Oocyte Quality in Patients with Increased FSH Levels

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ABSTRACT

Objective: The present study consists of quality comparison among oocytes retrieved from women under 37 years old showing increased levels of FSH (prone to premature ovarian insufficiency) and women at the same age with normal hormone levels.

Methods: Oocyte quality was accessed according to Lucinda L. Veeck parameters (1986) and the statistical analyses were carried out using Chisquared, SPSS for Windows 13.0 (SPSS, Inc., Chicago, IL). All pvalues were twotailed, and 95% confidence intervals (CIs) were calculated. A *P* value <0.05 was considered statistically significant.

Results: Eight morphologic changes variables were considered in the study and two of them showed statistically significant differences between cases and controls: granular cytoplasm (*P*=0.002) and presence of vacuoles (*P*=0.025), both more frequent among the study group patients.

Conclusion: As a conclusion, patients with increased FSH levels presented oocytes with worst quality variables than controls. This can be an indicative of ovarian aging and can impact negatively on oocyte development into viable embryos.

Keywords: Premature Ovarian Insufficiency, Oocyte quality, FSH levels, Ovarian aging

INTRODUCTION

Oocyte quality is one of the factors that directly influences the development of a viable embryo in assisted reproduction procedures. The oocyte is a central regulator of multiple aspects of female fertility, including ovarian follicular development and early embryogenesis. A deficit in oocyte competence can lead not only to a failure of fertilization but also to a lower developmental rate after fertilization (Moussa *et al.*, 2015).

According to Lucinda L. Veeck (1986) oocyte morphological aberrations can be classified according to the presence of granular cytoplasm, dark cytoplasm, vacuoles, debris, increased or absent perivitelline space, fragmented corpuscle, changes to oocyte shape, and zona pellucida alterations such as thick, thin, light and dark.

Ovarian aging is a major fact influencing oocyte quality. It is well known that the female reproductive capacity declines dramatically in the fourth decade of life as a result of an age-related decrease in oocyte quality and quantity. The primary causes of reproductive aging and the molecular factors responsible for decreased oocyte quality remain elusive (BenMeir, 2015).

However, some women experience ovarian failure effects prematurely, before 40 years of age. The condition is not rare and affects one in every 100 women between 30-39 years (Goswami & Conway, 2005). Before complete failure and menopause establishment, FSH levels start to increase and the ovary becomes less responsive.

In 2013, the ESHRE Premature Ovarian Insufficiency (POI) Consensus (Baat, 2013) determined a new cut off level for FSH: 25 mUI/ml; above which, patients can be classified with Premature Ovarian Insufficiency. The etiology

of this condition is complex and the dysfunction may be secondary to autoimmune diseases, infections, iatrogenic exposure and genetic alterations such as cytogenetic abnormalities of the X chromosome and gene mutations (Mattison *et al.*, 1984; Alper *et al.*, 1986; Conway, 1997; Anasti, 1998; Goswami & Conway, 2005).

Due to the large incidence of POI, in Human Assisted Reproduction clinics it is possible to find a significant number of women whose FSH levels range between 10 and 25 mUI/ml. Nevertheless, no information about oocyte condition in these cases is described in the literature.

Thus, the goal of our study is to compare the incidence of oocyte alterations in women with prematurely increased FSH levels with their counterparts in the control group.

MATERIALS AND METHODS

Cases

The study group encompassed women aged from 30 to 39 years with changes in menstrual cycles, increased FSH levels in at least two dosages on the first three days of the cycle (between 12 and 25 mUI/ml), and no other identified factor of ovarian damage such as surgery, chemo or radiotherapy, autoimmune diseases, endometriosis or PCOS.

Patients in the control group are women with normal FSH levels (< 10 mUI/ml), with no ovulatory factor of infertility. All patients read and signed the informed consent form and were subjected to treatment involving assisted human reproduction.

Oocyte quality assessment

We analyzed 48 mature (MII) oocytes from the case group and 81 MII oocytes from the control group morphologically according to Lucinda L. Veeck parameters (1986). The variables analyzed were:

- 1) Granular cytoplasm
- 2) Dark cytoplasm
- 3) Presence of vacuoles
- 4) Presence of Debris
- 5) Increased perivitelline space
- 6) Fragmented polar corpuscle
- 7) Changes in oocyte shape
- 8) Changes on the zona pellucida thickness

We measured basal levels of follicle stimulating hormone (FSH) in patients with premature ovarian failure, using 5 mL of peripheral blood and commercial kit (Vidas® FSH), using the Mini Vidas (bioMeriux SA, 69280 Marcy l'Etoile, France) device.

Statistical Analysis

Statistical analyses were carried out using Chisquared - SPSS for Windows 13.0 (SPSS, Inc., Chicago, IL). A *P* value <0.05 was considered statistically significant.

