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Research Article

## Does biochemical profile of follicular fluid in women undergoing assisted reproduction vary between the right and left ovaries?

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### ABSTRACT

**Background:** Follicular fluid (FF) provides a substantial micro-milieu for the development of the oocytes. Moreover, the content of FF may also reflect the circulating biochemical environment and the changes in blood serum. Despite the known embryological and histological similarities between right and left ovaries, differences do exist between their venous drainage, anatomical relations and cyclical physiological changes. The difference between the two ovaries in the response to controlled hyperstimulations (COH) in IVF regimens was later confirmed by recent studies. The aim of this study was to compare the biochemical differences between the FFs yielded from the right and left ovaries in patients undergoing standart IVF treatment.

**Methods:** A prospective observational study was conducted through the unexplained infertility patients with the age between 23-39 years, body mass index (BMI)  $\leq 28$  kg/m<sup>2</sup>, FSH  $\leq 10$  mIU/mL. FFs aspirated from the right and the left ovary of the patients were assayed for estradiol, calcium, phosphorus, uric acid, urea, total protein, AST, ALT, glucose, sodium, potassium and creatinine. A total of 10 patients undergoing IVF treatments were included.

**Results:** The investigated parameters were not significantly different between FFs yielded from the right and left ovaries.

**Conclusion:** According to the results of our study, biochemical profile of the aspirated FFs does not significantly differ between the right and the left ovary and consequently, either right or left ovaries provide similar micro-environments to the developing oocytes.

**Keywords:** Follicular fluid, Infertility, Right ovary, Left ovary, Biochemistry

### INTRODUCTION

Despite recent advances in the field of in vitro fertilization (IVF), success rates remain relatively low, with clinical pregnancy rates still at around 33%.<sup>1</sup> All attempts in assisted reproduction to improve pregnancy rates as well as to reduce the possibility of multiple pregnancy are to choose the best quality embryo for transfer and to allow implantation of a single embryo.<sup>2</sup> Despite the importance of oocyte quality, current

assessment of oocytes in assisted reproduction is limited and a variety of methods, including oocyte morphology studies,<sup>3</sup> molecular and genetic methods<sup>4</sup> and polar body biopsies<sup>5</sup> is being employed for oocyte selection. However, most of these techniques are not applicable in the current practice since they are complicated, expensive and time-consuming.<sup>6</sup>

The analysis of follicular fluid has recently been a matter of interest as a possible predictor of both oocyte

developmental competence and the subsequent potential to achieve fertilization and embryo viability.<sup>6-10</sup> Moreover, the content of follicular fluid (FF) may also reflect the circulating biochemical environment and the changes in blood serum.<sup>11</sup>

Despite the known embryological and histological similarities between right and left ovaries, differences do exist between their venous drainage, anatomical relations and cyclical physiological changes. Both ovaries receive arterial blood supply directly from the aorta, however, venous drainage differs as the right ovarian vein drains directly to the inferior vena cava (IVC), whereas the left drains firstly to the left renal vein then to the IVC.<sup>12</sup> On the other hand, intraovarian physiological control of side of ovulation has also been demonstrated to vary between right and the left ovaries, as both fertile and infertile women with two healthy ovaries ovulate significantly more often from the right ovary than the left and ovulation from the right ovary was more likely to achieve pregnancy than the left.<sup>13</sup> The difference between the two ovaries in the response to controlled hyperstimulations (COH) in IVF regimens was later confirmed by Lan et al. in 2010.<sup>14</sup> However, how the superior activity of the right ovary was mediated is poorly understood.

Given that the both ovaries are different in terms of cyclicity and frequency of the ovulation and the ovarian response to COH, it is reasonable to think that the components of FF surrounding the oocyte differ between the right and left ovaries. However, to the best to our knowledge, it has not been investigated whether the biochemical profile of FF vary between the right and left ovaries. Knowing whether, or not, any difference between the FFs yielded from right and left ovaries exist would let us estimate the reproductive destiny of the aspirated oocytes, especially in patients with one ovary or in those in whom the oocyte retrieval procedure is restricted to only one ovary due to anatomical or functional limitations. The objective of this study was to compare the biochemical differences between the FFs yielded from the right and left ovaries in patients undergoing standard IVF treatment.

