Original Article

Seasonal Influences on Different Stages of *In Vitro* Fertilization: Stimulation and Fertilization

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Abstract

Background: Effect of seasonal changes in human reproduction has been intensively researched. Some studies acknowledge influences of seasonal variation on natural conception, while others can not confirm them. The aim of this study was to investigate the effect of seasonal changes on different stages of assisted reproductive technology (ART) in Tehran.

Materials and Methods: This study was carried out on 268 cycles which were the first intracytoplasmic sperm injection (ICSI) cycle of the couples between July 2005 and March 2007. Multiple logistic regression was used to model the influence of different clinical factors on pregnancy rate and a bivariate Poisson regression model was used for modeling the number of mature oocytes and obtained embryos.

Results: Based on the logistic regression model, the duration of infertility, female age, number of mature oocytes, and transferred embryos were only the significant predictors. In this series, seasonal changes had no predictive value on the pregnancy rate.

Bivariate Poisson regression model showed that only the female age and duration of infertility were the factors simultaneously affecting the number of embryos and mature oocytes.

Conclusion: The suspected seasonal variability on the outcome of ICSI cycles has not been confirmed. Furthermore, among different seasons, there are no significant differences in the number of mature oocytes, obtained embryos, and transferred embryos.

Keywords: Oocyte, Embryos, ICSI, Pregnancy Rates, Seasonal Variation

Introduction

Every physician and biologist working in the field of reproductive medicine keeps wondering about variable pregnancy rates, because success rates are fluctuating without visible reason from excellent to very poor. Thus, the question of whether the different seasons of the year can have positive or negative influences on the characteristics of the oocytes, fertilization, or pregnancy rates in conjunction with assisted reproductive technologies (ART) in humans arises. Statisticians and epidemiologists have previously reported various *in vitro* fertilization (IVF) outcomes in correlation with or without seasonal and weather influences in different geographical areas. Some studies point to a variation in natural conception in humans (1-3); others cannot confirm the presence of the seasonal variations (4-7). Better sperm quality in the cooler seasons was observed by many studies (8), whereas other studies have been inconclusive (9, 10).

Different geographical areas with different climates may cause disparity in IVF outcomes. Thus, the aim of this study was to investigate the effect of seasonal changes on various embryological parameters in IVF patients in Tehran.

Materials and Methods

Study sample

A database containing information on all ICSI cycles was used. In total, 306 cycles from 268 couples were performed between July 2005 and March 2007 in Shayamehr Limited Surgical Center (Tehran). The Research was approved by Medical Ethics Commitee of Tarbiat Modares University. This study was carried out on 268 cycles which were the first ICSI cycle of the couples. The season was expected to influence the oocyte during its development at the time of IVF treatment; it was also expected to influence the spermatozoon during their development before sperm collection on the day of oocyte aspiration. Therefore, to study seasonality, the date of aspiration was taken as an approximation of the moment of etiological impact.

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The Iranian national regulations ensuring anonymity and privacy with respect to the patient data were followed. The ovarian stimulation protocol was as follows:
All of the patients in our study were underwent the Long protocol. All of them were down-regulated with the GnRH agonist and subsequently underwent controlled ovarian stimulation with gonadotrophins. When at least one follicle had reached a diameter of 18 mm and more than three had reached a diameter of greater than 15 mm, a single a single dose of 10,000 IU hCG via intramuscular injection was administered. Transvaginal ultrasound-guided follicular aspiration was performed 34-36 hours later. Oocytes were inseminated by the ICSI method 2-4 hours after follicular aspiration. According to embryo scoring, 1-3 embryos were transferred to each patient's uterus 48-72 hours post ICSI.
A clinical pregnancy was defined by the presence of at least one gestational sac in which a fetal heartbeat could be seen on transvaginal ultrasound examination 4 to 6 weeks after embryo transplantation .

Statistical Methods
One-way analysis of variance and chi-square were utilized to evaluate differences among the different seasons. A P value of less than 0.05 was considered to be statistically significant.
Multiple logistic regression was used to model the influence of the different clinical factors on pregnancy rate. All clinical, demographic, embryonic and temporal factors were entered into the analysis. Those factors were: the female age, duration of infertility, causes of infertility (male related, polycystic ovarian syndrome (PCOs), endometriosis, both male and female related, unexplained, etc.), season of treatment, abortion history, history of ectopic pregnancy, history of ovarian and/or uterine surgery, familial marriage, number of previous ART attempts, sperm characteristics (count, motility, and morphology rates), and number of previous intrauterine inseminations (IUI). Weather parameters including: temperature, relative humidity, atmospheric pressure and cumulative daylight hours were acquired from the National Weather Bureau station of Tehran, Iran. Statistical analysis was performed using SPSS version 14.0 (SPSS Inc., Chicago, IL).
Mature oocytes and obtained embryos are the outcomes of two important stages in IVF process: stimulation and fertilization. The number of mature oocytes and obtained embryos are likely to affect each other; hence their relationship must be analyzed with a simultaneous equation model. Because the number of mature oocytes and embryos are "counts" and they are correlated, the use of a standard method such as the standard linear regression or univariate Poisson regression was not thought to be suitable. Therefore, the bivariate Poisson regression model (11, 12) was alternatively chosen and adjusted to model the influence of seasonal variations on the number of mature oocytes, obtained embryos and other clinical factors. Because of the small sample size, the Bayesian approach was used for estimating the parameters and the Bayesian analysis was performed by using Winbugs v.1.4.3 (an open-source statistical computing environment described at http://www.mrc-bsu.cam.ac.uk/ bugs).

