		
Regular	154	64.7
Irregular	84	35.3
Previous reproduction system diseases		
Yes	89	37.4
No	149	62.6

Table 2: Distribution of women's infertility features

Features (N=238)	Number	%
State of having a previous pregnancy		
Yes	111	46.6
No	127	53.4
Knowing the reason of infertility		
Yes	164	68.9
No	74	31.1
History of treatment		
Those who had received no treatment before	70	29.4
Hormone therapy	56	23.5
Vaccination*	54	22.7
IVF	58	24.4
Recent stage of treatment		
Hormone therapy	17	7.1
Vaccination	65	27.3
IVF	156	65.6
Obtaining information about the infertility treatment		
Yes	169	71.0
No	69	29.0
Source of information (N=169)		
Medical staff	136	80.5
Friend, relative, environment	12	7.1
Internet-TV	21	12.4
	Mean	**Standard deviation**
Period to be continued for the infertility treatment (Months)	1.8	1.4
Elapsed time following the infertility diagnosis (Months)	44.6	47.1

IVF; In vitro fertilisation and *; Following special phases when the ovary is stimulated by drugs and ovulation occurs, sperm are prepared in the laboratory and are inserted into the female genital tracts by means of a catheter.

Table 3: Lowest and highest scores of BDI and MSPSS and mean scores of women

	Scales	Lowest and highest scores of scales	Minimum scores of scales	Maximum scores of scales	Mean scores of scales $\overline{X} \pm SD$
BDI	Total	0-63	0	42	12.55 ± 8.07
MSPSS	Friend	4-28	4	28	15.75 ± 8.53
	Family	4-28	4	28	21.52 ± 8.20
	Significant Other	4-28	4	28	15.62 ± 8.45
	Total	12-84	12	84	52.89 ± 21.75

BDI; Beck depression inventory and MSPSS; Multidimensional scale of perceived social support.

Table 4: Determination of the relationship between the mean scores of MSPSS and BDI

Scales			BDI total
MSPSS	Friend	r	-0.534*
		p	0.000
	Family	r	-0.555*
		p	0.000
	Significant Other	r	-0.456*
		p	0.000
	Total	r	-0.596*
		p	0.000

*; P<0.01, BDI; Beck depression inventory and MSPSS; Multidimensional scale of perceived social support.

Table 5: Some characteristics of women and comparison of mean scores of BDI and MSPSS

	BDI	Friend	Family	Significant other	Total
Features	$\overline{X} \pm SD$	$\overline{X} \pm SD$	$\overline{X} \pm SD$	$\overline{X} \pm SD$	$\overline{X} \pm SD$
Age					
19-30	12.23 ± 7.97	16.93 ± 8.28	21.90 ± 8.15	16.72 ± 8.36	55.56 ± 21.59
31 and older	12.80 ± 8.18	14.80 ± 8.64	21.21 ± 8.25	14.73 ± 8.45	50.75 ± 21.72
Test and p value	t: 0.53, df: 236, p>0.05	t: 1.92, df: 236, p>0.05	t: 0.64, df: 236, p>0.05	t: 1.81, df: 236, p>0.05	t: 1.70, df: 236, p>0.05
Educational status					
Low education level	13.05 ± 8.14	15.37 ± 8.53	21.08 ± 8.36	15.44 ± 8.24	51.91 ± 21.84
High education level	9.69 ± 7.16	17.86 ± 8.32	23.97 ± 6.81	16.61 ± 9.61	58.44 ± 20.63
Test and p value	t: 2.32, df: 236, p<0.05	t: 1.61, df: 236, p>0.05	t: 1.95, df: 236, p>0.05	t: 0.76, df: 236, p>0.05	t: 1.66, df: 236, p>0.05
Income state					
High	10.77 ± 7.40	16.13 ± 9.11	22.79 ± 7.88	16.51 ± 8.83	55.44 ± 21.78
Low	13.12 ± 8.22	15.62 ± 8.35	21.11 ± 8.28	15.33 ± 8.33	52.07 ± 21.74
Test and p value	t: 1.93, df: 236, p<0.05	t: 0.53, df: 236, p>0.05	t: 1.35, df: 236, p>0.05	t: 0.92, df: 236, p>0.05	t: 1.02, df: 236, p>0.05
Working condition of women					
Employee	10.09 ± 6.57	18.06 ± 8.78	25.21 ± 5.83	17.15 ± 9.59	60.43 ± 20.19
Unemployed	12.93 ± 8.23	15.39 ± 8.45	20.95 ± 8.37	15.38 ± 8.26	51.72 ± 21.80
Test and p value	t: 1.85, df: 236, p>0.05	t: 1.65, df: 236, p>0.05	t: 2.77, df: 236, p<0.05	t: 1.10, df: 236, p>0.05	t: 3.12, df:236, p<0.05
Mode of marriage					
Arranged marriage	12.48 ± 7.63	15.92 ± 8.16	21.49 ± 7.84	15.53 ± 8.01	52.95 ± 20.66
Love marriage	12.64 ± 8.65	15.52 ± 9.03	21.56 ± 8.68	15.73 ± 9.04	52.82 ± 23.20
Test and p value	t: 0.15, df: 236, p>0.05	t: 0.35, df: 236, p>0.05	t: 0.06, df: 236, p>0.05	t: 0.18, df: 236, p>0.05	t: 0.04, df: 236, p>0.05
Duration of marriage					
1-5	12.51 ± 8.05	16.95 ± 8.39	21.50 ± 7.74	16.69 ± 8.40	55.14 ± 21.04
6-11	11.48 ± 8.23	16.15 ± 8.16	22.97 ± 7.91	15.65 ± 8.81	54.78 ± 21.29
12 and above	14.06 ± 7.80	13.12 ± 8.82	19.58 ± 9.04	13.70 ± 7.82	46.41 ± 22.65
Test and p value	F: 1.72, df: 2, p>0.05	F: 3.93, df: 2, p<0.05	F: 2.90, df: 2, p<0.05	F: 2.32, df: 2, p<0.05	F: 3.48, df: 2, p<0.05
Type of infertility					
Primary infertility	13.33 ± 8.15	14.97 ± 8.62	21.90 ± 7.91	15.55 ± 8.26	52.43 ± 20.99
Secondary infertility	11.65 ± 7.92	16.63 ± 8.37	21.09 ± 8.52	15.70 ± 8.70	53.43 ± 22.67
Test and p value	t: 1.59, df: 236, p>0.05	t: 1.50, df: 236, p>0.05	t: 0.76, df: 236, p>0.05	t: 0.13, df: 236, p>0.05	t: 0.35, df: 236, p>0.05
Infertility period (Month)					
1-12	12.97 ± 7.62	14.87 ± 8.34	21.41 ± 8.34	15.07 ± 8.27	51.36 ± 21.47
13-24	9.92 ± 7.15	18.69 ± 8.59	24.07 ± 5.86	18.61 ± 8.09	61.38 ± 18.84
25-36	12.45 ± 7.77	15.37 ± 9.08	18.66 ± 9.30	13.95 ± 9.70	48.00 ± 24.71
37 and above	13.38 ± 8.74	15.26 ± 8.36	21.21 ± 8.48	15.15 ± 8.25	51.63 ± 21.77
Test and p value	F: 1.91, df: 3, p>0.05	F: 2.08, df: 3, p>0.05	F: 2.41, df: 3, p>0.05	F: 2.30, df: 3, p>0.05	F: 2.83, df: 3, p< 0.05

BDI; Beck depression inventory and MSPSS: Multidimensional scale of perceived social support.

Discussion

Diagnosis and treatment approaches used for infertile couples may hinder their coping skills and social support resources; consume their physical and emotional energy; cause sexual dysfunctions, depression, anxiety, and loneliness; and damage the couple's relationship (14, 26, 33-36). The results obtained from this study, conducted in order to determine the relationship between the perceived social support and depression in infertile women, are discussed in line with the relevant literature.

Examining the women's mean scores from the MSPSS and BDI (Table 3) determined that their BDI mean score was lower. This result is similar to that found in other studies where the mean score of depression is low (3, 14, 27, 31, 42-44). In the study conducted by Gurbuz, the women's mean score of depression was 21.11 ± 5.74. We determined that the women's total mean score on the MSPSS was close to the highest score that could be obtained from the scale where the highest perceived social support was from subscales "family", "friend" and "significant other", respectively (34). In Kus's study, the subscale mean scores and total mean scores of MSPSS also showed a similarity with the results of this study (4). A controlled study conducted by Upkong and Orji with 208 women in Nigeria revealed that receiving no support from the husband increased women's depression and anxiety scores (45). Similarly, in the study conducted by Matsubayashi et al. (44) with 101 infertile women in Japan, the researchers determined that women's anxiety and depression levels were very high and this was associated with lack of support from their husbands. In the light of such information, it could be stated that when social support meets individuals' expectations, especially the support of family, it enables individuals to cope with life's problems by showing a positive effect in terms of morale and coping.

After examining the relationship between the BDI total score with subscale mean scores and total mean scores of MSPSS shown in table 4, a statistically negative significant relationship was found between the scales at the level of $p<0.01$. It was determined that as the women's perceived social support increases, symptoms of depression decrease. Social support is a predictive factor for depression (43). The studies conducted with in-fertile women concluded that lack of social support caused a higher rate of anxiety and depression symptoms in infertile women (43-45). It is also reported that the lack of husband and his family's support results in the deterioration of mental health and depression (15, 45).

On examination of the BDI mean scores and educational status of women according to some of their features shown in table 5, it was observed that those with a high educational level (university graduate) had fewer symptoms of depression. As the educational level increases, it becomes easier for women to have a better economic status, social security and access to knowledge. Women who have access to full information might experience less worry, obscurity, and anxiety. Thus, it is possible to assert that women with higher educational levels have lower BDI scores. In the study conducted by Pinar, women's mean score of depression was 26.79 ± 10.90 (26).

Considering the income status of women, it is thought that those with a lower income status have a higher BDI mean score; in other words, those with a poor income status are negatively affected in terms of experiencing depression. In line with result of this study, there are some studies asserting that as the income status increases, the level of depression decreases (3, 4, 6, 26).

Examining the women's MSPSS mean scores also showed that the difference between women's working conditions with total mean score of MSPSS and mean score of its subscale "family" was found to be statistically significant ($p<0.05$). As the women's educational level increases, they are enabled to have a regular job and income; access positive information, attitudes and behaviours in terms of health; and also ensure their families attain positive information, attitudes and behaviours on this subject; it could, therefore, be asserted that the women's perception of social support is also affected positively.

The difference between marriage duration of women with total mean score of the MSPSS and mean score of its subscale "friend" was found to be statistically significant ($p<0.05$). As the marriage age increases, the perceived social support decreases-similar results were also found in the study conducted by Eren (27).

The difference between women's infertility pe-

riods and total mean scores of the MSPSS was found to be statistically significant ($p<0.05$). Similarly, Kus's study also observed that the difference between the elapsed time following the diagnosis of infertility and total mean scores of MSPSS was statistically significant (4).

Conclusion

In consequence of this study, it is observed that as the women's perceived social support decreases, their mean scores of depression increase. The recommendations made in line with results of the study are:

Informing midwives and nurses about the problems experienced by infertile women and interventions aimed at these problems through in-service training programs is important.

Enabling midwives and nurses to examine the social support mechanisms of women diagnosed with infertility, helping them to use the support involved in family and other social support systems positively, as well as making interventions that strengthen the social support systems of individuals with insufficient support, will improve overall care outcomes.

Evaluating the infertile woman both gynaecologically and psychologically, and providing her with contact with a psychologist or psychiatrist when required, can improve psychological health. Infertile women would benefit from close follow up in terms of depression risk.

By conducting comparative studies in groups where pregnancy is achieved or not achieved as a result of the treatment would help to determine the explicit effect of the perceived social support on depression in infertile women.

Acknowledgements

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Original Article

Infertile Individuals' Marital Relationship Status, Happiness, and Mental Health: A Causal Model

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Abstract

Background: This study examined the causal model of relation between marital relation-
ship status, happiness, and mental health in infertile individuals.

Materials and Methods: In this descriptive study, 155 subjects (men: 52 and women:
78), who had been visited in one of the infertility Centers, voluntarily participated in a
self-evaluation. Golombok Rust Inventory of Marital Status, Oxford Happiness Ques-
tionnaire, and General Health Questionnaire were used as instruments of the study. Data
was analyzed by SPSS17 and Amos 5 software using descriptive statistics, independent
sample t test, and path analysis.

Results: Disregarding the gender factor, marital relationship status was directly related to
happiness ($p<0.05$) and happiness was directly related to mental health, ($p<0.05$). Also,
indirect relation between marital relationship status and mental health was significant
($p<0.05$). These results were confirmed in women participants but in men participants
only the direct relation between happiness and mental health was significant ($p<0.05$).

Conclusion: Based on goodness of model fit in fitness indexes, happiness had a mediator
role in relation between marital relationship status and mental health in infertile individu-
als disregarding the gender factor. Also, considering the gender factor, only in infertile
women, marital relationship status can directly and indirectly affect happiness and mental
health.

Keywords: Infertility, Marital Relationship, Happiness, Mental Health

Introduction

Infertility is defined as 1 year unprotected in-
tercourse without pregnancy (1, 2). Although in-
fertility pertains to physical problems, today it is
considered not only as a gynecologic illness, but
also as a biopsycho-social health problem (3)." For
many couples, infertility is as much an emotional
and spiritual crisis as it is a physical challenge "(4).
Based on many psychological studies on infertility,
it is clear that infertility and its treatment procedure
are psychologically stressful. Based on the stud-
ies, although the majority of infertile individuals
do not show overt psychiatric disorders, rates of
anxiety, depressive symptoms, the low level of life
satisfaction, sense of guilt and inadequacy, inter-
personal problems, marital difficulties, and chang-
ing in sexual functioning were reported during or
after medical treatment of infertility (5-8).

