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Potential ability of circulating INSL3 level for the prediction of ovarian reserve and IVF success as a novel theca cell–specific biomarker in women with unexplained infertility and diminished ovarian reserve

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Abstract

Background Insulin-like peptide (INSL3), belonging to the insulin-like peptide family, is produced by theca interna cells within antral follicles and the corpora lutea. It is hypothesized that INSL3 is integral to the initial development and function of antral follicles, specifically through its regulatory effect on androgen biosynthesis in the thecal cells of these follicles. Moreover, INSL3 is implicated in the modulation of the ovarian microenvironment, which is essential for facilitating the maturation of oocytes. Our study investigates if circulating and follicular fluid INSL3 levels serve as biomarkers for ovarian reserve and IVF success in women with unexplained infertility (UI) and diminished ovarian reserve (DOR).

Methods This prospective study included 75 women (25 with DOR, 24 with UI) undergoing IVF and 26 controls with normal ovarian reserve. Serum and follicular fluid INSL3 levels were measured, and their association with ovarian reserve markers, pregnancy rates, and live birth rates (LBR) was analyzed.

Results Circulating ($p = 0.001$) and follicular fluid ($p < 0.001$) INSL3 levels, AMH levels ($p < 0.001$) and AFC ($p < 0.001$) were significantly lower and basal E2 level ($p < 0.001$) were significantly higher in DOR group compared to the UI and control groups. Circulating INSL3 positively correlated with serum anti-Müllerian hormone (AMH) and antral follicle count (AFC), and negatively correlated with follicle-stimulating hormone (FSH). Positive pregnancy rates and LBR were significantly lower in the DOR group. Basal FSH was identified as a significant predictor of LBR.

Conclusions The current study presents that although the serum and follicular fluid INSL3 levels are significantly lower in women with DOR, the narrow margin between the DOR and control groups indicates that INSL3measurement may be insufficient on its own to be of diagnostic value for DOR. Further research with larger sample sizes is needed to validate these findings and explore the role of INSL3 in ovarian aging and infertility treatment.

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Keywords Insulin-like peptide 3, Ovarian reserve, In vitro fertilization, Diminished ovarian reserve

Background

Couples who fail to achieve pregnancy for at least 12 months with regular and unprotected sexual intercourse are diagnosed with unexplained infertility (UI) after standard infertility investigations fail to identify abnormalities in the female and the male [1]. This somewhat controversial diagnosis is reached by exclusion according to the results of the diagnostic tests although the consensual standardization of diagnostic work-up remains unclear. The proportion of UI in infertile couples reach approximately 30% [2]. Several factors which remain undetectable with routine diagnostic tests may be crucial components of ovarian biology. These may prevent conception and predict IVF success in couples diagnosed with UI and are potential targets for further exploration. Diminishing ovarian reserve (DOR) is likely to be one of the underlying mechanisms of UI. However, there are still major uncertainties of the definition of DOR and its management. According to the Bologna criteria poor ovarian response (POR) is defined as presence of at least two of the following three conditions: (i) advanced maternal age (≥ 40 years) or any other risk factor for POR; (ii) a previous poor response with a conventional ovarian stimulation protocol (≤ 3 oocytes); and (iii) An abnormal ovarian reserve test (i.e. AFC 5–7 follicles or AMH 0.5 – 1.1 ng/ml) [3]. While there is a well described definition for POR as mentioned, a consensus is missing regarding DOR. DOR is generally defined as a decreased number or quality of oocytes and considered to have a significant impact on fertility in women as well as the quality of life [4–6]. Due to ongoing controversy about the definition and management of DOR, new markers that represent crucial components of ovarian biology and are related to pathophysiologic process of premature ovarian aging can shed further light on this subject.

Insulin-like peptide 3 (INSL3) may be one of these markers. INSL3 is a member of the insulin-like group of peptide hormones [7]. It is secreted by theca interna cells of antral follicles and the corpora lutea in females [8, 9]. It is postulated that INSL3 plays a key role in the early development and function of antral follicles by modulating androgen biosynthesis in thecal cells of antral follicles [10–12]. Moreover, it regulates the ovarian microenvironment by inducing expression of growth differentiation factor 9 (GDF9) by oocytes and it could facilitate oocyte maturation [10]. Therefore, INSL3 has been defined as a major regulator for reproductive physiology of women [7].