Results
The causes of infertility in the 268 cases which underwent ICSI cycles were male factor (38.1%), PCO (10%), endometriosis (3%), both male and female factor (12.3%), and Unexplained (8.2%). The mean duration of infertility and female age in our data were 8.53 4.83 and 31.59 5.32 years respectively.
Table 2 presents the numbers of mature oocytes, obtained embryos, transferred embryos and pregnancy rate in different seasons. However, the highest number of mature oocytes, obtained embryos, and

Table 1: Distribution of the clinical parameters of the patients in different seasons

Clinical Parameters	Spring	Summer	Fall	Winter
Number of Patients (%)	32 (11.9)	73 (28.0)	76 (28.4)	85 (31.7)
Female Age	32.91±5.69	31.02±4.67	31.08±5.72	32.06±5.32
Duration of Infertility	9.00 5.72	9.09±4.72	8.31±4.73	8.05±4.66
Diagnosis of Infertility				
Male Factor	13.7	27.5	26.5	32.4
PCO	15.4	8.5	30.8	42.3
Endometriosis	7.7	15.4	23.1	53.8
Both Male and Female Factor	9.1	9.1	33.3	48.5
Unexplained	0	45.4	31.8	22.7

Note: values are mean SD or percentages

Table 2: The number of mature oocytes, obtained and transferred embryos, and pregnancy rate among different seasons

Clinical Parameters (Mean±SD)	Spring	Summer	Fall	Winter	P-Value
Mature Oocytes	5.27±4.22	5.60±3.74	6.30±4.14	5.11±3.25	NS
Obtained Embryos	2.91±2.82	3.40±2.64	3.92±2.92	3.18±2.36	NS
Transferred Embryos	2.09±1.33	2.32±1.16	2.47±1.22	2.52±1.32	NS
Pregnancy Rate (%)	25.0	22.7	10.5	15.3	NS

NS = Not Significant

rarely transferred embryos were in the fall but it was not statistically significant.

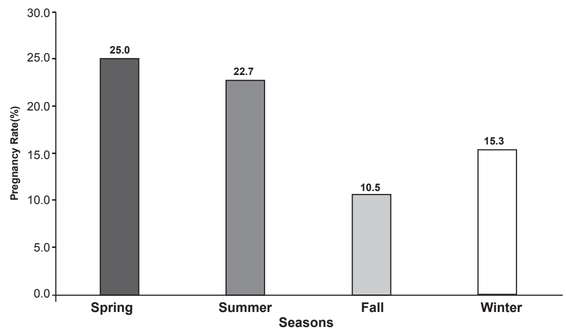

Fig 1: Pregnancy rate in different seasons in ICSI cycles

As shown in Fig 1, the highest pregnancy rate was in spring and the lowest in fall that was not statistically significant. Fig 2 shows the monthly weather parameter changes in Tehran. Maximum temperature was during the summer and was inversely related to the atmospheric pressure (2A and 2C). The relative humidity reached its peak from October to January (2B), and gradual declination of the cumulative daylight hours was observed from October to December (2D).

According to the logistic regression model on the outcome of pregnancy, among different clinical parameters only the duration of infertility, female age, number of mature oocytes, and transferred embryos were the significant predictors. Furthermore, seasonal changes had no predictive value on pregnancy rate in this series (Table 3).