Stresses of infertility and its treatment procedure
can damage the quality of relationships in infer-

tile couples. If the couples cannot appropriately cope with these stresses, those may lead to several psychological problems for each partner that can considerably affect their marital relationship with each other. Such stressful relationships per se can intensify stresses and distresses related to infertility. Thus, in this position a vicious circle develops that can negatively affect couple's mental health mutually. Moreover, it has been argued that the diagnosis of infertility may magnify and intensify the disappointments and conflicts that have been previously existed in couple's relationships with each other (5).

Although mental health of couples may be damaged by the diagnosis of infertility and couples are usually confused about the ways of handling this condition, infertility per se does not necessarily damage couple's mental health or quality of their relationship with each other (3). Even some studies show that infertile couples report more intimacy than fertile couples (9). In fact, the influence of infertility on the mental and marital relationship health of infertile couples may be variable in different couples. While in some couples infertility can destroy their marital relationships and consequently develops many stresses, some others have reported that this crisis improved their marital relationships and intimacy (5). It seems that some features of infertile couples' relationships have important negative or positive roles on their mental health, even when they confront with infertility. The quality of infertile couples' happiness can play such a role.

Happiness, which is as essential dimension of life and related to functioning and success (10), generally is considered to comprise three main components. These components are frequency and degree of positive affect or joy; absence of negative feelings, such as depression or anxiety; and the average level of satisfaction over a period (11). Studies show that happiness not only is considered as outcome of positive events and factors, but also considered as productive of positive outcomes especially in mental health (12). Some theoretician believes that happiness is related to satisfying social and interpersonal relationships especially marital relationship (13). Based on several theories and studies, marital happiness is a significant predictor for general sense of happiness (14, 15). This

relationship was even confirmed by multicultural studies (16). In fact a satisfying marital relationship by fulfilling intimacy needs of both partners enhances the rates of positive emotions between them such as happiness and consequently enhances physical and mental health of each partner (14). Studies on happiness consistently have showed a strong relationship between happiness and health, as happier people are healthier (17). This relationship has been confirmed through several studies in variety cultures and populations (18-21). Happiness and mental health are two key concepts in psychology that have considerably the overlap with each other, because both of them related to psychological well-being. However, these two concepts are regarded as two independent components in related conceptual details, mental health usually is recognized by behavioral and emotional status without any destructive dysfunctioning but happiness is usually recognized by positive and constructive emotional status (22).

Based on several studies and theoretical discussion, it is clear that there are significant relationships between marital relationship status with mental health (23-27), marital relationship status with happiness (15, 16), and happiness with mental health (19-21). But none of these studies were administrated in infertile individuals. Indeed, these studies suggest that marital relationship status and happiness are key variables to determine mental health in general population but many aspects of these relationships have remained vague so far, such as: 1. Does this relationships is significant in some special population such as infertile individuals that experience particular and different stresses and distresses compared with general population? 2. Does happiness can be considered as a mediator variable in relationship between marital relationship status and mental health in infertile individuals?

Based on several studies, that mentioned, it seems that infertile individuals may be more at risk for mental health problems, compared with fertile individuals, because they experience many social, cultural, and psychological stresses related to infertility (5). So it is necessary to identify any factors that may have a significant effect on mental health in this population. Identifying and regarding these conceptual components can be helpful to

consider some critical factors in treatment procedure of infertility.

According to our knowledge, so far no study investigated the mediator role of happiness in relationship between marital relationship status and mental health in infertile individuals through a causal model. Thus, the purpose of this study was to investigate the causal model of relation between marital relationship status, happiness, and mental health in infertile individuals. The theoretical path model for the relation between marital relationship status, happiness, and mental health is presented in the figure 1.

Fig 1: Theoretical path model for the relation between marital relationship status, happiness, and mental health.

Materials and Methods

The research method of this study was descriptive. The study population included all infertile men and women visited in the Isfahan Fertility and Infertility Center between August and September 2012. The sample of this study were 155 subjects (men: 52 and women: 78) whom had been visited in the Fertility and Infertility Center and selected by convenience sampling [a type of sampling in which members of the population are chosen based on their relative ease of access. This kind of sampling is common to use when applying other sampling methods accompanied by some difficulties. But in this kind of sampling there are limitations to generalization of data (28)]. Two psychologist (a man and a woman) conferred to the both men and women subjects (not with their comrades), separately in waiting room and explained the research, then if each of the subjects was volunteer to participate in the research, he/she would completed the instruments of the study (in Persian language). The Golombok Rust Inventory of Marital Status (GRIMS) is a self-report instrument contained 28 items. Each item concluded four options (0-3) based on the likert scale. This inventory was designed to measure the quality of marital relationships. It was created in 1998. The higher scores in this inventory indicate serious difficulties in marital relationships (29). The reliability of this inventory was confirmed thorough several studies (30). Psychometric components of the Persian form of this inventory were assessed by Besharat in infer-

tile couples and its reliability was assessed as 0.92 for women and 0.94 for men (31). One item of this inventory is, for example "I am dissatisfied from our marital relationship".

The Oxford Happiness Questionnaire (OHQ) is a self-report instrument with 29 items designed to measure intensity of happiness (32). It was created in 2002 (33). Each item concluded four options (0-3), constructed to reflect incremental steps defined as: unhappy or mildly depressed, a low level of happiness, a high level of happiness, and mania. The respondents were asked to "select the one statement in each group which best describes your feeling over the past week, including today." The higher scores in this questionnaire indicate higher levels of happiness (34). In the study of Abedi et al. (35) this questionnaire was standardized in Iranian population. Based on this study, for OHQ the reliability was assessed as 0.85, the factorial validity assessed as 0.74, and concurrent validity assessed as 0.73. One of items of this inventory is, for example "- I don't feel happiness - I somewhat feel happiness - I feel so happiness - I am extremely happy".

The General Health Questionnaire (GHQ-28) is a self-report instrument with 28 items designed to measure mental health (36). Each item concluded four options (0-3) based on the likert scale and the higher scores in this questionnaire indicate low levels of mental health (29). In the study of Ebrahimi et al. (37) the criterion validity of this questionnaire was assessed as 0.78 and its reliability

assessed as 0.97. One of items of this inventory is for example "Do you recently feel happiness in your life".

In this study, Cranach's alpha values for the three instruments were computed. In the GRIMS, Cranach's alpha was assessed as 0.935, in OHQ, as 0.934, and in GHQ-28, Cranach's alpha as 0.563.

Data was analyzed by SPSS17 and Amos 5 software using descriptive statistics and path analysis.

Ethical considerations in this study concluded:
1. each of subjects who not volunteer to participate in the research was disregarded for this study.
2. The information related to each participant was secret and no organization or person with the exception of the authors reaches to these data.

Results

Mean, standard deviation, and independent sample t test of all participants' scores in variables of the study are presented in the table 1.

According to table 1, there were no significant gender differences in marital relationship status and happiness but in mental health scores, a difference between men and women was significant. Accordingly, the levels of mental health of infertile women are significantly lower than infertile men.

To analysis of the causal model of relation between marital relationship status, happiness, and mental health, path analysis was used. The result of path analysis for all participants (including men and women) is presented in the table 2.

Based on the table 2, a direct path of marital relationship status to happiness and a direct path of happiness to mental health were significant. Marital relationship status had relatively the low direct effect on happiness but happiness had almost the high direct effect on mental health. Fitness of the theoretical presented model for all participants (including men and women) was investigated by fitness indexes (38, 39). These results are presented in the table 3.

Based on the results of table 3, fitness of the theoretical causal model of the study was confirmed for all participants including men and women. The model with path coefficients is presented in the figure 2. Based on this model, marital relationship status had indirectly effect on mental health through happiness (indirect effect=0.119).

Also, the model of the study separately investigated men and women. The result of path analysis for women is presented in the table 4.

Based on the table 4, in women participants, the direct path of marital relationship status to happiness and the direct path of happiness to mental health were significant. Marital relationship status had relatively the low direct effect on happiness but happiness had almost the high direct effect on mental health. Fitness of the theoretical presented model for women participants was investigated by fitness indexes. These results are presented in the table 5.

Table 1: Mean and standard deviation of participants' scores in marital relationship status, happiness, and mental health in men and women

Variables	Mean			SD			t
	Men	Women	Total	Men	Women	Total	
Marital relationship status	39.192	38.307	38.689	6.048	7.014	7.046	2.077*
Happiness	41.788	38.243	39.712	17.200	16.081	16.560	-0.500
Mental health	25.038	30.995	28.386	14.982	15.125	15.273	-0.849

*; P=0.040.

Table 2: Standard regression weights of paths between variables in men and women

Paths		Estimate	Standard error
Marital relationship status	Happiness	0.193*	0.197
Happiness	Mental health	- 0.621 **[1]	0.059

*[1]; Minus in this table were used because of differences between scoring the instruments, it is not indicate the negative relation between the two concepts, *; P=0.028 and **; P=0.001.*

Table 3: Fitness indexes for the theoretical path model for the relation between marital relationship status, happiness, and mental health in all participants (including men and women).

Fitness indexes	Value	Appropriate range for fitness	Position of model
CMIN	1.996*	Lack of statistical significance	Fitness
TLI	0.924	> 0.90	Fitness
NFI	0.976	> 0.90	Fitness
CFI	0.987	> 0.90	Fitness
RMSEA	0.080	> 0.05 - 0.08	Fitness

; P=0.158, CMIN; Chi-square value, TLI; Tucker lewis index, NFI; Normed fit index, CFI; Comparative fit index and RMSEA; Root mean square error of approximation.

Fig 2: The causal model of relation between marital relationship status, happiness, and mental health in all participants (including men and women).

Table 4: Standard regression weights of paths between variables in women

Paths		Estimate	Standard error
Marital relationship status	Happiness	0.254*	0.241
Happiness	Mental health	- 0.697**	0.072

*; P=0.026 and **; P=0.001.*

Based on the results of the table 5, fitness of the theoretical causal model of the study in women participants was confirmed. The model with path coefficients is presented in the figure 3. Based on this model, in women participants, marital relationship status had the indirectly effect on mental health through happiness (indirect effect= 0.177). The result of path analysis for men participants is presented in the table 6.

Based on the table 6, in men participants, the direct path of marital relationship status to happiness was not significant but the direct path of happiness to mental health was significant. So, in men participants, marital relationship status had not a significant direct effect on happiness but happiness had almost the high direct effect on mental health. Fitness of the theoretical presented model for men participants was investigated by fitness indexes. These results are presented in the table 7.

Based on the results of table 7, the model of the study for men participants had a poor fitness. The model with path coefficients is presented in the figure 4. Based on this model, in men participants, the direct effect of marital relationship status on happiness and indirect effect of marital relationship status on mental health, through happiness, had not been confirmed.

Table 5: Fitness indexes for the theoretical path model for the relation between marital relationship status, happiness, and mental health in women

Fitness indexes	Value	Appropriate range for fitness	Position of model
CMIN	0.039*	Lack of statistical significance	Fitness
TLI	1	> 0.90	Fitness
NFI	0.999	> 0.90	Fitness
CFI	1	> 0.90	Fitness
RMSEA	0.000	> 0.05 - 0.08	Fitness

; P=0.843,CMIN; Chi-square value, TLI; Tucker Lewis index, NFI; Normed fit index, CFI; Comparative fit index and RMSEA; Root mean square error of approximation.

0.254   - 0.697

| Marital relationship status | — | Happiness | — | Mental health |

Fig 3: The causal model of relation between marital relationship status, happiness, and mental health.

Table 6: Standard regression weights of paths between variables in men

Paths		Estimate	Standard error
Marital relationship status	Happiness	0.052*	0.376
Happiness	Mental health	- 0.559**	0.098

*; P=0.700 and **; P=0.001.*

Table 7: Fitness indexes for the theoretical path model for the relation between marital relationship status, happiness, and mental health in men

Fitness indexes	Value	Appropriate range for fitness	Position of model
CMIN	3.370*	Lack of statistical significance	Fitness
TLI	0.243	> 0.90	Fitness
NFI	0.864	> 0.90	Fitness
CFI	0.874	> 0.90	Fitness
RMSEA	0.197	> 0.05 - 0.08	Fitness

; P=0.066, CMIN; Chi-square value, TLI; Tucker lewis index, NFI; Normed fit index, CFI; Comparative fit index and RMSEA; Root mean square error of approximation.

0.052

| Marital relationship status | — | Happiness | — | Mental health |

Fig 4: The causal model of relation between marital relationship status, happiness, and mental health in men.

