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Seasonal variability in the fertilization rate of women undergoing assisted reproduction treatments

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The objective of this study was to evaluate whether seasonality affects human-assisted reproduction treatment outcomes. For this, 1932 patients undergoing intracytoplasmic sperm injection (ICSI) were assigned to a season group according to the day of oocyte retrieval: winter (n = 435), spring (n = 444), summer (n = 469) or autumn (n = 584). Analysis of variance was used to compare the ICSI outcomes. The fertilization rate was increased during the spring (winter: 67.9%, spring: 73.5%, summer: 68.7% and autumn: 69.0%; $p < 0.01$). In fact, a nearly 50% increase in the fertilization rate during the spring was observed (odds ratio 1.45, confidence interval 1.20–1.75; $p < 0.01$). The oestradiol concentration per number of oocytes was significantly higher during the spring (winter: 235.8 pg/mL, spring: 282.1 pg/mL, summer: 226.1 pg/mL and autumn: 228.7 pg/mL; $p = 0.030$). This study demonstrates a seasonal variability in fertilization after ICSI, where fertilization is higher during the spring than at any other time.

Keywords: Fertilization, ICSI, oestradiol, photoperiod, seasonality

Introduction

The seasonal distribution in human natural conception and birth rate has been extensively studied. Different theories have emerged to explain this phenomenon in animal species. It has been hypothesized that the reproductive system is responsive to changes in both seasonal environmental light intensity and photoperiod [1].

Photoperiodism is a process whereby organisms use the retina to measure day length and the direction of day length change; these measurements then serve as the basis for regulating seasonal changes in physiology and behaviour. Photoperiodic information is transduced into neuroendocrine changes through variations in melatonin secretion from the pineal gland. Melatonin triggers variations in the secretion of gonadotrophin-releasing hormone (GnRH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [2,3].

It has been suggested that all mammals have at least some elements of a pathway that starts with the retina and ends at the GnRH pulse generator. In some species, this pathway is complete and functional; in others, it is not [3].

Humans are probably an example of a species that retains only bits and pieces of this pathway. It has been shown that humans exhibit seasonal variation in a wide variety of behavioural and physiological processes, and numerous investigators have suggested that this might be because we are sensitive to seasonal variation in day length [4–6].

Epidemiological evidence suggests that inhibition of fertility by heat in men in summer contributes to seasonal variation in human reproduction at lower latitudes and that stimulation of fertility by lengthening of the photoperiod in spring contributes to the variation at higher latitudes [7].

Moreover, a new hypothesis has been suggested stating that some humans are indeed seasonally photoresponsive, but others are not; and individual variation may be the cause of the inconsistencies that have plagued the study of responsiveness to photoperiod in the past [8].

Nevertheless, there is no clear evidence that the annual cycle of births in humans reflects regulation by photoperiod as opposed to food availability, potent cultural variables or the effect of summer heat.

Human-assisted reproduction is a good model to investigate the relationship between the seasons of the year and the developmental capacity of the retrieved oocytes, because most variables are controlled *in vitro*. Therefore, this study retrospectively evaluated whether seasonality affects human-assisted reproduction treatment outcomes.

Methods

Experimental design

This is a retrospective observational cohort study of 1932 patients undergoing oocyte retrieval for intracytoplasmic sperm injection (ICSI) in the Fertility-Assisted Fertilization Centre, Brazil, between January 2005 and December 2009.

Patients were assigned to a season group according to the day of oocyte retrieval: winter (n = 435, 22.5%), spring (n = 444, 23.0%), summer (n = 469, 24.2%) or autumn (n = 584, 30.2%).

The percentage of retrieved metaphase II (MII) oocytes and high-quality embryos, as well as fertilization, implantation and pregnancy rates was compared among the groups.

Moreover, the influence of the each season on the fertilization rate and percentage of high-quality embryos was evaluated.

Serum oestradiol concentrations were compared among the groups, and to identify the effectiveness of the ovarian response, the oestradiol concentration per number of MII-retrieved oocytes was also evaluated.

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The fertilization rate was defined as the number of fertilized oocytes divided by the number of injected oocytes. The implantation rate was defined as the number of gestational sacs per number of embryos transferred per patient. The pregnancy rate was defined as the number of patients with confirmed clinical pregnancy per number of patients with embryos transferred.

Written informed consent was obtained in which patients agreed to share the outcomes of their cycles for research purposes. The study was approved by the local Institutional Review Board.

Controlled ovarian stimulation

Controlled ovarian stimulation was achieved by long pituitary downregulation using a GnRH agonist (Lupron Kit™ Abbott S.A Societé Française des Laboratoires, Paris, France). Pituitary downregulation WAS followed by ovarian stimulation with recombinant FSH (Gonal-F® Serono, Geneva, Switzerland). Oocyte retrieval was performed 35 hours after the administration of recombinant human chorionic gonadotrophin (rhCG, Ovidrel™ Serono) through transvaginal ultrasonography.