Several studies clearly demonstrated that there is a strong positive correlation between high levels of circulating INSL3 and high number of antral follicles in women with PCOS with a clear association to the total thecal cell mass in ovaries [13]. The data which evaluated the relationship between serum INSL3 and POI reported that serum INSL3 continuously decreases with the progress of ovarian insufficiency with a clear association to other ovarian reserve markers [14]. The functions of INSL3 are not limited with the reproductive system. INSL3 has also been shown to be involved in bone metabolism by promoting both bone turnover and accumulation [15]. Moreover, renal function may be affected by processes involving INSL3; Fu et al., demonstrated that INSL3 possibly affects mesangial cell proliferation [16]. Thyroid, vascular epithelium, breast and brain are some of the other tissues, where the influence of INSL3 is studied [7]. The role of INSL3 as a biomarker in women with UI and DOR is not yet known.

We hypothesized that circulating INSL3 levels may be considered as a good biomarker for the prediction of ovarian reserve and IVF success in women with UI and DOR. Circulating INSL3 levels might reflect oocyte impairment and androgen deficiency in these women. Therefore, we designed this study to investigate potential ability of circulating INSL3 levels for the prediction of ovarian reserve and IVF success as a novel theca cell–specific biomarker. We also studied the INSL3 concentrations in follicular fluid from dominant follicles containing oocytes during oocyte retrieval.

Methods

Participants

This prospective study was carried out at IVF center of Bezmialem Vakif University and Istanbul University School of Medicine. A total of 75 women with diminishing ovarian reserve ($n = 25$) and unexplained infertility ($n = 24$) between 20 and 40 years old and BMI < 30 kg/m^2 underwent IVF as well as 26 control women with normal ovarian reserve were included in this study. The protocol of our study was approved by the Ethical Committee of Bezmialem University (E-112926). Written informed consent was obtained from all participants. UI is defined as no abnormalities with female and male after standard infertility investigations including a tubal patency test, transvaginal ultrasound examination, AMH test and semen analysis and DOR was defined as serum AMH < 1.1 ng/ml.

Exclusion criteria were women with other endocrine abnormalities, such as polycystic ovary syndrome, hyperprolactinemia, hypo- and hyperthyroidism, cycle cancellation due to lack of a viable embryo or who had Mullerian anomalies not corrected by surgery, such as bicornuate, unicornuate, or didelphic uterus, those with

a history of recurrent miscarriage, and those with presence of a hydrosalpinx. Women taken oral contraception or luteal estradiol before the stimulation cycle were also excluded in this study. The collected data consisted of demographic characteristics of women including female and male age (year), BMI (kg/m2), the duration of infertility (years), infertility type (Primary/Secondary), previous IVF attempts, smoking, serum FSH, E2, AMH concentration (ng/mL) and AFC on the second or third day of the menstrual cycle, serum INSL3 concentration on the second or third day of the menstrual cycle, and INSL3 concentration in follicular fluid from dominant follicles containing oocytes (diameter > 14 mm) during oocyte retrieval, total dosage of gonadotropins (IU), maximum estradiol levels (pg/mL), the duration of stimulation (day), the number of oocytes retrieved, the number of mature follicles retrieved, the number of PN and endometrial thickness at day of transfer (mm). Outcome parameters were positive pregnancy test and live birth rate (LBR) (pregnancies ≥ 28 weeks of pregnancy).

Ovarian stimulation [17], endometrial preparation and embryo transfer [18] were performed as described previously. The gonadotropin-releasing hormone (GnRH) antagonist protocol and stimulation with recFSH were used for controlled ovarian stimulation (COS). The starting dose (between 150 and 450 IU) was dependent on the age of the woman, ovarian reserve and BMI. Transvaginal scans were performed on days 5, 7 and 9 of stimulation. If necessary, the dosage was adjusted on the basis of serial sonographic measurements of follicular development. A single dose of recombinant hCG (Ovitrelle, 250 mg; Serono) was administered for the triggering of the final oocyte maturation. Transvaginal ultrasound-guided oocyte retrieval was performed 36 h after hCG administration. The standard ICSI technique was applied to all oocytes for fertilization. The highest quality embryos based on morphology and cleavage criteria were selected for transfer.

Hormone measurement and ultrasonography

Serum FSH (IU/L) and estradiol (E2) (pg/mL) levels on the second or third day of the menstrual cycle were analyzed using Siemens Atellica Solutions (Siemens Healthineers AG, Forchheim, Germany) via chemiluminescent immunoassay (CLIA). Serum AMH level was evaluated by using CLIA on Maglumi (Snibe co. Ltd., Shenzhen, China). The AFC was evaluated on the second or third day of the menstrual cycle with transvaginal ultrasonography as the number of 2–10 mm in diameter of follicles in both ovaries. The intra-assay and inter-assay coefficients of variation were < 10% and < 10 for FSH, < 20% and < 10% for E2, < 10% and < 10 for AMH.

