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Influence of Seasonal Variations on In-Vitro Fertilization Success

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ABSTRACT

This study was designed to investigate the influence of seasonal variations on the number of retrieved ova, fertilization rate, embryo quality rates and pregnancy rate in IVF cycles. Prospective cohort study was conducted on a total number of 2140 cycles in infertile patients undergoing their first IVF cycle between 2000 and 2007 in IVF policlinic. χ^2-test, Pearson's correlation coefficient and one-way analysis of variance were used for statistical analyses. Overall fertilization rate was 77.96% and pregnancy rate 29.15±2.72% per cycle. Seasonal prevalence of pregnancy rate was highest during fall 33.8±4.5% and lowest during summer 23.4±6.2%, but these differences did not reach statistical significance. The study did not show any statistically significant differences in the number of retrieved oocyte, fertilization, and embryo quality according to season. Therefore seasonal changes should not be taken into account in everyday IVF practice.

***Key words**: assisted reproduction, embryo quality rate, fertilization rate, pregnancy rate, season*

Introduction

Several studies throughout the world have demonstrated seasonal changes in spontaneous fecundity among human population[1–3]. Seasonal effect on factors such as: ovulation[4], sperm quality[5], spontaneous abortions[6], births[7] and marriages or related to holidays[8] have been previously reported. Additionally, the recent war period in Croatia, marked with change in numerous aspects of human biology[9–13]. Influence of temperature and climate conditions on number, motility and sperm morphology has been proved very clearly in males[14–16]. Besides, there are some indications that fluctuation of hypothalamic-pituitary axis in women, through brain transmitters (serotonin, dopamine) has effect on pregnancy and embryo quality[17]. However, the impact of seasonal changes on in vitro fertilization cycles (IVF) remains unclear. Some authors reported apparent lack of seasonal variations in fertilization, implantation, conception[18] and pregnancy rates[19]. Additionally, one recent study failed to show any seasonal effect of the IVF-related parameters[20]. On the other hand, few studies demonstrated specific seasonal impact on pregnancy rates[21,22] fertilization rates and embryo quality[23].

The purpose of the present study was to investigate this controversy and to re-evaluate these parameters using a large number of IVF cycles conducted in a single IVF center. The authors sought to elucidate whether seasonality affects IVF parameters such as: the number of the retrieved ova, fertilization, embryo quality and pregnancy rate in women undergoing IVF-ET treatment.

Material and Methods

Patients and study protocol

A cohort of patients whose infertility has been proved and their written consent obtained were included in analysis. The patient's were consecutively enrolled according to exclusion criteria. Exclusion criteria were defined with prior IVF cycle (s), body mass index (BMI) > 25 kg/m^2, women's age >37 years and different protocol or procedures in use than standard described here. Those factors are known to have strong impact on IVF related parameters and their accumulation even by chance (for example in particular month or season) may represent a source of potentional biases.

Finally, study population consisted of 2140 women in an average age of (32.3±4.38) treated between 2000 and 2007 in IVF policlinic. The medical and biological staff of

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TABLE 1
THE SEASONAL VARIABILITY OF THE IVF TREATMENT CHARACTERISTICS

Treatment characteristics (mean±SD)	Winter	Spring	Summer	Fall
Woman's age	33.1±5.4	32.3±4.1	31.8±4.2	32.2±3.8
Number of retrieved oocyte	5.90±3.7	6.53±4.03	7.37±3.55	6.15±2.88
Number of embryos transferred	2.82±0.4	3.04±0.62	2.66±0.71	2.75±0.43
Number of grade 1 quality embryos	1.02±0.18	1.43±0.26	1.09±0.17	0.89±0.12

*p< 0.05

the laboratory was the same during the entire study period. Participants underwent routine ovarian down-stimulation protocol with GnRH agonists. After day 21 of the preliminary cycle, buserelin nasal spray (Suprefact; Aventis Pharma, Frankfurt, Germany) 2mg was used, 4 times daily.

Following day 2 of the ongoing cycle, rFSH (Gonal-F; Serono, Geneva, Switzerland) was subjoined in a dosage of 225 or 300 IU for a further 3 or 4 days. The dose was subsequently reduced to a total of 150 IU to the day of hCG (Ovitrelle; Serono) application.

The ovarian response was monitored by measuring the serum level of E_2 and ultrasound monitoring of the follicular maturation. When at least two follicles were the size of 16–17 mm in diameter, hCG (Ovitrelle®) 6 500 IU was administered. Oocyte aspiration was conducted 34–40 hours after hCG application, under transvaginal ultrasound control. Oocytes were cultivated in IVF or SPM media. Insemination took place 3–5h after oocyte aspiration and 3 days later, the high quality embryos were transferred into the uterus. Embryo quality was classified by 1–4 grades depending on number of blastomeres, shape and fragmentation. Embryos with equal-sized blastomeres, ideal cleavage rate (8 cells on the third day) with <10% were defined as grade 1. Luteal support was given by progesterone in a form of Crinone 8% gel (Fleet Laboratories Ltd. UK), 90 mg daily or Utrogestan vaginal capsules (Laboratories Besins International, France) 3x2 a100 mg. Pregnancy was determined by β hCG serum level, fourteen days after the embryo transfer took place. Values lower than 10 IU/L were considered negative. Pregnancy was confirmed with ultrasound findings of a gestation sack in 8th week. Study results were classified according to season: spring (March-May), summer (June-August), fall (September-November) and winter (December-February). Permission from Institutional review board was obtained before the onset of the study.