Discussion

Although the main purpose of this study was not to compare infertile men and women in dependant variables of the study but the results showed significant differences in infertile men's and women's mental health. These findings of this study were in the line of studies showed that infertile women demonstrated more health impairment, compared with infertile men (40, 41). Also, this study showed that when participants' scores in dependent variables of the study were separated based on the gender factor, the results of the model of the study were varied for two genders. This data showed that only in women and not men marital relationship status had direct and indirect effects on mental health. Thus, these results showed that infertile women had poorer mental health compared with infertile men and marital relationship status with its effect on happiness was effective on this position of mental health in the infertile women but mental health and happiness of infertile men was

not affected by their marital relationship status. It seems that some cultural factors intervene in this finding, such as high social pressures on women and extreme expectancies of them about infertility compared with men. There is the considerable evidence that infertility elicits different experience and reactions in men and women. Women tend to experience more worry about the infertility and its treatment and more tend to assume personal responsibility about this problem. Moreover, the experience of anxiety, depression, low levels of self-esteem, and psychological adjustment is more observable in women than men during infertility investigations or treatments. But it does not mean that infertile men don't experience any stress or distress related to infertility. Historical studies on psychological effects of infertility are often concerned on women experiences of infertility, while men's psychological stresses related to infertility have been less considered. Indeed, infertility as a crisis in couple's life can negatively affect both partners' health and their relationships (5). This

thought was confirmed by this study. Based on the results of this study, all of the scores of marital relationship status, happiness, and mental health of both men and women were not considerably appropriate as an idealistic health position. Based on the findings, both infertile men and women experience almost equal negative emotions in their personal and marital life, that indicated almost in their equal scores in marital relationship status and happiness. But it seems that for infertile women not only their marital relationship and happiness have impaired but also they experience significant mental health problematic issues that are affected by their marital relationship status with its effect on their happiness. These results indicated that infertile women experience more social and cultural stresses than infertile men about their marital role (2) while mental health and happiness of infertile men are significantly less affected by these stress factors. Some of these stresses that affect infertile women, related to sense of unsafety about stability of the marital relationship after infertility diagnosis. In many cultures divorce or even polygamy may be pursued by husband when infertility of a woman was confirmed. Although the rates of these choices are not very considerable worry about the issue can annoy the infertile women (5).

The findings of this study confirmed the causal model of relationship between marital relationship status, happiness, and mental health in infertile individuals without consideration the gender factor. This model also was confirmed in women participants but not confirmed in men participants. Based on this model in infertile women, marital relationship status directly is effective on mental health with mediation of happiness. Also the results showed that in both infertile men and women, happiness has the direct effect on mental health. The findings are the line of several studies that have showed significant relationships between these three variables (15, 16, 19, 20, 24-27) but this study was the first study that investigated these relationships through a causal model and among infertile population. There is considerable explanation for the key role of infertile couples' marital relationship status in their happiness and mental health. When a couple encounters with infertility, each of them needs to gradually experience this shocking crisis and regulates his or her emotional distress related to infertility. If the couples success to resolve these emotional conflicts, after short time, they can recover their individual mental health and emotional well being and then can look for appropriate solutions for treatment of infertility. But if the relationships between couples included several conflicts and dissatisfactions - especially before infertility diagnosis-, this recovery may be not acquired (5). Based on the results of this study, it seems that women more need to a satisfying and supportive marital relationship status than men to resolve the crisis of infertility that can due to their more experience of social and cultural stresses about infertility and their higher levels of affective needs in marital relationship. For both men and women, their experience of happiness significantly predicts their mental health in exposure of infertility crisis. Several studies have confirmed that positive emotions, especially happiness, have significant effects on several aspects of mental health in general population. When individuals experience happiness in long term and high levels, their personal and social functions in many fields of their everyday life improve but when they experience low levels of happiness, many aspects of their personal and social life are affected by difficulties (13).

In psychological theories and studies about close relationships, it has repeatedly reminded that happiness cannot be understood without understanding close relationships. Very studies in the field of well-being have confirmed that happy people have satisfying relationships. Based on these studies, satisfaction of marital relationships is a strong predictor of happiness (42). Even in some psychological theory such as the Choice Theory the concepts of happiness, satisfying relationships (especially in marital relationships), and mental health were considered as almost equal concepts (43). Based on the choice theory as an important and valid theory in psychology and counseling (44), happy people actually are people with satisfying relationships because based on the choice theory, basic psychological needs of human can be only satisfied in healthy and relationships. Only in this type of relationships, positive emotions, especially happiness, can be expected because positive emotions, such as happiness and pleasure, are developed when the basic physical and psychological human needs are satisfied (45). Thus, mental health is considered as the consequent of happily satisfying relationships (46). The fitness of the model of this study confirmed this psychological theoretical basis for the

relationship between marital relationship status, happiness, and mental health in infertile population disregarding gender differences and also in women participants. Although this model was not confirmed in infertile men but it is necessary to investigate this model in this population with large sample size because in this study the number of men was less than women and this position may affect the results. In fact also it is reasonable that marital relationship status has more significant effect on happiness and mental health in infertile women but it seems that infertile men somewhat are affected by marital relationship status too.

Conclusion

The findings of this study revealed the importance of attention to psychological health of infertile individuals, especially in infertile women. Also, the findings suggest that it is useful to enter some components of marital relationship status that can improve and enhance the experience of happiness in infertile couple's relationships to mental health in these individuals. So, in the field of psychotherapy and counseling with infertile individuals probably couple therapy or couple counseling is better and more appropriate than individual therapy or counseling. Moreover, the authors suggest that psychological interventions by psychologist or counselors integrate and accompany with medical treatments presented by professional infertility centers.

To generalize these findings to Iranian population, we suggest further researches to assess the model of this study in other population of infertile couples and individuals. One of the most important limitations of this study was disregarding demographic components and personal characteristics such as highest group of age, duration of infertility, type of treatment, main reason of infertility belong to which gender. Most of these limitations were limitation of sufficient number of professional examiner to interview with subjects and invite them to participate in the study. The examiners of this research were only two psychologists, so in spite of considerable time spent for the sampling, the sample size of the study was not completely sufficient.

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Original Article

Effects of *In Vitro* Zinc Sulphate Additive to The Semen Extender on Water Buffalo (*Bubalusbubalis*) Spermatozoa before and after Freezing

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Abstract——————

Background: The objective of the study was to investigate the effects of *in vitro* zinc sulphate additive to semen extender on sperm parameters (progressive motility, viability, membrane integrity and DNA stability) after cryopreservation.

Materials and Methods: In this Prospective longitudinal laboratory study, semen samples of 5 buffalo bulls of 3-5 years old were collected at 5 different occasions from Iran, Urmia during summer and autumn 2011, 25 samples were used in each treatment. Sperm progressive motility, viability and abnormal morphology were measured before and at 0.5 (T_0), 1(T_1) and 2(T_2) hours after diluting semen(1:10 v/v) in Tris-citric acid based extender (without egg yolk and glycerol) at 37˚C containing none (control group), 0.072, 0.144, 0.288, 0.576 and 1.152 mg/L zinc sulphate to investigate dose and time effects. Next, a Tris-citric acid-egg yolk-glycerol extender (20% egg yolk and 7% glycerol) containing the same amount of zinc sulphate was prepared, diluted semen (1:10 v/v) was cooled and kept into a refrigerated chamber (4˚C) for 4 hours to equilibrate. Sperm progressive motility, viability, abnormal morphology, membrane integrity and DNA damage were estimated.The equilibrated semen was loaded in 0.5 ml French straws and frozen in liquid nitrogen. Later, the frozen semen was thawed and the same parameters as well as total antioxidant capacity (TAC) of the frozen-thawed semen were determined.

Results: The results showed that zinc sulphate additive at the rate of 0.288 mg/L gave a higher protection of sperm progressive motility (53.7 ± 1.8% vs. 40.5 ± 1.7%), viability (70.8 ± 1.8% vs. 60.1 ± 1.5%), membrane integrity (67.3 ± 1.6% vs. 56.6 ± 1.7%), DNA stability (10.1 ± 0.47% vs. 11.8 ± 0.33% damaged DNA) through the process of dilution, equilibration and freeze-thawing and caused a higher TAC level (81 ± 3.3% vs. 63 ± 3.2 µmol/L) after freez-thawing compared to the control group. Adding 0.576 and 1.152 mg/L zinc sulphate, however, was deleterious to the sperm and significantly reduced the studied sperm parameters.

Conclusion: Adding 0.288 mg/L zinc sulphate to the extender, compared to the control group, gives a better sperm preservation upon freezing processes which in turn, may results in higher semen fertility. But, addition of higher zinc sulphate concentrations (0.576 and 1.152 mg/L) are detrimental to buffalo spermatozoa.

Keywords: Semen, Zinc, Buffalo

Introduction

Semen cryopreservation is an important section of artificial insemination programs (1), it allows preservation of semen fertility for a long time. In this procedure, however, many spermatozoa lose their motility and other parameters which lead to a low fertility. A number of studies have demonstrated membrane lipid peroxidation as one of the causes of defective sperm function in liquid semen preserved at 4˚C (2) and in cryopreserved semen (3, 4). Some attempts (5-7) have been made to preserve sperm parameters, particularly sperm motility, by adding some elements (zinc, copper and selenium) (8) and materials (vitamins, antioxidants, amino acids and coenzymes) to the semen before freezing.

Zinc is one of the important trace elements in the body, while its deficiency causes infertility in most animals due to disorders of testes development, spermatogenesis (9), steroidogenesis through gonadotropic hormones secretion (10), genetic expression of steroid receptors (11), testosterone synthesis and function through Zn-dependent metalloenzyme 5α-reductase (12) and serum cholesterol level adjustment (13). The total zinc content in semen from mammalians is high and zinc has been found to be critical to spermatogenesis (14, 15) and sperm concentration (16).

Zinc ions affect the expression of some germ-cell specific-genes during the course of spermatogenesis in sheep (17). Zinc supplementation leads to improved fertility in zinc deficient animals by increasing concentration and motility of spermatozoa (18) and sperm membrane integrity and reducing sperm DNA damage in human subjects (19). Zinc also contributes to the stability of sperm chromatin and repair of DNA damage (20). Mechanisms responsible for DNA strand break and changes in the histone to protamine ratio in DNA of zinc deficient men has been described in detail (20). Zinc influences the fluidity of lipids, and thus the stability of biological membranes. It is involved in the formation of free oxygen radicals and may play a regulatory role in the process of capacitation and the acrosome reaction (21), sperm nuclear chromatin condensation and acrosinactivity (22). Zinc, at high concentrations in particular, may depress oxygen uptake (cell respiration) in the sperm cell and influence the sperm motility (23), zinc antioxidant capacity has been reported by many authors (24, 25). Alavi-Shoushtari et al.

observed that buffalo semen samples with higher seminal plasma zinc content (161.07 ± 1.36 vs. 136.42 ± 4.96 mg/L) had higher sperm motility, viability as well as lower abnormal morphology (26) and total antioxidant capacity (1.57 ± 0.01 vs. 1.23 ± 0.05 mmol/L) (27).

Information on the *in vitro* effects of zinc sulphate on buffalo bull spermatozoa is scarce. This prospective longitudinal laboratory study was conducted to investigate the effects of *in vitro* zinc supplementation on the progressive motility, viability, sperm membrane integrity and DNA stability of the spermatozoa in buffalo bulls' semen before and after semen equilibration and freezing with the aim of finding a practical protocol to have an improved semen quality after freeze-thawing.

Materials and Methods

Semen collection and processing

In this prospective longitudinal laboratory study, semen samples of 5 buffalo bulls of 3-5 years old kept in Buffalo Breeding Center of North-West of Iran, Urmia (37° 33´ N, 45° 4´ E), were collected by artificial vagina at 5 different occasions at weekly intervals during the late summer and autumn 2011. A total number of 25 samples were used in each examination. Semen samples were immediately transferred into a 37˚C water bath after a physical (appearance, homogeneity, density, color and volume) examination. Sperm gross and progressive motility, viability, and abnormal morphology of semen were evaluateded. Thin, watery specimens with low quality were discarded. These parameters (except gross motility) were measured within $0.5(T_0)$, $1(T_1)$ and $2(T_2)$ hours after diluting semen (1:10 v/v) in the Tris-citric acid extender traditioallyused in the center [(without egg yolk and glycerol), pH=7.1, osmotic pressure≡300 mosmolkg^{-1} and all the chemicals were purchased from Merck Co., Germany]. The extender consisting of Tris 2.66 g, glucose 1.2 g, citric acid 1.39 g, cysteine 0.139 g and distilled water in total volume of 100 ml that contained none (control, without zinc sulphate), 0.072, 0.144, 0.288, 0.576 and 1.152 mg/L zinc sulphate ($ZnSO_4$, $7H_2O$, ScharlauChemie S.A., Sentimental, Spain). Next, a Tris-citric acid-egg yolk-glycerol extender (above mentioned extender with 20% egg yolk and 7% glycerol, added in one step, as protectant and without antimicrobial agents) containing the same amounts of zinc

sulphate was prepared at room temperature. The semen samples were diluted at a rate of 1:10 v/v, (an approximate count of 12×10^6 sperm cells/mL), cooled to 4˚C within 2 hours, transferred to the equilibrium chamber of 4˚C and left for 4 extra hours to equilibrate. Sperm parameters (progressive motility, viability, membrane integrity and DNA damage) were estimated in equilibrated semen and semen was then loaded in 0.5 ml French straws and frozen over liquid nitrogen in static vapor up to –120˚C within 25 minutes before being plunged in the liquid nitrogen (28) and stored until further analysis. Later, the frozen semen was thawed in 37˚C water bath for 30 seconds, and the same parameters, as well as total antioxidant capacity (TAC) of the frozen-thawed semen, were determined.

Sperm functional assays

Sperm progressive motility, viability and abnormal morphology

Sperm progressive motility was estimated using a computer assisted system of analysis (CASA) [Hoshmand Fannavar (HF) CASA, version 6, Amirkabir Medical Engineering Co, Tehran]. After the samples were stained using eosin-nigrosin staining method (29), sperm viability was estimated by counting live and dead spermatozoa using a light microscope (Olympus BX41, Japan), at least 200 sperm cells were examined on each slide. Sperm cell abnormal morphology was also estimated.

Plasma membrane integrity

Hypo-osmotic swelling test (HOST) was used to examine membrane integrity of spermatozoa before and after freezing according to a method of Jeyendran et al. (30) as described by El-Sisy et al. (31). In brief, the hypo-osmotic solution (osmotic pressure≡190 mosmol kg^{-1}, Osmomat 030, Nr. 951211, Gonotec, Germany) was prepared by dissolving 0.73 g sodium citrate and 1.35 g fructose in 100 ml of distilled water. Hypo-osmotic solution (500 µl) was mixed with 50 µl of semen and incubated at 37˚C for 40 minutes. After incubation, a drop of semen sample was examined under a phase-contrast microscope (×400) (Olympus BX31, Olympus Optical Co., Japan) and 200 spermatozoa were counted in at least 5 different fields for their swelling characterized by coiled tail

indicating intact plasma membrane, which could be differentiated from abnormally coiled tails previously estimated.