Preparation of oocytes

Retrieved oocytes were maintained in human tubal-cultured medium (Irvine Scientific, Irvine, CA) supplemented with 10% human synthetic albumin (Irvine Scientific) covered with mineral oil for 4–5 hours before cumulus cell removal. Surrounding cumulus cells were removed by exposure to a HEPES-buffered medium containing hyaluronidase (80 IU/mL; Irvine Scientific). The remaining cumulus cells were then mechanically removed by gently pipetting with a hand-drawn Pasteur pipette (Humagen Fertility Diagnostics, Charlottesville, VA).

Intracytoplasmic sperm injection

Intracytoplasmic sperm injection was performed for all MII oocytes according to the technique described by Palermo et al. [9]. Oocytes were transferred to a microinjection dish, prepared with 4-μL droplets of buffered medium (HEPES; Irvine Scientific) and covered with mineral oil on the heated stage (37.0 ± 0.5°C) of an inverted microscope.

Assessment of fertilization, embryo quality and embryo transfer

Fertilization was assessed 18 hours after ICSI, and normal fertilization was declared when two clearly distinct pronuclei were present. The quality of the embryo was evaluated under an inverted microscope. The following parameters were recorded: (i) the number of blastomeres, (ii) the fragmentation percentage, (iii) variation in blastomere symmetry, (iv) the presence of multinucleation and (v) defects in the zona pellucida and the cytoplasm.

High-quality embryos were defined as those having all of the following characteristics: either four to six cells on the second day or eight to ten cells on the third day of development, less than 15% fragmentation, symmetric blastomeres, absence of multinucleation, colourless cytoplasm with moderate granulation and no inclusions, absence of perivitelline space granularity and absence of zona pellucida dysmorphism. Embryos lacking any of the above characteristics were considered to be of medium or low quality.

Embryo transfer was performed on the third day of development. One to three embryos from each couple were transferred.

Statistical analysis

Continuous variables are given as means ± SD, and proportions (%) are used for categorical variables.

Analysis of variance (ANOVA) was used to compare the percentage of retrieved MII oocytes and high-quality embryos, as well as fertilization, implantation and pregnancy rates.

Serum oestradiol concentration and oestradiol concentration per number of MII-retrieved oocytes were evaluated through ANOVA. When statistically significant differences were found, analyses were complemented by Tukey's test.

To evaluate the influence of the each season on the fertilization rate and percentage of high-quality embryos, linear regression analyses were performed, and results were expressed as odds ratios, 95% confidence intervals and *p* values.

Results were considered significant at the 5% critical level ($p < 0.05$). Data analysis was carried out using Minitab (Minitab Inc., Pennsylvania, USA) v.14, a statistical analysis program.

Results

The causes of infertility were ovarian disorders (28.9%), endometriosis (8.4%), tubal-uterine factor (5.4%), polycystic ovarian syndrome (4.3%), male factor (27.2%), idiopathic causes (10.9%) and combined causes of infertility (14.9%). It was equally distributed among the groups

Demographic and clinical data of patients listed according to experimental group are presented in Table I.

The groups were equivalent regarding age, body mass index, total dose of FSH administered, number of follicles and number of oocytes recovered.

The percentage of retrieved MII oocytes and high-quality embryos, as well as implantation and pregnancy rates, did not differ among the groups.

In addition, all the seminal parameters were equal among the groups; nevertheless, the fertilization rate was significantly increased during the spring compared to any other season (Table I). In addition, no significant difference was noted regarding the serum oestradiol concentration; however, the oestradiol concentration per number of MII retrieved oocytes was significantly higher during the spring (Table I).

Regression analysis demonstrated a nearly 50% increase in the fertilization chance in cycles performed during the spring. However, no influence of any other season on the fertilization rate and on the percentage of high-quality embryos was noted (Table II).

Discussion

It has been well documented that most mammals are seasonal breeders, and as day length increases, gonadotropin secretion increases, to anticipate reproduction in the spring and summer months [10]. There is intriguing evidence that there is a seasonal variance in human reproduction, but the data are not entirely consistent.

The present study evaluated whether seasonality affects human-assisted reproduction treatment outcomes, because variables are most controlled *in vitro.* A significant seasonal variability in the fertilization rate after ICSI, where the fertilization rate is higher during the spring than at any other time, was demonstrated here. However, no relationship between the season and any other ICSI outcome could be observed.

Studies in a wide range of organisms have led to the concept that transition from winter to spring or summer phenotypes occurs when day length exceeds a minimum threshold, termed the "critical day length " [11]. This threshold varies within individuals as a function of prior photoperiodic exposure and between

Table I. Demographic and clinical data of patients undergoing intracytoplasmic sperm injection.