Serum and follicular fluid collection for analysis of INSL3

Peripheral blood samples were collected on the second or third day of the menstrual cycle and serum samples were stored at -80^0 C until the evaluation. Follicular fluid from dominant follicles containing oocytes (diameter > 14 mm) during oocyte retrieval was centrifuged and stored at -80^0 C until the evaluation. INSL3 levels in serum and follicular fluid (pg/mL) were measuredusing an Enzyme-linked Immunosorbent Assay (ELISA) kit (MyBioSource) according to the manufacturer's instructions. The intra-assay and inter-assay coefficients of variation was < 10% and < 12.

Statistical analysis

The data were analyzed using SPSS Statistics for Windows, Version 29 (IBM Corp, Armonk, NY, USA). Data were reported as mean ± SD or number and percentage. $P < 0.05$ was considered significant. Normally distributed parametric variables were tested by the Shapiro–Wilk test. The Mann–Whitney U-test with Bonferroni correction was used to compare two independent groups. Non-normally distributed metric variables were analyzed by Kruskal Wallis test. The relationships between the variables in the quantitative structure were examined using the Spearman correlation coefficient. Correlation between different variables was analyzed by Pearson correlation analysis. Univariate and multivariate evaluations were performed for the evaluation of variables affecting LBR.

Results

Baseline characteristics of patients are shown in Table 1. The mean age of the patients was 28.4 ± 3.5 years in the unexplained infertility group, 32.4 ± 4.2 in the DOR group and 29.6 ± 3.9 in the control group. There was no significant difference regarding male age, infertility type and smoking status between the groups. A statistically significant difference was found between the groups in terms of female age, BMI, duration of infertility, previous IVF attempts, basal FSH, E2, AMH levels and AFC. Female age ($p = 0.001$), BMI ($p = 0.049$), duration of infertility ($p = 0.006$), previous IVF attempts ($p < 0.001$), basal FSH ($p < 0.001$) and basal E2 level ($p < 0.001$) were higher, and AMH levels ($p < 0.001$) and AFC ($p < 0.001$) were lower in DOR group. IVF cycle characteristics of patients are presented in Table 2. No statistically significant differences in endometrial thickness on the day of transfer. Although the total dosage of gonadotropins required was higher in the DOR group, it did not reach statistical significance. There were significant differences regarding maximum estradiol levels, duration of stimulations, number of oocytes retrieved, number of mature follicles retrieved and number of PN among groups. Duration of stimulations ($p = 0.017$) was higher in DOR group while

Table 1 Baseline characteristics of patients

Characteristics	Unexplained (*n* = 24)	DOR (*n* = 25)	Control (*n* = 26)	*P* value
Female age (years)	28.41 ± 3.5	32.40 ± 4.21[a]	29.65 ± 3.91	0.001*
Male age (years)	35.50 ± 3.70	36.12 ± 4.10	34.76 ± 3.55	0.191
Female BMI (kg/m^2)	23.36 ± 3.01	24.39 ± 2.60	22.10 ± 2.98	0.049*
Duration of infertility (years)	3.45 ± 1.41	5.70 ± 2.81	4.21 ± 2.05	0.006*
Infertility type	21	23	24	0.78
Primary	3	2	2	
Secondary				
Previous IVF attempts	1.16 ± 0.38	1.80 ± 0.89	1.64 ± 0.75	< 0.001*
Smoking female	5/24	3/25	6/26	0.57
Smoking male	10/24	7/25	12/26	0.38
Basal FSH level (IU/L)	6.49 ± 1.25	11.46 ± 3.80	7.14 ± 2.10	< 0.001*
Basal E2 level (pg/mL)	34.85 ± 13.68	50.12 ± 10.34	29.13 ± 10.86	< 0.001*
Serum AMH level (ng/mL)	3.01 ± 0.90	0.59 ± 0.25	3.29 ± 0.93	< 0.001*
AFC	10.70 ± 2.80	3.64 ± 1.68	11.19 ± 2.60	0.002*
Serum INSL3 level (pg/mL)	1775.52 ± 238.18	1512.98 ± 184.9	1742.54 ± 215.14	0.001*
INSL3 level in follicular fluid (pg/mL)	1193.91 ± 148.55	958.67 ± 175.23	1043.53 ± 257.31	< 0.001*