Sperm DNA damage

DNA damage was detected using acridine orange staining technique, according to the method of Katayose et al. (32). Briefly, first, spermatozoa were smeared on the glass slide. After being air dried, the samples were treated with acid alcohol (methyl alcohol-glacial acetic acid 3:1, vol/vol) for 2 hours. Immediately after air drying, approximately 1 mL working solution including 0.019% acridine orange [3, 6-bis (dimethylamino) acridine and hemi (zinc chloride) salt (Sigma Chemical Co., St. Louis, MO, USA)] was mounted on each glass slide for 5 minutes at room temperature and the samples were then washed with distilled water. The samples were observed under a fluorescence microscope (Model GS7, Nikon Co., Japan) immediately after placing a coverslip on the slide. A total of 100 to 200 spermatozoa were observed and classified, like green (intact DNA) or red (damaged DNA), based on differences in their fluorescent color.

Total antioxidant capacity (TAC)

TAC of the frozen-thawed semen was assayed in duplicates using a commercial kit (Antioxidant Capacity Assay Kit, Cayman Chemical Co., Ann Arbor, MI, USA).

Statistical analysis

The data obtained from 25 semen samples from 5 bulls (total number of 125 assays for each estimation) were analyzed using SAS (Version 9.2, SAS Institute Inc., Cary NC.). Prior to analyses, data were checked using box plots to examine for errors and outliers. No outlier was detected. The parameters were analyzed using a linear mixed model (PROC MIXED) with the REPEATED command. The Kenward-Roger procedure was used to approximate the denominator degrees of freedom. The residuals were assessed visually by quantile-quantile plots for testing of the normality and also predicted values were plotted versus residuals to assess the homogeneity of variance. To meet the assumptions of the tests, the data for all parameters, except than that for TAC, were transformed by applying square root transformation. The initial

model for each parameter included treatment, stage and their interactions as fixed effects and buffaloes nested in treatment were considered as random effect. When a non-significant interaction term was detected, the model was re-run with the interaction effect excluded from the model. For each parameter several covariance structure between repeated measures were examined and the model which provided better fit according to minimum Akaike's Information Criterion (AIC) was used. Differences between least squares means were determined using the DIFF option and Bonferroni's correction was applied to pair-wise comparisons. All reported values are least squares means and statistical mean and standard error of mean (SEM). Effects were declared significant at p<0.05. This study was approved by the Ethic Committee of Urmia University.

Results

Freshly diluted semen

The results are summarized in tables 1 and 2. Adding 0.288 mg/L zinc sulphate to the extender preserved the sperm motility at T_1 and T_2 compared to controls (85.4 ± 1.2% vs. 84.8 ± 1.5% at T_0, 85.5 ± 1.1% vs. 80.8 ± 1.3% at T_1 and 85.7 ± 1.1% vs. 77.4 ± 1.5% at T_2) which was significant

(p<0.05) at T_1 and T_2 indicating that the highest value belongs to only at T_2, whereas shows a significant effect on sperm viability (86.3 ± 0.9%, p<0.05, Table 1). Immediately after adding 0.576 and 1.152 mg/L zinc sulphate (T_0) to the extender, the sperm motility (from 84.8 ± 1.5% in control to 80.7 ± 1.7% and 75.2 ± 1.2 % respectively) and viability (from 86.1 ± 1.2% in control to 83.5 ± 1.6% and 77.8 ± 1.2% respectively) were significantly (p<0.05) decreased as compared with the controls and continued decreasing as time passed (p<0.001, Table 1). Sperm abnormal morphology did not show significant variation.

Equilibrated diluted semen

In equilibrated diluted semen the highest sperm progressive motility (80.7 ± 1.9%), viability (84.1 ± 1.0%) and sperm membrane integrity (83.3 ± 0.9%) values were observed in 0.288 mg/L zinc sulphate, the number of intact DNA cells (green cells) in this diluted solution was not statistically different from control. Adding 1.152 mg/L zinc sulphate to the extender, however, significantly (p<0.001) reduced motility, viability and membrane integrity while increasing sperm cell DNA damagerate (Table 2).

Table 1: Effects of different zinc sulphate concentrations (mg/L) on the motility and viability (LS mean ± SEM) of spermatozoa in freshly diluted semen

Parameter	Time	Treatment						SE	P value		
		Control	0.072	0.144	0.288	0.576	1.152		TRT	STG	TRT×STG
Motility	T_0	84.8 ± 1.5[ab]	84.2 ± 1.4[ab]	85.0 ± 1.8[ab]	85.4 ± 1.2[a]	80.7 ± 1.7[b]	75.2 ± 1.2[c]	0.0025	<0.0001	<0.0001	<0.0001
	T_1	80.8 ± 1.3[ac]	81.0 ± 1.4[ac]	83.5 ± 1.6[ab]	85.5 ± 1.1[b]	78.2 ± 1.7[c]	71.7 ± 1.2[d]	0.0025			
	T_2	77.4 ± 1.5[a**]	78.9 ± 1.2[bc**]	81.6 ± 1.4[b]	85.7 ± 1.1[c]	72.7 ± 1.0d[***+]	67.9 ± 1.2e[***+]	0.0025			
Viability	T_0	86.1 ± 1.2[ab]	86.3 ± 1.2[ab]	87.3 ± 1.5[a]	87.2 ± 1.0[ab]	83.5 ± 1.6[b]	77.8 ± 1.2[c]	0.0016	<0.0001	<0.0001	0.0220
	T_1	83.8 ± 1.5[ab]	84.2 ± 1.2[a]	85.6 ± 1.4[a]	86.8 ± 1.0[a]	80.2 ± 1.6[b]	74.0 ± 1.3[c*]	0.0016			
	T_2	81.7 ± 1.1[ac*]	80.2 ± 1.4[ac**+]	83.5 ± 1.2[ab]	86.3 ± 0.9[b]	78.7 ± 1.2[c*]	71.7 ± 0.9[d***]	0.0016			

T_0; 0, T_1; 60, T_2; 120 minutes after diluting semen, [a, b, c, d, e]; Signify a significant difference (p<0.05) within the same row, *; Signifies a significant difference (p<0.05) with T_0 values, **; Signifies a significant difference (p<0.01) with T_0 values,***; Signifies a significant difference (p<0.001) with T_0 values, +; Denotes a significant difference (p<0.05) with T_1 values, LS; Least square, TRT; Treatment and STG; Stage.

Table 2: Effect of different zinc sulphate concentrations (mg/L) on sperm parameters (LS mean ± SEM) after equilibrium and thawing* time

Parameter	Stage	Treatment						SE	P value		
		Control	0.072	0.144	0.288	0.576	1.152		TRT	STG	TRT×STG
Motility	EQ	71.4 ± 1.9ᵃ	73.5 ± 1.8ᵃᵇ	78.3 ± 1.6ᵇᶜ	80.7 ± 1.9ᶜ	64.1 ± 1.6ᵈ	56.9 ± 1.3ᵉ	0.0081	<0.0001	<0.0001	<0.0001
(%)	TW	40.5 ± 1.7ᵃ	43.2 ± 2.0ᵃᵇ	47.8 ± 1.9ᵇ	53.7 ± 1.8ᶜ	32.9 ± 1.7ᵈ	27.6 ± 1.6ᵉ	0.0081			
Viability	EQ	78.0 ± 1.5ᵃ	79.1 ± 1.3ᵃ	80.4 ± 1.2ᵃ	84.1 ± 1.0ᵇ	77.4 ± 1.1ᵃ	73.5 ± 1.3ᶜ	0.0036	<0.0001	<0.0001	<0.0001
(%)	TW	60.1 ± 1.5ᵃ	62.0 ± 1.6ᵃᵇ	65.7 ± 1.8ᵇ	70.8 ± 1.8ᶜ	59.6 ± 1.8ᵃ	53.7 ± 1.9ᵈ	0.0036			
Membrane	EQ	79.0 ± 1.2ᵃᶜ	79.4 ± 1.3ᵃᶜ	81.7 ± 0.9ᵃᵇ	83.3 ± 0.9ᵇ	77.8 ± 1.1ᶜ	73.8 ± 1.5ᵈ	0.0036	<0.0001	<0.0001	<0.0001
Integrity (%)	TW	56.6 ± 1.7ᵃ	58.9 ± 1.7ᵃ	62.1 ± 1.8ᵇ	67.3 ± 1.6ᶜ	54.9 ± 1.7ᵃ	48.8 ± 2.2ᵈ	0.0036			
Damaged	EQ	3.0 ± 0.25ᵃ	3.4 ± 0.21ᵃ	3.4 ± 0.23ᵃ	3.0 ± 0.25ᵃ	3.5 ± 0.24ᵃᵇ	4.14 ± 0.21ᵇ	0.0025			
DNA (%)	TW	11.8 ± 0.33ᵃ	11.6 ± 0.43ᵃ	10.3 ± 0.49ᵇ	10.1 ± 0.47ᵇ	12.2 ± 0.33ᵃᶜ	13.3 ± 0.26ᶜ	0.0025	<0.0001	<0.0001	0.3084
TAC (μmol/L)	TW	63 ± 3.2ᵃ	64 ± 3.7ᵃ	77 ± 3.4ᵇ	81 ± 3.3ᵇ	57 ± 2.7ᵃᶜ	49 ± 2.4ᶜ				

*; 30 seconds in a 37˚C water bath, EQ; After equilibrium, TW; After thawing, TAC; Total antioxidant capacity, ᵃ, ᵇ, ᶜ, ᵈ, ᵉ; Signify a significant difference (p<0.05) within the same row, LS; Least square, TRT; Treatment and STG; Stage.

Frozen-thawed semen

In frozen-thawed semen, the highest sperm motility (53.7 ± 1.8%, p=0.000), viability (70.8 ± 1.8 %, p=0.001) and sperm membrane integrity (67.3 ± 1.6%, p<0.05) values, were observed in 0.288 mg/L zinc sulphate, indicating that this concentration is significantly higher than the other treatments. Furthermore, the least number of DNA damaged cells (p<0.01) was obtained in this treatment (Table 2). Moreover, adding 0.144 and 0.288 mg/L zinc sulphate to the freezing extender, significantly (p<0.05) increased the total antioxidant capacity of the frozen-thawed semen as compared to the control (77 ± 3.4 and 81 ± 3.3 μmol/L vs. 63 ± 3.2 μmol/L, respectively).

Discussion

Sperm motility and fertility is reduced by sperm processing and cryopreservation involved in the semen preservation in artificial insemination programs (33-35), while the antioxidant defense capacity of the semen is also decreased (36-38). Furthermore, antioxidants supplementation of semen extenders during liquid storage or cryopreservation of the bull (5) and the boar (39) spermatozoa and its beneficial effects have been reported. Therefore, this study was designed to investigate the effects of different doses of zinc sulphate supplementation added tothe extender on the quality of the sperm

during freezing.

The extender used in this study was Tris-citric acid base extender that had been used in the Buffalo Breeding Center for some years but the egg yolk, glycerol and antibiotics were not added to it in the first stage, in order not to interfere with the examination and in the view that they might have some effect on the sperm parameters. Andrabi (40) in a review of several studies concluded that Tris–citric acid might provide the best satisfactory buffering system to improve the post-thaw freezability and motion charactericties of buffalo spermatozoa. Akhter et al. (41) compared effects of soya lecithin based extender (Bioxcell®), Tris-citric acid egg yolk, egg yolk-citrate and skim milk extenders on buffalo spermatozoa stored at 5˚C for 5 days and observed that motility, viability and plasma membrane integrity of buffalo bulls were similar in Tris-citric acid egg yolk, milk and Bioxcell on the first and third day of storage but on the fifth day they were better in Bioxcell. However, Rasul et al. (42) reported that Tris-citrate was better than Tris-citric acid, Tris-Tes and Tris-HEPES buffers in terms of improving post-thaw motion characteristics of buffalo spermatozoa.

In this study a CASA was used for the sperm progressive motility evaluation, but since the system was not calibrated for buffalo sperm, progressive motility of 25 semen samples were examined

visually coincident with the system evaluation and the results were compared. The difference of two readings was less than 3%. So, only this parameter of motility was used in the study. The other parameters of motility were not used because their reliability for buffalo semen was not assured.

Sperm progressive motility, viability and abnormal morphology were evaluated immediately after the semen being diluted with the extender containing different dozes of zinc sulphate at different time intervals in order to assess the dose and time effect of zinc sulphate on spermatozoa quality. In all the examinations, all the treated samples were compared with their own control which had no zinc sulphate in its extender.

In selecting zinc sulphate dose two points were considered: 1. molecular weight (MW) of "zinc sulphate, $7H_2O$" is nearly 288 (287.57) grams with 65 gram zinc content, 2. Storage media, commercially prepared for *in vitro* fertilization (IVF) (Ham's F-10), usually contains 0.0288 mg/L (1:10000 MW) zinc sulphate, $7H_2O$ in their composition (43). The dose of 0.288 mg/L (1:1000 MW) zinc sulphate, $7H_2O$, 10 times more than that in storage media, was selected as a base to creat a zinc sulphate dose high enough to affect the spermatozoa;it was two times divided into halves [0.288:2(0.144) and 0.288:4(0.072) mg/L) or doubled 0.288×2(0.576) and 0.288×4 (1.152) mg/L] for dose selection in this study to gain a reasonable range of zinc sulphate concentrations in the extender. This selection worked well in the study since low doses (0.072 and 0.144 mg/L) were not as effective as 0.288 mg/L dose and high doses (0.576 and 1.152 mg/L) showed detrimental effects.