Statistic analysis

SPSS software, version 10.0 for Windows was used for statistical analysis. χ^2-test was used to estimate statistical differences between variables on each specific period of season. Pearson's correlation coefficient was introduced to explore the possible correlation between various parameters. One-way analysis of variance with three degrees of freedom was used to compare seasonal frequencies and sample means. A value of $p<0.05$ (two tailed) was considered statistically significant and unless otherwise stated, the results are being expressed as mean number±SD.

Results

This study was introduced to investigate the influence of seasonal changes on the number of retrieved ova, fertilization rate, embryo quality and pregnancy rate on a total number of 2140 cycles. Women were in average age of 32.3±4.38; no significant variability in this parameter was observed either between seasons or between the different years of study. The number of retrieved oocytes

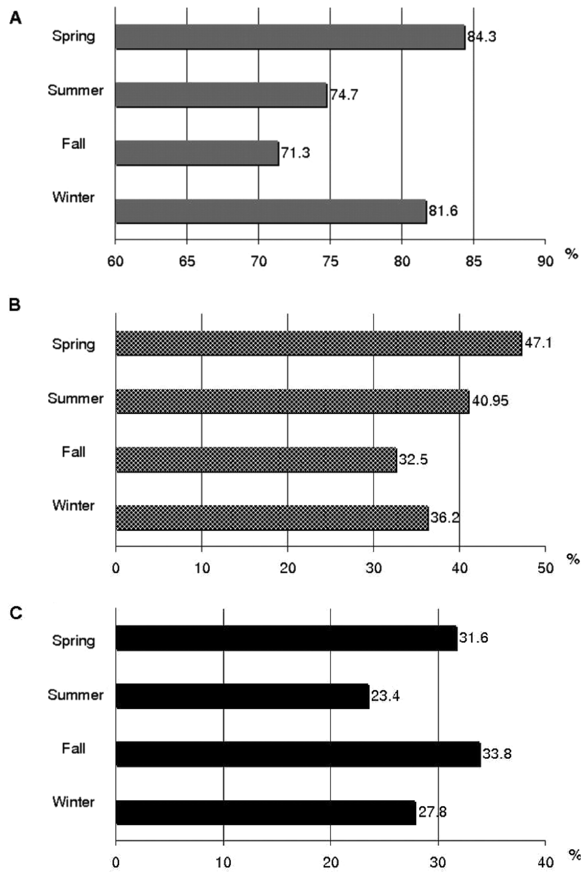

Fig. 1. a) Seasonal variations in fertilization rate, F=0.614; p= 0.625 (N.S); b) Seasonal variations in embryo quality – grade 1, F=0.601; p=0.632 (N.S); c) Seasonal variations in pregnancy rate (per cycle), F= 0.402; p=0.295 (N.S).

(6.24±3.66) was lowest in winter and highest in summer but also without statistical significance. The mean number of embryo transferred *per* cycle was 2.83±0.35 with the increase tendency from fall to spring and decrease in summer. The mean number of grade 1 quality embryos transferred was 1.11±0.19, higher in spring and lower in fall. Neither the embryo transferred nor top quality embryos-grade 1, differed between seasons (Table 1).

Results did not show any significant changes related to a specific seasonal period (Figures 1a-c). Fertilization rate was 77.96%. Fertilization rate showed a tendency of increasing during winter with the highest peak in spring (84.3%) and decreasing with the lowest point reached in fall (71.3%) but without statistical difference (Figure 1a). Embryo quality (grade 1) was 40.94%. The seasonal distribution of the embryo quality grade 1 showed increasing rates during summer period (40.9%), reaching a spring climax (47.1%) and declining during cold months, winter (36.2%) and fall (32.5%) but without significant variation (Figure 1b). The overall pregnancy rate of the study population was 29.15±2.72% *per* cycle. Seasonal prevalence of pregnancy outcomes was highest during fall 33.8±4.5%. Lowest conception rate was notified in summer 23.4±6.2%, but these differences did not reach statistical significance (Figure 1c). Gemini pregnancy rate occurred with 44.44% during May, so every second pregnancy in May was actually twin-pregnancy (data not shown).

Discussion and Conclusion

The influence of seasonal variations on human natural conception and birth rates has been demonstrated by previous studies[1–3] conducted in different geographical areas.