## METHODS

A prospective observational study was initiated at the Assisted Reproduction and Infertility Department of Zeynep Kamil Training and Educational Hospital, Istanbul, Turkey. Ethical Board approval was obtained prior to the initiation of the study. Patients seeking treatment for solely unexplained infertility were recruited, in a 6 month period between June and December 2013. Inclusion criteria were as follows; age between 23-39 years and body mass index (BMI)  $\leq 28$  kg/m<sup>2</sup>, follicular stimulating hormone (FSH)  $\leq 10$  mIU/mL, unexplained infertility patients who

underwent either agonist or antagonist protocol. Patients with male or tubal factor infertility were excluded.

All patients underwent our standardized IVF treatment protocol. Gonadotropin doses were individualized according to age, BMI, basal hormone levels, antral follicle count (AFC) and previous experiences. Gonadotropin stimulation was achieved by either rFSH or hMG. All patients were administered acetyl-salicylic acid 100 mg daily and folic acid 400 mcg daily simultaneously with the start of the protocol.

Serial ultrasonographic controls and estradiol (E2) level measurements were made until 3 follicles  $\geq 17$  mm and a serum E2 level  $>500$  pg/ml were detected. Choriogonadotropin alpha 250  $\mu$ g s.c. (Ovitrelle®; Merck Serono, Italy) was administered to induce final follicular maturation. Transvaginal ultrasound-guided oocyte retrieval was performed 35-36 hours after hCG administration. In both left and right ovary, FFs collected from the first punctured, mature ( $> 17$  mm) follicle at the first entry without contamination, were analyzed; whereas FFs contaminated with blood were not used. All patients were implemented a single dose of cefazolin sodium (Sefazol, Mustafa Nevzat İlaç San., Turkey) 1 gr, i.m. at the course of OPU procedure and were given doxycycline 100 mg capsule (Tetradox capsule, Fako İlaç, Turkey) twice daily and methylprednisolone 16 mg capsule (Prednol tablet, Mustafa Nevzat İlaç San., Turkey) once daily orally and continued for 4 days.

FFs aspirated from the right and the left ovary of the patients were centrifuged for 10 minutes at 2000xG at room temperature and separated supernatant fluids were assayed for estradiol, calcium, phosphorus, uric acid, urea, total protein, AST, ALT, glucose, sodium, potassium and creatinine. The results of the right and left ovaries were compared. Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows 15.0 software (SPSS, Chicago, IL., USA). Descriptive statistics were given as mean and standard deviation. Non-parametric comparisons were performed using Mann-Whitney U test. Statistical significance was defined as  $p < 0.05$ .

## RESULTS

A total of 10 patients undergoing IVF treatments were included and the FFs yielded from these patients were compared. According to the results of our study, levels of E2, calcium, sodium, glucose and ALT were higher in the FFs yielded from the left ovary, while phosphorus, urea and total protein were higher in the FFs of the right ovary. However these differences were not statistically different. Meanwhile, potassium, uric acid, creatinine and AST levels were the same between the two ovaries. Consequently, biochemical profile of the aspirated FFs does not significantly differ between the right and the left ovary (Table 1).

**Table 1: Comparison of the biochemical parameters in the FFs yielded from the right and left ovaries.**

	Right ovary	Left ovary	p*
Estradiol, ng/ml	1286.6 ± 589.1	1440 ± 743.9	0.796
Calcium, mM	7.8 ± 0.6	7.9 ± 0.3	0.683
Phosphorus, mg/dl	3.4 ± 0.4	3.3 ± 0.4	0.791
Sodium, mM	139.6 ± 1.3	140.4 ± 1.4	0.138
Potassium, mM	3.8 ± 0.8	3.8 ± 0.8	0.909
AST, IU/l	28.5 ± 5.1	28.5 ± 4.9	0.940
ALT, IU/l	12.9 ± 6.0	13.3 ± 5.1	0.910
Glucose, mg/dl	56.3 ± 15.7	58.3 ± 12.9	0.734
Uric acid, mg/dl	3.5 ± 0.6	3.5 ± 0.5	0.940
Urea, mg/dl	23 ± 5.1	22.8 ± 4.4	0.999
Creatinine, mg/dl	0.8 ± 0.1	0.8 ± 0.1	0.912
Total protein, g/dl	5.6 ± 0.6	5.5 ± 0.5	0.970