The present study revealed that supplementation of zinc sulphate improved the quality of freshly diluted and frozen semen of buffalo bulls as compared to the non-supplemented control group. An earlier study on buffalo semen by Alavi-Shoushtari et al. (26) showed significant correlations between zinc content of seminal plasma and sperm parameters including progressive motility and viability, suggesting that zinc ions may be positively correlated with semen quality.Some authors have suggested that zinc, as an antioxidant agent, particularly as a co-factor of copper/zinc superoxide dismutase (Cu/Zn SOD), plays a major role in the protection of spermatozoa against peroxidative damages of and reactive oxygen species (ROS) (44). This is shown by adding Zn to the testicular cell

culture and increased enzymatic activity of Cu/Zn SOD (45). Our results revealed that adding zinc sulphate to semen extender improves semen TAC after freezing, in a dose dependant manner. These results were in consistent with the study of Omu et al. (19), who reported zinc therapy-associated improvement in sperm parameters includes an increase in the seminal antioxidant capacity and reduction of oxidative stress status. Alvarez and Storey (46) demonstrated that cryopreservation enhanced lipid peroxidation due to the loss of SOD activity during the freezing process. A better preserved sperm progressive motility, viability and membrane integrity after semen equilibration and freezing observed in this study could be attributed to increased antioxidant capacity of zinc ions, as reported by Hidiroglou et al. (47). Furthermore, this antioxidant activity might have been responsible for lower cases of DNA damage observed in this study. This could also be the effect of zinc antioxidant capacity on ROS released from the sperm mitochondria or on preventing production of lipid peroxidation products during freezing, which could cause DNA damage (DNA strand breakage) (20).

As most of sperm functions originated from the membrane, zinc may improve sperm functional capacity by creating a favorable environment (19, 48). In this study, the higher membrane integrity rate after adding 0.288 mg/L of the extender before and after semen freezing may be due to membrane stabilizing effect of zinc which is the result of interacting with some functional group of the intrinsic component of sperm membrane that has been reported by Sorensen et al. (14). We also observed a high percentage of membrane integrity, progressive motility and viability in test group of 0.288 mg/L, before and after freezing. As reported by Kumar et al. (49), sperm motility and viability are dependent upon membrane transport rate affected by zinc concentration.

A low percentage of motile sperms after adding high levels of zinc sulphate (0.576 and 1.152 mg/L) to the extender observed in this study could be due to an elevated free zinc fraction and its subsequent uptake by spermatozoa (50) and reduction of oxygen consumption, since high levels of zinc in semen impairs the oxygen consumption of sperms (14). This elevated free zinc fraction may be accounted for lower viability and membrane integrity rate and for higher

cases of DNA damage as recorded in this study.

In this study zinc is believed to be important for membrane and chromatin stability and sperm motility (47). The high sperm viability values obtained by adding 0.144 and 0.288 mg/L of zinc sulphate may be due to the membrane stabilizing action of zinc to prevent leakage of enzymes, proteins and other vital components of the sperm in order to extend the functional life of sperm (49). Betteger and O'Dell (51) have reported that zinc stabilizes ribosomes, lysosomes, DNA and RNA that result in survival and normal functioning of the sperm. They further found that zinc protects sperm from free radicals that induce damages through their scavenging properties.

Storage time affected quality of the freshly diluted semen by deteriorating sperm progressive motility and viability which was more evident in the control group and low doses of zinc sulphatesupplementation groups, while adding 0.288 mg/ml zinc sulphate to the extender showed significantly better result. In equilibrated semen some spermatozoa did not tolerate the process. This was evident by lower semen quality (Table 2). Many sperms were damaged during the process of freezing which led to lower percentage of sperm progressive motility, viability, and membrane integrity, lower total antioxidant capacity and higher DNA damaged cells. Adding 0.288 mg/ml zinc sulphate to the extender significantly reduced the cell damage during freezing process, while the lower doses were not so effective and higher doses had adverse effect.

Conclusion

The results showed that 0.288 mg/L zinc sulphate improve to the sperm quality (progressive motility, viability, membrane integrity and total antioxidant capacity) preservation upon freezing processes, however, we suggest a bigger sample population to achieve a definite statement. Our finding also revealed that zinc affects the cell membrane and leads to a lower degree of sperm DNA damage after semen freeze-thawing, which in turn, results in higher semen fertility. However, addition of higher zinc concentrations (0.576 and 1.152 mg/L) are detrimental to spermatozoa.

Acknowledgements

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Original Article

Expression of RFamide-Related Peptide-3 (RFRP-3) mRNA in Dorsomedial Hypothalamic Nucleus and KiSS-1 mRNA in Arcuate Nucleus of Rat during Pregnancy

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Abstract

Background: RFamide-related peptide-3 (RFRP-3) and kisspeptin (KiSS-1) are known to respectively inhibit and stimulate gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in rat. The aim of the present study was to evaluate the relative mRNA expression of RFRP-3 and KiSS-1 in the hypothalamus of pregnant rats.

Materials and Methods: In a randomized controlled experimental study, the exact pregnancy day of 18 Sprague-Dawley rats were confirmed using the vaginal smear method and were equally assigned to three groups of days 7, 14 and 21 of pregnancy. Four non-pregnant female rats were ovariectomized and assigned as the control group. All rats were decapitated, and the dorsomedial hypothalamic nucleus (DMH) and the arcuate nucleus (ARC) for detection of KiSS-1 mRNA were separated from their hypothalamus to detect RFRP-3 and KiSS-1 mRNA respectively. Then, their relative expressions were compared between control and pregnant groups using real-time polymerase chain reaction (PCR).

Results: The relative expression of RFRP-3 mRNA in DMH did not change significantly during pregnancy (p>0.01). However, the relative expression of KiSS-1 mRNA in ARC was at its highest in day 7 of pregnancy and decreased until day 21 of pregnancy (p<0.01).

Conclusion: Decrease in GnRH and LH secretion during the pregnancy of rat may be controlled by constant expression of RFRP-3 mRNA and reduced expression of KiSS-1 mRNA in hypothalamus.

Keywords: KiSS-1, RFamide-Related Peptide-3, Pregnancy, Dorsomedial Hypothalamic Nucleus, Arcuate Nucleus

Introduction

There is no follicular development during pregnancy in the rat compared to the changes during the estrous cycle (1). During pregnancy, it has been documented that serum luteinizing hormone (LH) levels tend to decrease, reaching its lowest at mid-pregnancy and tend to recover by the end of gestation (2, 3). During the first 11 days of pregnancy in rat, serum LH concentration was shown to be higher than the period between days 13-19. A progressive increase then occurs beginning on day 20 and continuing to term, but is not contiguous with the postpartum ovulation inducing surge of LH (3). Kisspeptin and RFamide-related peptide-3 (RFRP-3) were recognized as regulators of gonadotropin releasing hormone (GnRH) and LH secretion in several species, including the rat (4).

Kisspeptins belong to a family of peptides which are encoded by the *KiSS-1* gene and are natural ligands of the G protein-coupled receptor 54 (GPR54). Kisspeptin has a fundamental role in the control of gonadal axis (5, 6). It has been shown that kisspeptin neurons stimulate LH release by affecting GnRH neurons (7). This is based on the observation that the excitatory effect of kisspeptin on gonadotropin secretion was inhibited by GnRH antagonists (8). Almost all GnRH neurons express GPR54 (9) and many kisspeptin neurons in rats express estradiol receptor α (ERα) (10). Thus, it is possible that estrogen effects on GnRH neurons are mediated through these cells. KiSS-1 mRNA and encoded peptide were detected in the arcuate nucleus (ARC) and anteroventral periventricular nucleus (AVPV) of rodents using immunohistochemistry and in situ hybridization (11, 12). Kisspeptin neurons of the ARC might be the GnRH pulse generating center while AVPV might have a role in preovulatory GnRH/LH surge (13). KiSS-1 mRNA levels in AVPV was highest during the proestrus and lowest during metestrus (14). Furthermore, the level of KiSS-1 mRNA in ARC was highest during diestrus and lowest during proestrus (14), milking (15, 16) and malnutrition condition (17).

Gonadotropin-inhibitory hormone (GnIH) is a novel hypothalamic neuropeptide was discovered in birds as an inhibitory factor for gonadotropin release (18). RFRP-3 is a mammalian GnIH ortholog that inhibits gonadotropin synthesis and release in mammals through actions on GnRH neurons and gonadotropes, mediated via the GnIH receptor (GnIH-R), GPR147 (19). This peptide, was identified in the brain of rodents, modulates the negative feedback effect of estrogen on gonadotropin secretion (20). The RFRP-ir cells, clustering in the dorsomedial nucleus of the hypothalamus (DMH), were identified in hamsters, rats and mice (20-22). Inhibitory effects of RFRP on pituitary gonadotropins decreases reproductive activity of male and female rats (23, 24), and sheep (25, 26). Axons of RFRP neurons are projected to GnRH neurons in rodents (20) and RFRP-3 peptide has an inhibitory effect on GnRH neurons in mouse (27) and rat (28). Moreover, RFRP cells in hamster (20) and mouse (27) express ERα and administration of estradiol 17β can dramatically decrease prepro-RFRP mRNA in ovariectomized rats (27).

The aim of the present study was to evaluate the relative expression of RFRP-3 and KiSS-1 mRNAs in the hypothalamus of pregnant rats on days 7, 14 and 21 after mating. To achieve this goal, we need to determine pregnancy earlier than day 7 after mating with high accuracy and without using hormonal estrous synchronization. Hitherto, different non-invasive methods such as vaginal plug observation (day 1 after mating) (29), ultrasonography (day 8 after mating) (30), observation of abdominal distention and fetal palpation (day 13 after mating) (31) were presented for pregnancy detection in rat. For the first time, we present a novel low-cost and noninvasive method to increase the chances of making rats pregnant on day 5 after mating, and to determine the exact time of their pregnancy.

Materials and Methods

Study 1: early pregnancy detection

In a randomized controlled experimental study, 40 mature female Sprague-Dawley rats (body weight 150-250 g) were selected and housed in The Laboratory Animal Center of

Shiraz University of Medical Sciences, Shiraz, Iran. The animals were housed in standard cages, six per cage, in a controlled temperature room (22˚C), with a 12 hours light and 12 hours dark cycle. Standard laboratory chow and tap water were available ad libitum.

Phases of their estrous cycle were determined by microscope observation of their vaginal smears (32). The rats at the proestrous or estrous stage were transferred to the cage of mature male rats (body weight 250-350 g) with 3:1 ratio and left overnight. The presence of vaginal plug was recorded the next morning and female rats were separated from the males. On days 4 and 5 after estrus, vaginal smear was evaluated once again and their cellular characteristics were determined under light microscope. Finally, all females were checked for pregnancy. Abdomen enlargement of female rats on day 16 after mating and/or post-parturition observation of their litters were considered as positive pregnancy. All the above was repeated three times.

The rats were assigned into three groups. The first group was the rats with diestrous cell characteristics in day 4 and metestrous/diestrous cell characteristics in day 5. The second group was the rats with metestrous cell characteristics in day 4 and metestrous/diestrous cell characteristics in day 5. The other cell characteristics in days 4 and 5 were assigned to the third group.

Study 2: expression of RFRP-3 and KiSS-1 mRNA in hypothalamus

Animals, experimental groups, and sampling

Twenty two adult (3-4 months old) female Sprague-Dawley rats (*Rattus norvegicus*) weighing between 170 and 220 g were used in the present randomized controlled experimental study. The rats were randomly selected and housed in The Laboratory Animal Center of Shiraz University of Medical Sciences, Shiraz, Iran under controlled temperature (22˚C) and lighting (12:12 light to dark ratio; light on at 7:30 AM) conditions. The rats were housed in compliance with the recommendations of The Animal Care Committee of the Shiraz University of Medical Sciences. All experimental procedures were carried out between 12.00-2.00 PM. The exact

pregnancy day of the 18 rats was confirmed using the vaginal smear method (study 1). The rats were then randomly assigned in three equal groups of 7, 14 and 21 days of pregnancy (n=6).

Four ovariectomized rats, selected randomly, were used as the control group. The rats were anesthetized by an intraperitoneal injection of ketamine (100 mg/kg, Woerden, Netherlands) and xylazine (7 mg/kg, Alfazyne, Woerden, Netherlands) and ovariectomized through ventral midline incision. Further procedures were carried out after a 2-week recovery period.

The pregnant and ovariectomized rats were decapitated and brains were removed immediately. Pregnancy of 18 rats was confirmed with certainty by observing their pregnant uterus. The diencephalon was dissected out by an anterior coronal section, anterior to the optic chiasm, and a posterior coronal cut at the posterior border of the mammillary bodies. To separate ARC from AVPV, a third coronal cut was made through the middle of the optic tract, just rostral to infundibulum (33). The specimens consisting of ARC and DMH were stored in liquid nitrogen until further analysis.