All values are expressed as mean±Standard Deviation or number. *$p < 0.05$, significant difference

comparison with unexplained group; $p < 0.05$

DOR: Diminished ovarian reserve

BMI: Body Mass Index

IVF: In vitro fertilizasyon

FSH: Follicle stimulating hormone

E2: Estradiol

AMH: Antimullerian hormone

AFC: Antral follicle count

INSL3: Insulin-like peptide 3

Table 2 IVF cycle characteristics of patients

Variables	Unexplained (*n* = 24)	DOR (*n* = 25)	Control (*n* = 26)	*p* value
Total dosage of gonadotropins (IU)	1831.25 ± 554.19	2193.00 ± 625.72	1817.30 ± 527.05	0.093
Maximum estradiol levels (pg/mL)	2220.58 ± 739.21	724.08 ± 405.72	2350.00 ± 746.43	< 0.001*
Duration of stimulations (day)	7.62 ± 1.13	8.76 ± 1.42	8.11 ± 1.39	0.017*
Number of oocytes retrieved	11.08 ± 3.77	3.72 ± 1.79	11.73 ± 3.02	< 0.001*
Number of mature follicles retrieved	9.58 ± 3.79	2.92 ± 1.38	10.53 ± 2.95	< 0.001*
Number of PN	7.37 ± 3.56	1.92 ± 1.03	7.69 ± 2.49	< 0.001*
Endometrial thickness at day of transfer (mm)	8.27 ± 1.17	15.47 ± 23.96	7.99 ± 1.07	0.155

All values are expressed as mean±Standard Deviation. *$p < 0.05$, significant difference

IVF: In vitro fertilizasyon

DOR: Diminished ovarian reserve

PN: Pronucleus

maximum estradiol levels ($p < 0.001$), number of oocytes retrieved ($p < 0.001$), number of mature follicles retrieved ($p < 0.001$) and number of PN ($p < 0.001$) were lower in DOR group.

Circulating and follicular fluid INSL3 levels were found to be lower in the DOR group than the other groups ($p < 0.001$). Correlation between different variables was analyzed to determine the relationship between circulating and follicular fluid INSL3 level and ovarian reserve indicators (basal FSH level, serum AMH level, AFC and age), as well as positive pregnancy rates and LBR. While no correlation was found between the follicular fluid INSL3 level and ovarian reserve indicators, positive correlation was found between the circulating INSL3 level and serum AMH level ($p < 0.001$) and AFC ($p = 0.006$). Furthermore, there was negative correlation between the circulating INSL3 and basal FSH levels ($p = 0.023$).

The pregnancy rates were shown in Table 3. The positive pregnancy rates after the first fresh cycle were 13/24 (54.1%) in the unexplained infertility, 6/25 (24%) in the DOR and 14/26 (53.84%) in the control groups. The LBR were 13/24 (54.1%) in the unexplained infertility, 5/26 (20%) in the DOR and 11/26 (42.3%) in the control groups. After adding the results of consecutive frozen

Table 3 Pregnancy outcomes

Variables	Unexplained (*n* = 24)	DOR (*n* = 25)	Control (*n* = 26)	*p* value
Positive pregnancy test	13/24 (54.1%)	6/25 (24%)	14/26 (53.84%)	0.04*
Live birth rates	13/13 (54.1%)	5/6 (20%)	11/14 (42.3%)	0.03*
Cumulative pregnancy rates	14/24 (58.3%)	7/25 (28%)	15/26 (57.7%)	0.04*

All values are expressed as number and percentage. *$p < 0.05$, significant difference

DOR: Diminished ovarian reserve

embryo transfers, cumulative pregnancy rates were 14/24 (58.3%) in the unexplained infertility, 7/25 (28%) in the DOR and 15/26 (57.7%) in the control groups ($p = 0.04$). Statistically significant differences in positive pregnancy test ($p = 0.04$) and LBR ($p = 0.03$) were found between the groups, both being lower in the DOR group.

Univariate and multivariate analyzes were performed to evaluate predictors of LBR (Table 4). According to the results of the univariate analysis, of the included potential independent variables, DOR ($p = 0.03$), the duration of infertility ($p = 0.03$), basal FSH level ($p = 0.01$), serum AMH level ($p = 0.03$), AFC ($p = 0.03$), the number of oocytes retrieved ($p = 0.03$) and the number of PN ($p = 0.03$) were noted to be the significant predictors of LBR. There was a negative correlation between LBR and DOR, the duration of infertility and basal FSH level. There was a positive correlation between LBR and serum AMH level, AFC, number of oocytes retrieved and number of PN. Variables with a significant effect in univariate evaluations were included in the multivariate analysis as independent variables. Basal FSH level ($p = 0.01$) was found to be the only significant variable. The probability of LBR decreased with the increase in basal FSH level.