RESULTS

The mean age of the study group patients was 37.1 ± 15.0 years; and it was 36.6 ± 4.5 years old in the control group.

Table 1. Study group X Control group. Analyses of morphological variables

Morphological Variables	Study group N=48	Control group N=81	P value
Granular Cytoplasm	45	57	0.025
Dark Cytoplasm	16	38	
Presence of vacuoles	10	6	0.020
Presence of debris	13	22	
Increased perivitelline space	7	6	
Fragmented polar corpuscle	13	20	
Change in oocyte shape	0	2	
Changes on zona pellucida thickness	45	79	

The mean levels of FSH were 15.09 ± 5.0 mUI/ml in the study group, and 6.7 ± 4.1 mUI/ml in the control group.

The morphological variables were analyzed and compared between the groups. The granular cytoplasm and presence of vacuoles variables in the study showed statistically significant differences from the control group, with P values of $P = 0.025$ and $P = 0.02$, respectively (Table 1).

DISCUSSION

Human reproduction presents a number of challenges that need to be better understood. One of them refers to factors that can impact oocyte quality. Some conditions, such as PCOS are widely studied and its impact on oocyte quality is not fully understood. However, there are many conditions that still are poorly investigated as POI.

Premature Ovarian Insufficiency is a polygenic and multifactorial disease, which etiology can be attributed to several factors and is still being studied. According to Vilodre *et al.* (2007), various causes can lead to POI or reduced number of follicles and/or defects in the follicular stimulation mechanism. However, there is no study about the events affecting oocytes previously to ovarian arrest.

Here we investigated patients with ovarian dysfunction presenting difficulties in oocyte retrieval, due to increased FSH levels, looking for oocyte morphological alterations. Eight variables of morphologic alterations were considered in the study and two of them showed statistically significant differences between cases and controls: granular cytoplasm and the presence of vacuoles, both more frequent in the study group.

One of the most fundamental determinants of oocyte developmental competence is nuclear maturity. This complex process involves: germinal vesicle breakdown, chromosome segregation, asymmetric meiotic division, expulsion of the first polar body and organization of oocyte cytoskeletal structure. The MII spindle, constructed of microtubules arranged around centrosome poles, plays an essential role in fertilization. Sperm penetration through the zona pellucida triggers resumption of meiosis and expulsion of the second polar body. Once the male and female pronuclear envelopes fuse, mixing maternal and paternal DNA, the chromosomes align along the newly formed mitotic spindle in preparation for segregation into separate blastomeres during embryonic cleavage (Wallbutton and Kasraie, 2010).

Cytoplasmic abnormalities that occur during oocyte maturation, especially those that interfere with meiotic spindle and intricate cytoskeletal structure, can adversely affect the ability of oocytes to undergo normal fertilization and embryo development.

Oocyte vacuolization is a common observation in clinical IVF. Ebner *et al.* (2005) found vacuoles in 3.9% of

oocytes at collection, of which 66% had single, 21.3% had double and 12.7% had multiple vacuoles. De Sutter *et al.* (1996) and Alikani *et al.* (1995) suggest a slightly higher oocyte vacuolization rate (5.7% and 12.4%, respectively). Ebner *et al.* (2005) found no significant difference in clinical pregnancy rate but significantly higher miscarriage rates, lower birth weights and greater obstetric problems in pregnancies from cycles where some oocytes collected exhibited sERCs. Rienzi *et al.* (2008) identified significantly lower fertilization rates in vacuolated oocytes. Ebner *et al.*, (2005) observed lower than normal fertilization rates in vacuoles up to $14 \mu\text{m}$ (51.6% for single and 43.8% for multiple vacuoles) and suggest that fertilization cannot be expected in vacuoles $>14 \mu\text{m}$.

Granularity is one of the abnormalities observed in the perivitelline space. These abnormalities are among the most important dimorphisms of the extracytoplasmic component. Hassan *et al.* (1998) found an association between the perivitelline space granularity and lower numbers of MII oocytes; however, perivitelline space granularity was not associated with fertilization rate, cleavage rate, and embryo quality. Interestingly, they found that granularity was positively associated with the dosage of human menopausal gonadotropins administered during stimulation. Our study suggests that FSH levels also have a negative impact on oocyte granularity.

In conclusion, higher FSH levels seem to impact negatively on oocyte morphology. Future studies with a larger population and investigating the association of these morphological abnormalities with clinical pregnancy rates would be interesting.

CONCLUSIONS

As a conclusion, patients with increased FSH levels presented oocytes with worst quality variables than controls. This can be indicative of ovarian aging and can affect negatively on the oocytes development into viable embryos.

CONFLICT OF INTERESTS

No conflict of interest have been declared.

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