The Bivariate Poisson regression model showed that only the female age and duration of infertility were

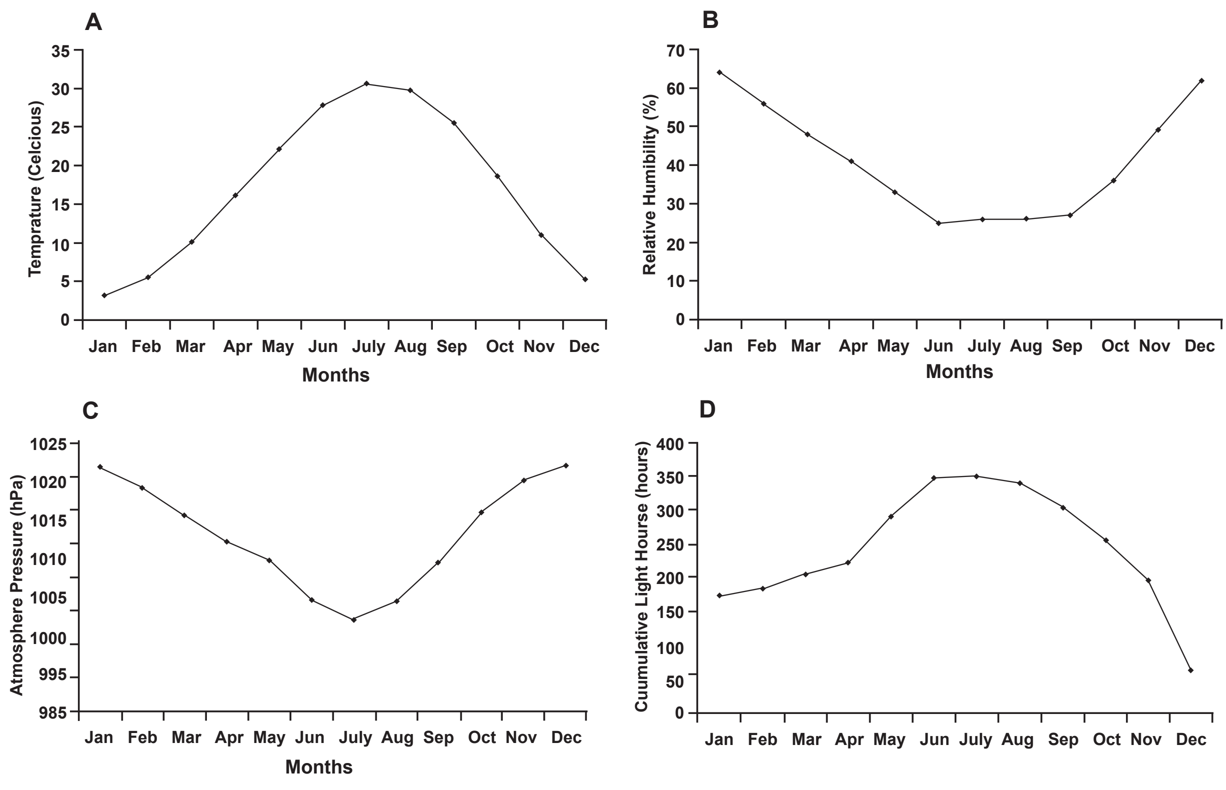

Fig 2: Monthly weather parameter changes

Table 3: Variables selected as predictive of pregnancy by using multiple logistic regression

Parameters	Estimation	OR	95% CI
Female age	-0.040	0.954	0.714-00.982
Duration of infertility	-0.055	0.946	0.785-00.971
The number of mature oocytes	0.131	1.140	1.006-01.291
The number of transferred embryos	0.462	1.590	1.022-64.300

Table 4: Summary statistics of fitting the Bivariate poisson regression

Variable definition	Mean	Std.Error	Lower	Upper
Response variable: Mature Oocytes				
Constant	2.282	0.013	1.783	2.822 [a]
Female age	-0.017	0.004	-0.035	-0.004 [a]
Indicator For season of treatment: 1=Fall, 0=otherwise	0.165	0.004	-0.021	0.348
Familial marriage	0.091	0.002	-0.111	0.290
Female history	0.146	0.003	-0.063	0.355
Duration of infertility	-0.025	0.002	-0.044	-0.005 [a]
Indicator for infertility diagnostic: 1=Tubal factor, 0=Male factor	-0.203	0.02	-0.381	0.027
Indicator for previous ART attempts:1=2 twice, 0=1 twice	-0.105	0.003	-0.341	0.129
Indicator for previous ART attempts:1=3 twice or more, 0=1 twice	0.258	0.003	-0.017	0.491
History of ovarian problem	0.258	0.006	-0.132	0.636
Response variable: Obtained embryos				
Constant	1.459	0.029	0.272	2.447 [a]
Female age	-0.013	0.004	-0.035	-0.008 [a]
Indicator for season of treatment: 1= Fall, 0= otherwise	0.163	0.004	-0.064	0.390
Female history	0.121	0.004	-0.137	0.372
Duration of infertility	-0.025	0.003	-0.049	-0.001[a]
Sperm motility	0.004	0.001	-0.004	0.012
Sperm morphology	0.006	0.001	-0.007	0.009

♦ "Lower" and "Upper" denote the 2.5th percentile and the 97.5th percentile of the simulated draws, respectively
a: Significant at 0.05 level.

the factors simultaneously affecting the number of embryos and mature oocytes (Table 4).