Ever since assisted reproduction technologies were introduced into practice scientist are trying to find the similar connection, but the existence of a seasonal pattern on the results of *in vitro* fertilization remains questionable. Significant seasonal fluctuations in the results of IVF have been reported by several authors[22–25], but have not been supported by others[18,20,26]. Occurred differences between studies may be explained by various stimulation protocols in use, climate conditions and studies design along with different statistical approach. Considering stimulation protocols in use some authors decided to include down-regulation but others did not. Second, studies have been conducted in various parts of the world and consequently different environment and climate conditions such as temperature and daylight changes that could affect their results. Therefore, the studies are not directly comparable. In a published data reporting seasonality the observed differences among results are remarkable. For example, Stolwijk et al.[22] in the Dutch study found better pregnancy rates during the period from November to December and February to March. On the contrary, Vahidi et al.[25] (Iran) demonstrated significantly higher pregnancy rates during the March-July period. Others presented the lowest pregnancy rates in the months of December[21], January[21,27], February[21,27] and March[21,23]. There was also no agreement in inclusion criteria of factors known to affect the IVF success rate such as: the woman's age, number of previous attempts and infertility cause. Therefore, result dispersion exists and seasonality is still debating.

In the current study we considered several IVF-related parameters: the number of retrieved ova, fertilization, embryo quality and pregnancy rates and analyzed their potential changes according to four seasons.

Our study did not show any significant seasonal variations in the fertilization rate, embryo quality nor pregnancy rate (Figures 1a-c). The number of the retrieved oocytes proved to be highest during summer and lowest during winter, similarly to Israel study by Rojansky et al.[23] who have reported the highest number of retrieved oocytes during summer months and lowest during winter months. However, the differences did not significantly differ during the seasons (Table 1), suggesting very consistent ovarian responsiveness to gonadotropin stimulation as reported by Revelli et al.[20] Fertilization rate in our study showed some fluctuation with the highest peak reached in spring (84.3%) and the lowest in fall (71.3%) but without statistical significance (Figure 1a). Some investigators have reported a seasonal variability of IVF treatment related to embryo quality[22,23,28], pregnancy[22,24,28] and implantation rates[28]. Despite some annual variations in embryo quality – highest during spring (47.1%), and lowest during fall (32.5%) along with pregnancy rate with the highest peaks reached in fall (33.8%) and lowest in summer (23.4%), our study did not show any correlation in respect to seasonal changes (Figures 1b and 1c). Our findings uphold other studies[14–16,26] also emphasizing no statistical differences made by seasonal changes in the results of *in vitro* fertilization. Results from one recent study that analyzed seasonality of births in Croatia, although naturally conceived, showed an increase in the summer birth proportion[29] that correspondent to higher pregnancy rate in fall found in our study.

The effect of seasonality in the light/dark cycle on spontaneous conception has been established among humans[3] but it seems to be belittled in IVF, most likely by pituitary suppression and the administration of the exogenous supplement that allows complete control to be taken over the ovulation. It has been suggested that melatonin, a hormone with higher secretion during darkness[30] may influence reproduction[31] in both maturation and fertilization rates[32,33]. However, the differences in present study concerning the number of retrieved ova, fertilization, embryo quality and pregnancy rate were rather small according to four year season and did not reach statistical significance pointing out that even if a direct mediator's effect on the end organ (ovary) exists, it seems to be diminished by the supplements used in induced cycles.

In conclusion, results of our study did not show significant influence of the seasonal changes in assisted reproduction of the outcomes studied: number of retrieved oocytes, fertilization, embryo quality or pregnancy rates.

It seems likely that medicaments used in order to control ovarian and endometrial function in IVF cycles as well as sophisticated laboratory equipment and procedures in use, lower the influence of seasonal variations on IVF-related parameters.

To minimize the effect of seasonal changes on IVF results, the authors recommend a quality system management based on the principles of good clinical practice.

Specific technical and quality requirements should be set out in each IVF unit and corresponding laboratory to cover organization, materials, documentation records, staff education, unit management and quality review.

Recently, as the next European Union (EU) member to be, the EU guidelines 2004/86/EU and 2006/86/EU have been implemented in Croatian legislative (from 17, July, 2009) to ensure high standards of quality and safety.

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UTJECAJ SEZONSKIH VARIJACIJA NA USPJEH IZVANTJELESNE OPLODNJE

SAŽETAK

Cilj ovog rada bio je istražiti utjecaj promjena u godišnjim dobima na broj aspiriranih jajnih stanica, postotak oplodnje, kvalitetu embrija i postotak trudnoća u IVF ciklusima. Prospektivna kohortna studija provedena je na ukupno 2140 ciklusa u neplodnih pacijenata, koji su bili podvrgnuti svom prvom IVF ciklusu od 2000 do 2007. u IVF poliklinici. χ^2-test, Pearsonov koeficijent korelacije i jednosmjerna analiza varijance bili su korišteni u statističkoj analizi podataka. Ukupni postotak oplodnje po ciklusu bio je 77,96%, a trudnoća 29,15±2,72%. Gledajući po godišnjim dobima postotak trudnoća bio je najviši tijekom jeseni 33,8±4,5%, a najniži tijekom ljeta 23,4±6,2%, ali te razlike nisu dosegle statističku značajnost. Ovaj rad nije pokazao nikakve statistički značajne razlike u broju aspiriranih jajnih stanica, postotku oplodnje i kvaliteti embrija koja bi korelirala s promjenama godišnjih doba. Stoga sezonske varijacije ne bi trebalo uzimati u obzir u svakodnevnoj IVF praksi.