Real-time polymerase chain reaction (PCR)

Total RNA was extracted, using the RNX-Plus buffer (Cinnagen, Tehran, Iran). Briefly, the tissue (100 mg) was ground in liquid nitrogen, transferred to RNX-Plus buffer (1 mL) in an RNase-free microtube, mixed thoroughly, and kept at room temperature for 5 minutes. Chloroform (0.2 mL) was added to the slurry and mixed gently. The mixture was centrifuged at $12,000 \times g$ (4˚C) for 20 minutes and the supernatant was transferred to another tube and precipitated with an equal volume of isopropanol for 15 minutes. The RNA pellet was washed with 75% ethanol and quickly dried and re-suspended in 50 µL RNase-free water. The purified total RNA was quantified by Nano-Drop ND 1000 spectrophotometer (Nano-Drop Technologies, Wilmington, DE, USA). The DNase treatment was carried out using the DNase kit (Fermentas, St. Leon-Roth, Germany) according to the manufacturer's instructions. The DNase-treated RNA (3 µg) was used for the first strand cDNA synthesis, using 100 pmol oligo-dT, 15 pmol dNTPs, 20 U RNase inhibitor,

and 200 U M-Mulv reverse transcriptase (Fermentas, St. Leon-Roth, Germany) in a 20 µL final volume. Primers were designed using Allele ID 7 software (Premier Biosoft International, Palo Alto, USA) for the reference gene, KiSS-1 (NM_181692) and RFRP-3 (NM_023952). The rat *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) gene (M32599) was used as the reference gene for data normalization (Table 1). Relative real-time PCR was performed in a 20 µL volume containing 1 µL cDNA, 1X Syber Green buffer and 4 pmol of each primer. The amplification reactions were carried out in a Line-Gene K thermal cycler (BIOER Technology Co., Ltd, Hangzhou, China) under the following conditions: 2 minutes at 94˚C, 40 cycles of 94˚C for 10 seconds, 57˚C for 15 seconds, and 72˚C for 30 seconds. After 40 cycles, the specificity of the amplifications was tested by analyzing melting curves with the temperature ranging from 50˚C to 95˚C. All amplification reactions were repeated 3 times under identical conditions, including a negative control and 5 standard samples. To ensure that the PCR products were generated from cDNA, but not the genomic DNA, proper control reactions were implemented in the absence of reverse transcriptase. For quantitative real-time PCR data, the relative expression of KiSS-1 was calculated based on the threshold cycle (C_T) method. The C_T for each sample was calculated, using Line-gene K software (34). Accordingly, the fold expression of the target mRNAs over the reference values was calculated by the equation $2^{-\Delta\Delta CT}$ (35), where ΔC_T is determined by subtracting the corresponding *GAPDH* C_T value (internal control) from the specific C_T of the target (KiSS-1 or RFRP-3). The $\Delta\Delta C_T$ was obtained by subtracting the ΔC_T of each experimental sample from that of the control (ovariectomized rats).

Table 1: Sequences of real time polymerase chain reaction (PCR) primers for evaluation of the relative expression of RFRP-3 and KiSS1 genes in rat

Primer	Sequence	Amplicon length (bp)
KiSS1-F	TGCTGCTTCTCCTCTGTG	116
KiSS1-R	CCAGGCATTAACGAGTTCC	
RFRP-3-F	CTCAGCAGCCAACCTTCC	165
RFRP-3-R	AAACCAGCCAGTGTCTTG	
GAPDH-F	AAGAAGGTGGTGAAGCAGGCATC	112
GAPDH-R	CGAAGGTGGAAGAGTGGGAGTTG	

KiSS1; Kisspeptin, RFRP-3; RFamide-related peptide-3 and GAPDH; Glyceraldehyde-3-phosphate dehydrogenase.

Statistical analysis

In study 1, relationship between the cell characteristics of vaginal smear (proestrus, estrus, metestrus and diestrus) on days 4 and 5 after mating and positive and negative results of pregnancy were compared using the Chi-square test (SPSS for Windows, version 11.5, SPSS Inc, Chicago, Illinois). Percent of pregnant rats between three groups were analyzed using one-way analysis of variance (ANOVA) (SAS 9.1 SAS Institute Inc., Cary, NC). Tukey post hoc test was used for comparison of means within the groups. Values of p≤0.05 were considered significant.

In study 2, the data on relative expression of *KiSS-1* and *RFRP-3* genes were subjected to the test of normality and analyzed by one-way ANOVA, and mean separation was performed by Tukey's test at p=0.01. Group means and their standard errors are reported in the text and figures (GraphPad Prism v 5.01, GraphPad software Inc., San Diego, CA, USA).

Results

In study 1, there was a positive association between pregnancy of rats and vaginal smear cell characteristics of metestrus (the same proportion among leukocytes, cornified and nucleated epithelial cells) or diestrous (a predominance of leukocytes) stages observed in days 4 and 5 after mating (p=0.001). If diestrous cell characteristics were observed in vaginal smear on day 4 after mating, and metestrous or diestrous cell characteristics were detected on day 5 after mating, 78.4% of rats would be pregnant. The cell characteristics of vaginal smear on days 4 and 5 were compared with cell characteristics of vaginal smear in different stages of estrous cycle in rat is shown in fig 1. Moreover, if metestrous cell characteristics were observed on day 4 after mating and metestrous or diestrous cell characteristics were detected on day 5 after mating, accuracy of pregnancy detection dropped to 60% which was not significantly different with the previous case (diestrous on day 4 and metestrous/diestrous on day 5). Only 16.7% of pregnant rats showed other cases of cellular characteristics of estrous cycle on days 4 and 5 after mating with chances to be pregnant was less than the two previous cases (p<0.05).
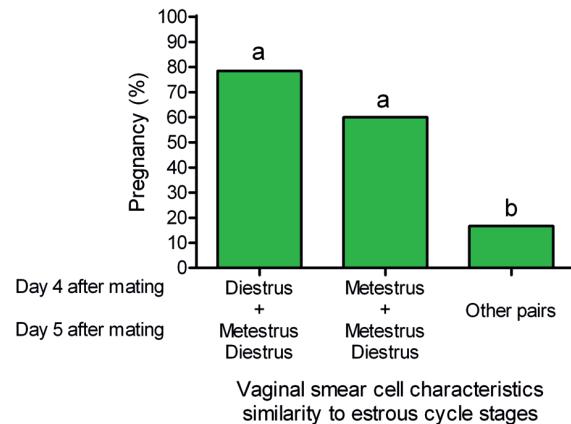

Fig 1: Accuracy of rat pregnancy detection by vaginal smear evaluation on days 4 and 5 after mating. a, b; Bars labeled with different letters are significantly different from each other at p<0.05.

In study 2, the mean and standard error of relative expression of RFRP-3 mRNA in DMH did not change during pregnancy (p>0.01, Fig 2). However, the relative expression of KiSS-1 mRNA in ARC was at its highest on day 7 of pregnancy and decreased until day 21 of pregnancy (p<0.01, Fig 3).
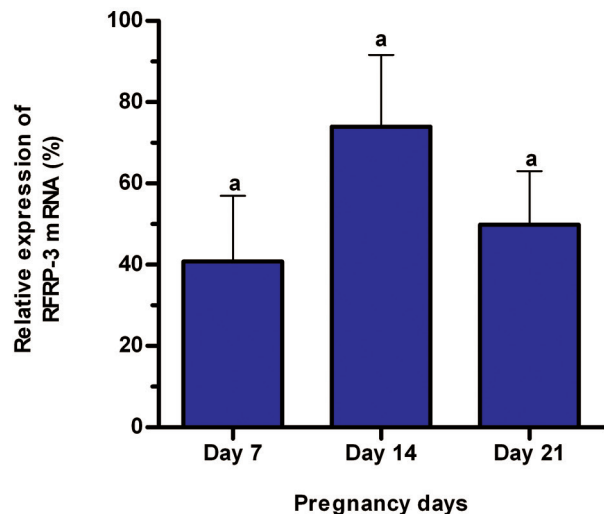
Fig 2: Effect of pregnancy on the relative expression of RFamide-related peptide-3 (RFRP-3) gene (mean ± SE) in the dorsomedial hypothalamic nucleus (DMH) rats (n=6 for each pregnancy day). a; Different letters indicate significant difference (p<0.01).
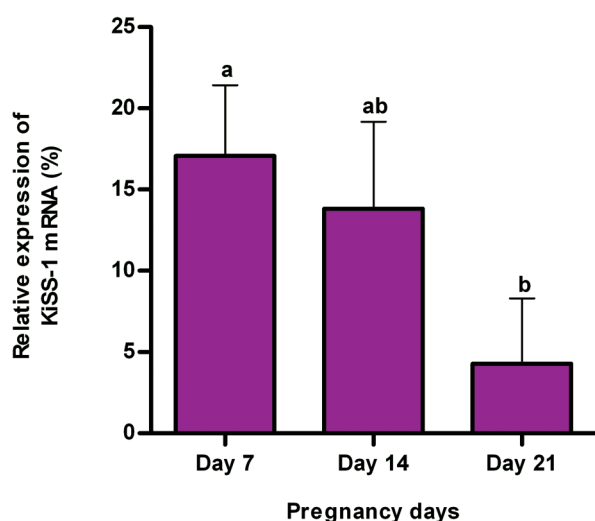
Fig 3: *Effect of pregnancy on the relative expression of KiSS-1 gene (mean ± SE) in the hypothalamic arcuate nucleus (ARC) of rats (n=6 for each pregnancy day). a, b; Different letters indicate significant difference (p<0.01).*

Discussion

The relative expression of KiSS-1 mRNA in ARC was at its peak in the first week of pregnancy and decreased 4-fold in the third week of pregnancy in rat. In contrast to our results, Roa et al. (36) reported that KiSS-1 mRNA increased during the pregnancy in rat brains. In humans, kisspeptin levels increased by 940-fold in the first trimester in comparison with non-pregnant woman and further increased to some 7000-fold higher in the third trimester (37). Level of expression of KiSS-1 mRNA is higher in the first trimester placenta than in term placenta in humans (38), apparently contrasting with higher circulating kisspeptin levels reported during pregnancy (37). Considering the ability of systemically delivered KiSS-1 peptides to release LH (8, 39-42), this phenomenon seems to be at odds with the reported increase in serum kisspeptin concentrations in human pregnancy (37). Although human and rat differ with regard to length of gestation and placental structure, the spatial and temporal expression of KiSS-1 mRNA are similar. Kisspeptin and its receptors are detected in rat trophoblast (43). In specific, KiSS-1 mRNA is expressed in the trophoblast giant cells of the rodent placenta (44), which are responsible for early invasion of spiral arteries and replacement of the endovasculature. These cells have the same functional phenotype as the human extravillous trophoblasts. As in humans, levels of KISS-1 and its receptor gradually decline during placental maturation and are not detectable at embryonic day 18.5 (44). The finding of the highest expression level of KISS-1 mRNA in trophoblast cells during the first trimester in humans and at day 12.5 of pregnancy in rodents and decrease in day 15.5 to no detectable expression on day 18.5 of pregnancy in rat coincides with the time of peak trophoblast invasion when regulation of this process is of critical importance (45, 46).

It has been shown that the number of GnIH neurons have a positive correlation with plasma progesterone concentration (47) and that GnIH neurons are regulated by progesterone (48). Responsiveness to RFRP-3 mRNA at pregnancy may derive from the combined exposure to high levels of progesterone and suppression of LH levels during pregnancy in rat (49). On the other hand, total cortisol and progesterone increased significantly from one trimester of pregnancy to the next in humans (50). Increase of glucocorticoids caused an increase in RFRP that contributes to hypothalamic suppression of reproductive function in rat (51).

Conclusion

Decrease of GnRH and LH secretion during rat pregnancy may be controlled by constant expression of RFRP-3 mRNA and reduced expression of KiSS-1 mRNA. On the other hand, methods of early detection of pregnancy and exact determination of pregnancy time in rats are widely applied on pregnant rats. Our presented method, in addition to increasing the probability of rat pregnancy after the first mating event, can detect pregnancy with an accuracy of more than 60% on day 5.

Acknowledgements

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Case Report

Early Pregnancy Loss Following Laparoscopic Management of Ovarian Abscess Secondary to Oocyte Retrieval

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Abstract
Severe pelvic infections following ultrasound-guided transvaginal oocyte retrieval (TVOR) are rare but challenging. Ovarian abscess formation is one of the consequences and management of such cases as highly debated in pregnant patients. In this case report, an early fetal loss following laparoscopic management of ovarian abscess is described and possible etiologies are discussed.

Keywords: Abscess, Laparoscopy, Oocyte Retrieval, Pelvic Infection

Introduction

Pelvic infections following ultrasound-guided transvaginal oocyte retrieval (TVOR) are rare complications with an incidence of 0.6% (1). The procedure is generally considered safe; however, vaginal flora acts as the main reservoir for microorganisms and bacterial inoculation via retrieval needle is possible.. Pelvic abscess formation is very rare, and until now, 8 cases have been reported in the literature (2-9). There is no standard approach available and management becomes more complicated, especially in pregnant patients. Pelvic infections could be a potential threat to an early pregnancy, and hence an early diagnosis and a thorough management are of paramount importance both for the patient and the ongoing pregnancy. This case report intends to discuss different treatment strategies and to question their reliabilities, especially during early pregnancy period.