Discussion

Carefully studying the literature on the seasonal variations in the outcome of ART cycles, the extent of discrepancies between these studies is remarkable. Some show the best pregnancy rates in the months of November thru February (13), others show the worst pregnancy rates in the months of January, February, and March (5) in IVF cycles. Consistent with the results of Rojansky et al (14) and Vahidi et al (15), we found an upper pregnancy rate in the spring and a lower rate in the fall (25.0% vs. 10.5%), but no statistical significances were observed. Our findings are in agreement with other studies showing no differences in the results of assisted reproductive techniques done in different seasons of the year (4-7).

Better fertilization rates, embryo quality and pregnancy rates during the cold months have been observed (16, 13). In our study we showed no significant differences in the number of mature oocytes and embryos and transferred embryos among seasonal variations that this fact is disagreement with above-mentioned studies. However, maximum value of those variables is in the fall.

Melatonin, a hormone produced during darkness, may be one of the factors influencing gonadal function (17). The involvement of melatonin in the steroid genesis of human ovaries has been documented (18, 19). Webley and Luck (18) reported direct stimulation of melatonin on hCG. Melatonin is also thought to enhance hCG stimulation of progesterone production by human granulose cells in culture (19). Yie et al. (20, 21) reported higher concentra-

tions of melatonin and progesterone during the dark season of the year and that of oestradiol during the light season in pre-ovulatory follicular fluids.
Rojansky et al. (14), showed the correlation between the absolute number of daylight hours and fertilization rate. Their result was highest in spring and lowest in autumn. Furthermore, Chang et al. (22) showed the cumulative daylight hours negatively correlated with the implantation and pregnancy rates of day 3 embryo transfers.
The higher pregnancy rate in spite of low oocyte and embryo numbers during spring may only be explained by increased endometrial receptivity during this season.
Fig 2 shows the maximum values for pressure and humidity, minimum as well as values for daylight hours and temperature in fall in Tehran, Iran. Furthermore, our results show the highest number of mature oocytes and obtained embryos in fall versus the other seasons. These results together confirm the conclusions of the previously mentioned studies.
Better sperm quality in the cooler seasons was observed by many authors (9). For those with severe poor sperm quality, normal fertilization could be easily achieved by ICSI. Thus, the impacts of poor sperm quality on ART outcome may be largely eliminated (23, 24). Therefore, the impact of the sperm quality was removed from the model in this study.
Multiple logistic regression showed that that the significant predictors influencing the outcome of ICSI cycle are the female age, duration of infertility, and the number of mature oocytes and transferred embryos.
Our results show that pregnancy rate increased with an increase in the number of transferred embryos and mature oocytes as well as with decreased patient age, and duration of infertility.
the significant predictors influencing the outcome of the ICSI cycle are the female age, duration of infertility, and the number of mature oocytes and transferred embryos.
When we used the bivariate Poisson regression model for studying the relationship between seasonal changes and two outcomes (mature oocytes and obtained embryos), we found no significant association. Our findings are in agreement with the studies showing no differences in the results of assisted reproductive techniques done in different seasons of the year (4-7). This may be due to the fact that seasonal changes should be taken into account together with other factors when evaluating infertility data (15). Furthermore, in addition to different ICSI variables, we found that the correlation factor between the two variables (the number of mature oocytes and embryos) should also be taken into account. More investigations using appropriate statistical modeling are needed in this regard.
This study has some limitations. We could not compare implantation rate among different seasons, therefore whether endometrial receptivity also played a role needs further investigations. The cross-sectional estimates of seasonal variation of hormone levels are not true measures of longitudinal changes, and individual variations cannot be identified. A longitudinal design following up the same participants throughout the year would have been preferable. However, true seasonal variations would be expected to be evident in this study due to a large sample size despite the cross-sectional design. Furthermore, the analysis included information from the first cycle which is likely to deviate from that in other cycles. In the next phase of our research, we will study the effect of the number of referring cycles on the outcomes in the presence of other factors. In addition, we will study the effect of different geographical areas with different climates on different embryological parameters with a nationwide multi-center study.

Conclusion

In conclusion, by the analysis of the first ICSI cycles, we confirmed that significant predictors for ICSI outcomes are the duration of infertility, female age, number of mature oocytes, and transferred embryos. However, the suspected seasonal variability of the outcome of ICSI cycles has not been confirmed. Furthermore, there are no significant differences in the numbers of mature oocytes, obtained embryos, and transferred embryos among different seasons.
A change in routine fertility treatments concerning different seasons should therefore not be considered.

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