	Winter	Autumn	Spring	Summer	*p*
Number of patients	435	584	444	469	
Age (years)	35.3 ± 0.2	35.3 ± 0.2	35.6 ± 0.2	35.2 ± 0.2	0.169
BMI (kg/m^2)	23.1 ± 0.4	22.5 ± 0.5	23.0 ± 0.5	23.7 ± 0.4	0.660
Total dose of FSH (IU)	2,475.6 ± 37.3	2,473.8 ± 31.0	2,479.1 ± 34.5	2,525.9 ± 35.5	0.664
Serum oestradiol concentration (pg/mL)	1779 ± 104.8	1713 ± 117.0	1763 ± 96.8	1985 ± 85.9	0.312
Oestradiol concentration/retrieved oocytes (%)	235.8[a]	228.7[a]	282.1[b]	226.1[a]	0.030
Number of follicles	17.0 ± 0.6	17.9 ± 0.6	16.8 ± 0.7	17.0 ± 0.6	0.340
Number of retrieved oocytes	11.7 ± 0.4	12.0 ± 0.4	11.3 ± 0.5	11.7 ± 0.4	0.555
Percentage of retrieved MII (%)	71.7	74.3	72.4	73.0	0.480
Sperm concentration (M/mL)	30.8 ± 4.0	27.3 ± 5.2	32.4 ± 4.4	26.4 ± 3.2	0.365
Sperm motility (%)	28.2 ± 2.4	26.6 ± 0.4	30.4 ± 3.2	25.2 ± 4.4	0.858
Sperm morphology (%)	5.2 ± 0.4	4.3 ± 0.7	6.1 ± 0.1	5.4 ± 0.6	0.635
Fertilization rate (%)	67.9[a]	69.0[a]	73.5[b]	68.7[a]	<0.01
High-quality embryos rate (%)	61.1	58.9	57.2	61.7	0.360
Pregnancy rate (%)	31.4	32.4	32.6	35.5	0.639
Implantation rate (%)	20.6	20.3	20.5	20.7	0.907

a≠b: $p < 0.05$.

Table II. Regression analysis of the influence of the season of the year on the fertilization and high-quality embryos rate.

	Fertilization rate			
	OR	CI lower	CI upper	*p*
Winter	1.11	0.78	2.12	0.234
Autumn	1.21	0.81	1.69	0.186
Spring	1.45	1.20	1.75	<0.01
Summer	1.12	0.69	1.82	0.666
	High-quality embryo rate			
	OR	CI lower	CI upper	*p*
Winter	0.98	0.27	2.01	0.845
Autumn	1.65	0.76	3.05	0.872
Spring	0.73	0.38	1.41	0.346
Summer	1.02	0.82	1.78	0.576

OR, odds ratio; CI, confidence interval.

individuals in a manner reflecting the latitude of origin. This phenomenon implies that a genetically controlled mechanism determines the working range of environmental photoperiods over which an organism can precisely time seasonal transitions in physiology and behaviour [7].

The relationship among fecundity, daylight and temperature during different seasons has been previously suggested in different animal species, including humans [12–18] and similar seasonal changes in pregnancy rates have been reported from several studies of assisted reproduction [19–22].

Human reproduction varies somewhat by season [23,24], and it has been reported that there are two birth peaks per year in some human population data sets [25].

How seasonal influence may be responsible for alterations in the oocyte and sperm fertilization functions is still unknown, but some evidence points to the involvement of endocrine system.

It has been suggested that fertility is stimulated by lengthening of the photoperiod, and increasing day length is associated with changes in the brain that are responsible for mediating reproductive activities [4].

In mammals, photoperiodic information is transformed into a melatonin secretory rhythm in the pineal gland. Melatonin exerts its effects in discrete hypothalamic areas, modulating the GnRH secretion [26], which initiates the cascade of reproductive hormones in mammals [27]. Increased release of GnRH into hypothalamo-hypophyseal portal vessels results in increased secretion of LH and FSH, which in turn regulate gonadal function [28].

In fact, it has been described that human melatonin secretion has a drastic variation during the year [15,29,30], as well as variations in LH secretion that have been observed according to the season [31,32].

In the present study, not only the fertilization rate but the effectiveness of the ovarian response to the hormonal treatment was also higher during the spring in comparison with the other seasons.

In fact, final follicular growth is associated with an increase in LH secretion. Final follicular growth is encompassed by a transient elevation in LH, which is required for the production of oestradiol and free insulin-like growth factor-I. Insulin-like growth factor-I synergizes with FSH in stimulating granulosa cell proliferation and steroidogenesis [33]. Moreover, the preovulatory follicle responds on the LH peak with final oocyte maturation and ovulation [34,35].

Our findings suggest that the increased fertilization rate observed in assisted reproduction cycles performed during the spring, in which the day length is longer, may be associated with an increased melatonin–hypothalamus–gonadotrophin activity, leading to the production of high-quality gametes and resulting in higher fertilization potential.

***Declaration of Interest*:** The authors report no conflict of interest.

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