Case Report

A 26-year-old, nulliparous woman underwent *in vitro* fertilization (IVF) for male factor subfertility in an assisted reproduction unit, Ankara, Turkey. Her medical history was unremarkable and physical examination was normal. Basal ultrasound examination on the 3rd day of menstrual cycle revealed a normal pelvic anatomy without appearance of an ovarian cyst including endometrioma. Following a standard ovarian stimulation with gonadotropins, TVOR was performed at the 36th hour of ovulation trigger using 250 μg of recombinant human chorionic gonadotropin (rhCG, ovitrelle, Merck Serono, Turkey). Retrieval process was uneventful without any complications. The patient was prescribed a 5-day course of oral doxycycline (Monodoks, Deva, Turkey) (100 mg, twice daily) as a part of routine medication after retrieval process and vaginal micronized progesterone (Progestan, Kocak, Turkey) (200 mg, three times a day) for luteal phase support. On the 14th day of transfer, pregnancy was confirmed with a quantitative beta-hCG (ß-hCG) value of 240 mIU/ml. A subsequent doubling in serum hCG levels was also observed suggestive of an early ongoing intrauterine pregnancy. Three weeks after the retrieval procedure, the patient was admitted to the emergency unit with mild abdominal pain and elevated body temperature (38.3˚C). Her physical examination revealed rebound tenderness in the lower abdomen and tenderness during bimanual pelvic examination. Pelvic sonography revealed a 4.5×4 cm echogenic cystic mass on the left adnexa with mild fluid in the Douglas pouch. Laboratory tests were within the normal range except for leukocyto-

sis (12.500/mm³) and elevated C-reactive protein (8 mg/L) (CRP). An ultrasound-guided drainage of the mass was considered, but due to the anatomic position of the mass and poor cooperation from the patient, the procedure was not successful. Thus an initial empirical antibiotic therapy was started within travenous (IV) ceftriaxone sodium (Iesef, Ulagay, Turkey) 1gr twice a day and metronidazole 500 mg (Flagyl, Sanofi Aventis, Turkey) three times a day, as we were also unable to obtain satisfactory amount of culture sample from Douglas pouch. Despite full course of antibiotics for 48 hours, mild fever (38˚C) and abdominal tenderness persisted and a laparoscopic drainage was decided. On initial evaluation, formations of left ovarian abscess and diffuse pus were confirmed (Figs 1, 2). Drainage and excision of the abscess wall were performed and whole pelvis was irrigated with 3 liters of saline (Fig 3). Antibiotics and progesterone supplementation were continued following laparoscopy. Following an uneventful recovery, the patient was discharged five days after the surgery. Escherichia coli, bacteroides and peptostreptococcus species were reported to be isolated in the abscess culture. A dichorionic-diamniotic twin pregnancy with cardiac activities was confirmed during her routine obstetric follow-up at the 5th week of gestation. However, three weeks later, unfortunately ultrasound investigation failed to confirm both cardiac activities. There were no signs and symptoms suggestive of either persisting or recurrence of infection in the patient at that stage. An informed consent was obtained from patient before submitting the case into the journal.
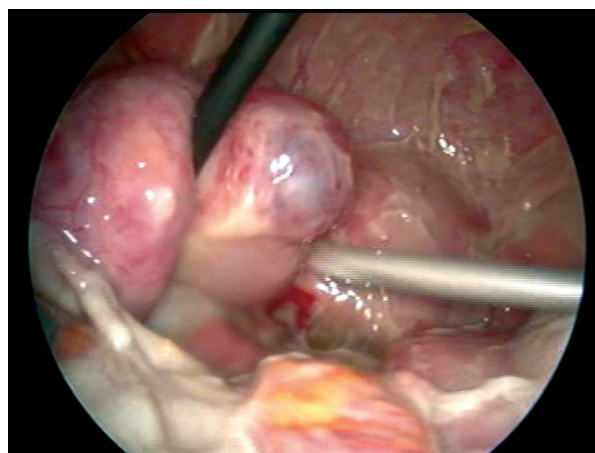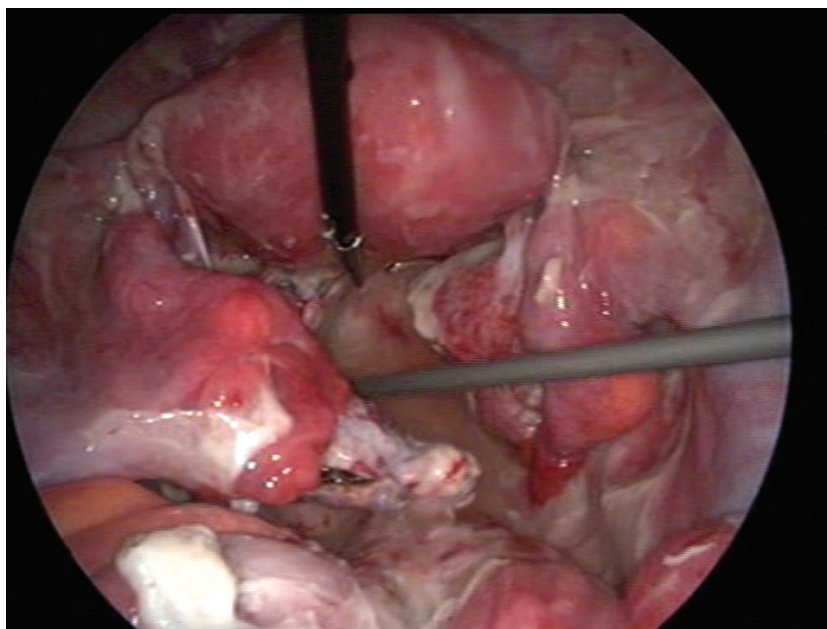

Fig 1: The initial view of the pelvis, depicting a left ovarian mass consistent with abscess, disseminated purulent fluid.
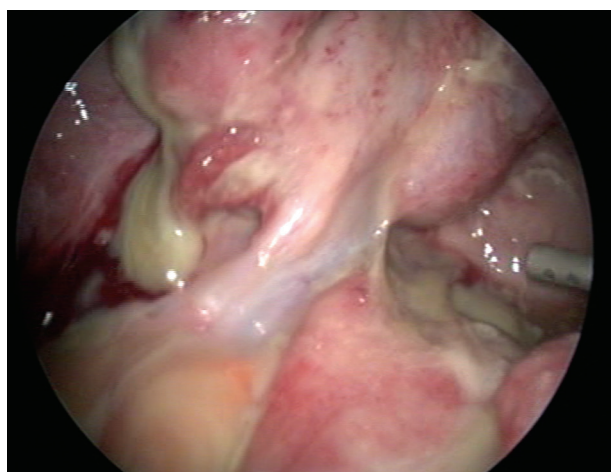
Fig 2: Drainage of purulent fluid from the left ovarian mass.

Fig 3: Appearance after excision of the mass and irrigation of the pelvis.

Discussion

Oocyte retrieval process is an invasive procedure with the potential risk of inoculation of the vaginal microorganisms into abdomen. The risk of pelvic infection after TVOR is estimated as 0.6% (1). It may be caused not only by inoculation of vaginal microorganisms, but also by reactivation of a latent pelvic inflammation or direct intestinal injury (4). The interval between the procedure and occurrence of symptoms is variable. According to the literature (Table 1), this was reported as shorter than 25 days in almost half of the patients; however, prolonged intervals have also been reported (3, 4). This interval might differ according to the virulence of microorganisms or immune response of the patient. Use of prophylactic antibiotics following oocyte retrieval is controversial as pelvic inflammation is uncommon and these medications may not prevent all associated infections. Hence, antibiotics should be considered for at-risk patients, such as those with endometriosis or history of pelvic infection or surgery (1, 3). On the other hand, presence of vaginal-cervical microbial contamination at the time of embryo transfer is associated with significantly decreased pregnancy rates (10). Therefore, empirical antibiotic decision mostly correlates with clinician's experience and opinion. In our case, none of the above-mentioned risk factors were present; however, a prophylactic doxycycline was prescribed due to relatively high prevalence of chlamydial infections among reproductive age patients in our population.

Topical antiseptic usage for vaginal preparation before TVOR is another controversial issue in the presence of risk factors. However, there has been no such accepted universal approach. Povidone-iodine or chlorhexidine are commonly used to sterilize the vagina, whereas other options include saline irrigation, careful removal with dry swabs, or avoiding them completely by cleansing the vagina with only saline solutions. Tsai et al. (11) reported that vaginal douching with the addition of aqueous povidone-iodine is effective in preventing the infection without compromising the outcome of the IVF treatment. In the present case, povidone-iodine and further vaginal irrigation with saline was performed.

Table 1: Review of cases with Pelvic abscess formation following oocyte retrieval in the literature

Reference	Age	OPU procedure	Time of symptoms	Possible risk factor(s)	Treatment	Maternal / fetal outcome
Biringer et al. (2)	32	NA	16th week of gestation	Unilateral salpingectomy for ectopic pregnancy	Antibiotherapy, delivery of a first fetus at 16th week and laparotomic drainage	Delivery of second fetus without complications at 30th week
Den Boon et al. (3)	36	NA	25 week 4 days of gestation	Surgery for endometriosis and presence of endometrioma during OPU	Laparotomic bilateral multiple ovarian abscess drainage and antibiotherapy	Delivery at 26 weeks, hypoalbuminemia , pulmonary edema, re-laparotomy for peritonitis and ileus 1. Baby: After treatment for prematurity related complications, he is well and 8 months old; 2. Baby: died at 9th week with severe brain damage
Sharpe et al. (4)	35	Vagina cleansed with saline	13th week of gestation	Endometriosis and aspiration of an endometrioma during OPU	Antibiotherapy and observation	Delivery at 31st week by C-section. Drain left to pelvis and abscess resolved completely There were no neonatal complications
Matsunaga et al. (5)	35	NA	16th week of gestation	Presence of endometriosis and endometrioma	Antibiotherapy at 16th and 20th week. Delivery of unviable fetus at 22nd week and laparotomic left salpingoophorectomy for large abscess	Full postoperative course
Younis et al. (6)	29	NA	22 days after OPU	Bilaterally endometriomas	Antibiotherapy without surgical intervention	Delivery at term without neonatal or maternal complications
Padilla et al. (7)	34	Vaginal iodinization followed by saline irrigation	21 days after OPU	Aspiration of endometrioma during OPU	Antibiotherapy and L/S drainage of abscess	7 weeks of ongoing pregnancy
Jahan and Powell (8)	27	NA	23 rd day of IVF cycle	Presence of endometrioma	Antibiotherapy and L/S drainage (interval of 5 days between 2 L/S)	Delivery at 37th week of gestation without any maternal complications. Newborn was operated for cardiac anomaly and well after the operation
Zweemer et al. (9)	34	NA	36th week of gestation	Surgery for tubal pregnancy	NA	Delivery at 38th week of gestation without any maternal complications
Present case	26	Vaginal iodinization followed by saline irrigation	21 days after OPU	No	Antibiotherapy and L/S drainage of abscess	Missed abortus at 8th week of gestation

NA; Not available, OPU; Oocyte pick up, L/S;Laparoscopy and IVF; In vitro fertilization.

Life threatening complications as a result of assisted reproductive techniques obviously require close surveillance and active management. Ideally, less invasive and conservative approaches should be the first option; nevertheless, severe complications such as pelvic abscess sometimes require more aggressive treatment. Trans-vaginal ultrasound-guided drainage of pelvic abscess is a relatively easy, safe and effective procedure. It has also been proven to be significantly more effective than medical therapy and has been associated with a low surgical morbidity (12). It is, therefore, suggested as the first-line procedure for the treatment of tubal-ovarian abscess. In this case, drainage was considered unsafe due to anatomic malposition of the mass, as it was visualized just behind the corpus uteri in vaginal sonography. As conservative medical management was not successful, laparoscopic surgery was decided and successful excision of the abscess wall along with whole abdomen irrigation was performed. For non-urgent conditions, the second trimester of pregnancy has classically been considered as the safest period for surgical intervention. Utero-placental oxygenation, adequate fetal perfusion and avoidance of teratogenic drugs are essential factors to be considered when embarking on an endoscopic surgery in a pregnant patient (13, 14). During the procedure, physiologic outcomes of pneumo-peritoneum to the fetus must be carefully considered, since carbon-dioxide (CO_2) is highly diffusible and may induce fetal tachycardia and acidosis (15). Amos et al. (16) reported four fetal deaths in seven pregnant women who underwent laparoscopic cholecystectomy or appendectomy, and authors also suggested that fetal demise could have been due to prolonged respiratory acidosis, despite maintaining end-tidal carbon dioxide ($EtCO_2$) in the physiologic range. On the other hand, Steinbrook and Bhavani-Shankar (17) reported a case series of ten pregnant women, with gestational ages of 9 to 30 weeks undergoing laparoscopic cholecystectomy and no adverse maternal or fetal outcomes were noted. Additionally, a recent guideline has already showed that laparoscopy can safely be performed during any trimester of pregnancy and has no disadvantage compared to laparotomy (18).

Management of tubal-ovarian abscess is quite complicated in women of reproductive age, and especially in pregnant patients. Today, in the light of growing evidence, majority of clinicians choose to perform fertility-sparing procedures in management of pelvic abscess. Laparoscopy provides direct visualization of pelvis and allows clinicians to perform additional procedures such as adhesiolysis, salpingotomy, and excision of necrotic tissues, simultaneously.

Even though the exact cause of fetal loss is unclear in this case report, pelvic infection can be assumed to be the plausible cause as there was evidence of inflammation reported in the pathological examination of the fetal material. As the duration between surgery and fetal loss was relatively long, it is really hard to say that fetal loss may be linked with the surgical procedure. Long duration of parenteral antibiotic usage is another questionable issue in this case for its toxic consequences on the fetus. However, beyond the etiology of the loss, this report was aimed at raising the issue of alternative management of an abscess when the case is not suitable for ultrasound-guided drainage. In seeking for a more accurate and safe method, especially applicable in early pregnancy, further studies are definitely required.

Conclusion

In sum, formation of pelvic abscess following TVOR is a rare, but a serious complication and laparoscopy may be a feasible option when less invasive approaches are unsuccessful during early pregnancy.

Acknowledgements

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Case Report

Placenta Percreta Resulting in Incomplete Spontaneous Abortion in First Trimester

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Abstract

Placenta percreta is a rare complication potentially fatal to fetus and the mother. We present here a 41-year-old female patient who underwent curettage for incomplete abortion at 6th week of pregnancy. She had persistent vaginal bleeding for 2 months after the curettage, for which she was treated with hysterectomy. Preoperative ultrasonography and magnetic resonance imaging (MRI) made the diagnosis of placenta percreta. Postoperative pathological examination confirmed this diagnosis.