\*Mann Whitney-U test.

## DISCUSSION

The right and the left ovaries are not the same regarding venous and lymphatic drainage and regional anatomy, in addition to ovulatory cyclicity. The control of the side of ovulation has been demonstrated in humans firstly in 1987 by Potashnik et al.,<sup>15</sup> who suggested that ovulation tended to occur more frequently in the right ovary. Following studies reported similar results which indicated the difference between the right and left ovaries regarding the frequency of ovulation and the destiny of the ova from the different sides.<sup>13,14</sup> Lan et al also reported statistically significant differences between the right and left ovarian responses in IVF patients with healthy ovaries and that the right ovarian responses were superior to the left ovarian responses.<sup>14</sup>

The biochemical composition of fluid in mature Graafian follicle is of great importance, since this medium bathes developing oocytes and the composition of FF may have an impact in the oocyte quality, which is a crucial parameter in women undergoing assisted reproduction. FF contains a large variety of metabolites, including hormones, growth factors, interleukins, proteins, peptides and prostanoids that are presumably involved in oocyte growth and development.<sup>6</sup> The identification and quantification of FF composition can provide valuable information about the oocyte state which can be helpful in distinguishing those oocytes that have a greater capacity to be fertilized and to

develop properly. In this context, numerous metabolites have been investigated in recent years. Wallace et al. identified a number of metabolites, including glucose, lactate, choline, phosphocholine, proline, leucine/isoleucine, glutamine and HDL, as being important indicators of whether a fertilized oocyte will divide and develop into an early cleavage stage embryo or fail to cleave.<sup>11</sup> Some other studies investigated the composition of free fatty acid and triglyceride in FF in order to clarify whether the levels of lipid content in FF could be predictive to select the best quality oocyte.<sup>16-19</sup> Peptide and protein composition of FF was analyzed to identify molecules that could be used as markers of good follicular development in order to optimize oocyte selection process.<sup>20</sup> D'Aniello et al.<sup>21</sup> demonstrated that D-aspartic acid concentrations in FF correlated with percentage of good morphology, MII oocytes and with the fertilization rate. Józwick et al.<sup>22</sup> investigated amino acid, ammonia and urea concentrations in FF and reported that their concentrations were higher in FF than in blood. Although differences between the right and left ovaries, regarding the anatomical relations and cyclical physiological changes are well-established, the biochemical profile of FF yielded from the two ovaries has never been compared. In the present, which is a preliminary study in this context, we have investigated the quantitative levels of a total of 12 parameters in the FF, none revealed a statistically significant difference between the FFs from right and left ovaries. Our result might be important in patients with one ovary or in those in whom oocyte pick-up is available only from one ovary due to various limitations. In such cases, we suggest that either right or left ovaries provide similar micro-milieu to the developing oocytes.

One major limitation of this study is that the number of women included in the study is low. However, since the biochemical analysis of the FF is not a routinely applied procedure, we considered to conduct a preliminary study to provide us an opinion on this issue. Further studies in larger cohorts to evaluate the composition of FF are required. In addition, due to the increasing evidence of the relationship between FF metabolite composition and oocyte developmental competence, it is necessary to understand the main influencing factors and how its composition may be modulated to improve oocyte quality.

## CONCLUSION

FFs yielded from the right and left ovaries do not vary in terms of biochemical profile, including estradiol, calcium, phosphorus, uric acid, urea, total protein, AST, ALT, glucose, sodium, potassium and creatinine.

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