Keywords: Incomplete Abortion, Magnetic Resonance Imaging, Placenta Percreta, Ultrasonography

Introduction

Placenta accreta (abnormal placentation) is characterized by a regional or insufficient diffusion of decidua basalis. It has three types: placenta accreta where the villi are superficially attached to, but do not invade the uterus; placenta increta where villi invade myometrium; and placenta percreta where placenta crosses full thickness of myometrium and reaches the serosa (1, 2).

Placenta percreta is the most severe form as it invades the serosal layer of the uterus and has a potential to invade adjacent pelvic organs. Its incidence has been increasing with each passing day as a result of an increase in the number of surgical deliveries (3). Hysterectomy is usually needed to control life-threatening bleeding. In selected hemodynamically stable patients, a conservative approach may be tried both to preserve the fertility and to reduce morbidity and the amount of blood transfusion (4).

We report a patient with placenta percreta who presented with continued vaginal bleeding following curettage for incomplete abortion at 6 weeks of pregnancy.

Case Report

41-year-old female G3 P2 L1 A1 with history of 2 previous cesarean deliveries, presented to our clinic with protracted vaginal bleeding. It was learnt that she had undergone curettage for incomplete abortion at an outside center 2 months ago, following which she had persistent vaginal bleeding. She did not apply to any healthcare facility because her sociocultural level was low and she resided in a rural area of İzmir, Turkey. She could not have any sexual intercourse as a result of protracted bleeding. Her hemoglobin was 7.1 g/dl, and beta-human chorionic gonadotropin (β-HCG) was 130 mIU/ml. Transabdominal and transvaginal ultrasonography (USG) was used to rule out abortion imminens and extrauterine pregnancy. Transvaginal color Doppler ultrasonography revealed a 20 mm solid mass lesion with smooth contour compressing endometrium anterior to uterine isthmus as well as a dense fluid collection within the

cavity (Fig 1). T2-wieghted (T2W) magnetic reso-
nance imaging (MRI) showed hyperintense lesions
extending to endometrial cavity at the anterior part
of isthmus (Fig 2A). Fat suppression axial T2W
images demonstrated fluid collection in the cav-
ity and a hyperintense lesion in the myometrium
(Fig 2B). Pre and post contrast T1W sagittal im-
ages showed a myometrial mass lesion with local-
ized contrast uptake and a hematoma compress-
ing the cavity (Fig 2C). Considering the elevated
β-HCG level, it was suggested that the mass le-
sion in myometrium may be secondary to residual
placenta. A discussion was made with the patient
and hysterectomy was planned. Explorative op-
eration showed that urinary bladder was adhered
to anterior uterine wall at the lower uterine seg-
ment and there was a formation consistent with
placenta percreta extending beyond the serosa and
invading the urinary bladder at the site of previ-
ous caesarean section. Placenta was detached from
urinary bladder and hysterectomy was performed.
Urinary bladder was repaired. The hysterectomy
material was sent for pathological examination,
which revealed a lesion consistent with placenta
localized to myometrium and extending to serosa
at the level of isthmus, and a hematoma opening
to endometrial cavity medial to this lesion (Fig 3).
Histopathological examination revealed that cho-
rionic villi invaded myometrium and extended to
serosa (Fig 4), thus confirming the diagnosis of
placenta percreta.
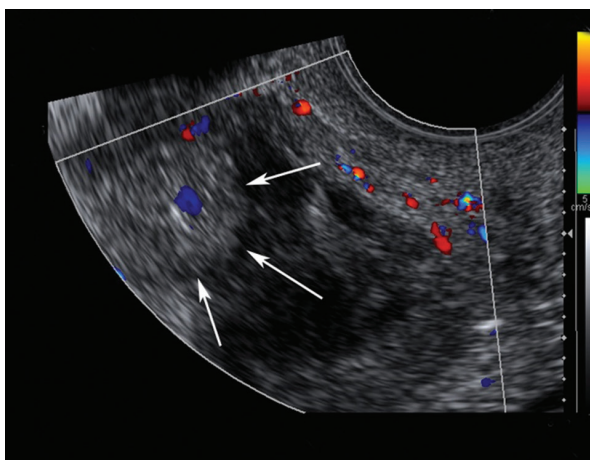
Fig 1: Transvaginal color Doppler interrogation demon-
strates a solid mass lesion with smooth contour and a cen-
tral vascular flow at the anterior wall of the uterine isthmus.
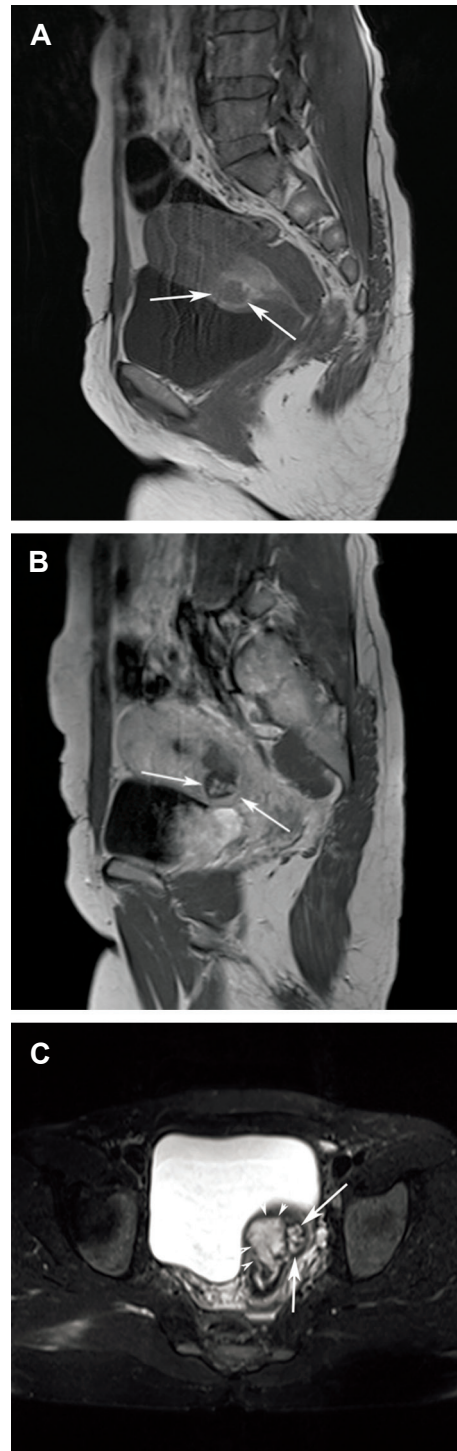
Fig 2: T1W sagittal precontrast image (A) shows residual
placental (white arrows) and a hyperintense area consistent
with subacute hemorrhage around it at the posterior seg-
ment of uterus. T1W sagittal postcontrast image (B) shows
contrast uptake in myometrium and placental residue (white
arrows). Axial fat suppression T2W image (C) shows placen-
tal residue (white arrows) and hemorrhage (arrow heads).
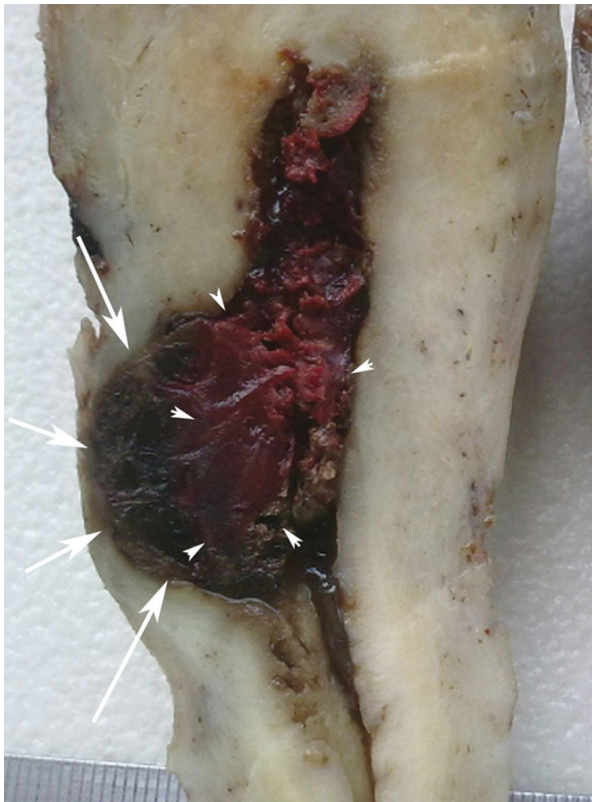
Fig 3: Postoperative hysterectomy material reveals placenta percreta extending to serosa (white arrows) at the level of uterine isthmus and a hematoma opening to endometrial cavity medial to it (arrow heads).
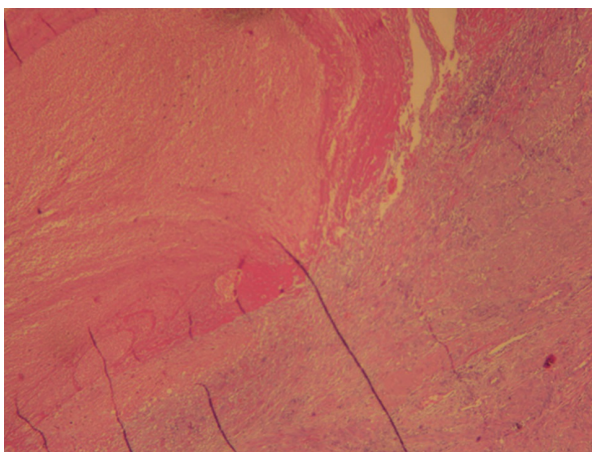
Fig 4: Macrophotography demonstrates hemorrhagic placental residue containing necrotic villi interspersed in muscle tissue.

Discussion

Placenta percreta is a disorder that results from regional or insufficient diffusion of decidua basalis and is characterized by placenta passing beyond myometrium to reach serosa. Approximately, 5% of the cases with abnormal placentation consist of placenta percreta. Nearly all cases of placenta percreta are diagnosed in 3rd trimester. Its incidence has been on the rise with each passing day as a result of the increase in the number of caesarean section operations (3).

Risk factors for placenta percreta include previous surgery (caesarean section, myomectomy or curettage), abnormal placental localizations, advanced maternal age, grand multiparity, Asherman's syndrome, endometritis, adenomyosis, endometriosis, and submucous leiomyoma. Our patient had 2 of these risk factors, including advanced age and previous caesarean sections. Early use of USG and MRI to establish an early and accurate diagnosis, and to determine the appropriate treatment modality are of paramount importance in reducing the morbidity (3, 5, 6). Ultrasonographic findings that may aid in diagnosis include loss of normal hypoechoic zone of the retroplacental myometrium, thinning and interruption of the hyperechogenicity between uterine serosa and urinary bladder, and presence of a focal exophytic mass suggesting neighboring organ invasion, especially of the urinary bladder. MRI is frequently used in combination with USG. The sensitivity and specificity reported for MRI in detecting abnormal placentation are 80-88% and 65-100%, respectively (7). However, the ability of MRI to diagnose placental invasion of myometrium is still dependent on the experience of the interpreter. Sometimes, even combined use of USG and MRI may fail to diagnose abnormal placentation.

Two strategies have been proposed for treatment of placenta percreta, namely hysterectomy and conservative treatment. Although, hysterectomy is the first line treatment modality, it may prove insufficient in achieving hemostasis in cases with advanced and severe invasion of adjacent structures. Thus, hemodynamically stable patients with placenta percreta may be conservatively treated with methotrexate (8). Uterine artery embolization is another conservative method that may be used for patients that wish to preserve their fertility. As our patient had no desire to preserve her fertility, we proceeded with hysterectomy after discussion about various treatment modalities. The anterior

wall of urinary bladder to which placenta was adhered, was repaired. No complication developed during the operation.

Our literature search yielded very few cases with placenta percreta diagnosed in the first and second trimester of pregnancy. Massive bleeding may develop following curettage performed after incomplete abortion and hysterectomy may be required to stop the bleeding (9, 10). Gupta et al. reported that all patients with placenta accreta underwent hysterectomy, after curettage was performed for incomplete abortion in such patients (10). While iatrogenic uterine rupture may develop due to curettage performed for the treatment of incomplete abortion, spontaneous rupture may ensue as pregnancy progresses (11, 12) in the patients with placenta percreta.

In our case, incomplete abortion at the 6th week of pregnancy, leading to an early curettage, may have prevented progressive placental growth and further complications. No massive bleeding was observed during curettage and an urgent hysterectomy was not needed. Furthermore, an increased serum β-HCG level in the preoperative period, history of curettage for incomplete abortion 2 months back, no history of sexual intercourse after curettage, history of 2 previous cesarean deliveries and demonstration of placental residue in the uterine cavity on USG and MRI all led to the correct diagnosis. To our knowledge, there is no case diagnosed with placenta percreta as early as 6 weeks of gestation, in English literature.

In conclusion placenta percreta is one of the most important complications of pregnancy with serious morbidity and mortality. First trimester diagnosis is quite difficult. USG and MRI are diagnostic adjuncts. Unexplained protracted vaginal bleeding after curettage for incomplete abortion should raise the suspicion of placenta percreta.

More importantly, uterine rupture may take place in these patients during curettage, leading to shock secondary to abundant hemorrhage. For this reason, it is recommended to perform the curettage in a fully equipped healthcare facility where blood transfusion and hysterectomy can be carried out.

Acknowledgements

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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.

Conferences:
Name(s) of editor(s). Conference title; Holding date; Holding place. Publication place; Publisher name; Publication date (year).
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Internet References
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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 Journal($_{Yakhteh}$).2008; 10 (2): 87-92. Available from: http://www.celljournal.org. (20 Oct 2008).

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

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