Discussion

Our study is the first study to evaluate potential of circulating INSL3 along with INSL3 concentration in the follicular fluid in women with UI and DOR which are most controversial clinical entities in daily practice. As part of the search for new markers of declining ovarian function we hypothesised that changes in circulating INSL3 might be a useful indicator. We observed that circulating and follicular fluid INSL3 levels in women with UI were similar to the control group. We also observed that circulating and follicular fluid INSL3 levels were significantly

Table 4 Results of univariate and multivariate factors in relation to the live birth rate

Variables	Univariable		Multivariable	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Female Age	0.92 (0.829, 1.035)	0.18		
Duration of infertility	0.79 (0.638, 0.985)	0.03*		
Type of infertility (DOR vs. UI)	0,285 (0.089, 0.918)	0.03*		
Previous IVF attempts	0.938 (0.514, 1.713)	0.83	0.803 (0.677, 0.953)	0.012*
Basal FSH level	0.803 (0.677, 0.953)	0.01*		
Serum AMH level	1.446 (1.035, 2.021)	0.03*		
AFC	1.137 (1.013, 1.277)	0.03*		
Total dosage of gonadotropins	1 (0.999, 1,001)	0.846		
Duration of stimulations	0.764 (0.540, 1.080)	0.128		
Estradiol levels on hCG day	1 (1, 1.001)	0.212		
Number of oocytes retrieved	1.114 (1.005, 1.234)	0.03*		
Number of mature follicles retrieved	1.120 (1.005, 1.248)	0.125		
Number of PN	1.169 (1.022, 1.336)	0.02*		
Endometrial thickness at day of transfer	1 (0.968, 1.033)	1		
Serum INSL3 level	1.001 (0.999, 1.003)	0.365		
INSL3 level in follicular fluid	1.000 (0.997, 1.002)	0.694		

*$p < 0.05$, significant difference

OR: Odds ratio

PN: Pronucleus

hCG: Human chorionic gonadotropin

AFC: Antral follicle count

DOR: Diminished ovarian reserve

BMI: Body Mass Index

IVF: In vitro fertilizasyon

FSH: Follicle stimulating hormone

E2: Estradiol

AMH: Antimullerian hormone

UI: Unexplained infertility

lower in women with DOR. The decrease in circulating INSL3 was strongly associated with other ovarian reserve markers including FSH, AMH and AFC. A positive correlation was found between circulating INSL3 level and serum AMH level and AFC. In contrast, there was negative correlation between the circulating INSL3 level and basal FSH level. This suggests that a relative decrease in circulating and follicular fluid INSL3 might indeed reflect concurrent accelerated aging of ovary from a quantitive perspective in women with DOR. The finding that low INSL3 level in follicular fluid is accompanied by low circulating INSL3 might explain that decreasing circulating INSL3 levels might reflect impaired ovarian physiology in women with DOR.

In contrast to the serum AMH levels, which showed substantial differences between the control and DOR groups, the range of INSL3 had a much narrower range. In fact, differences between the DOR patients and UI and control were similar to the standard deviation within groups. Moreover, when compared to the reduction of serum AMH level, the relatively high baseline value of INSL3 in women with DOR shows that the reduction of circulating INSL3 does not scale in the same way as AMH. It is possible that ovaries with little or no follicular activity still produce INSL3. As such, measurement of INSL3 in any single patient may provide values that cannot clearly be interpreted as low or normal.

Another interesting finding among our results is that the serum INSL3 were higher than the follicular fluid levels. This is in contrast to serum and follicular fluid AMH levels which are at least an order of magnitude higher in follicular fluid relative to serum. INSL3 exhibits the reverse trend, with serum values greater than follicular fluid but less that 2x greater. This likely reflects the disparity in expression patterns, with AMH expressed by granulosa cells and INSL3 expressed by theca interna. Higher circulating INSL3 levels might be explained with another source of INSL3 that enters circulation when compared to follicular fluid INSL3. INSL3, previously called relaxin-like factor, is a member of the insulin/relaxin gene family and was originally described as a male hormone. The potential additional source of INSL3 remains unknown.

Recently, INSL3 has emerged as a potential marker of ovarian physiology. INSL3 secreted by the theca cells can induce follicle growth via two pathways. The first one is that INSL3 is responsible for the production of androstenedione in theca cells in an autocrine/paracrine manner on its receptor, most probably by stimulating thecal androgen production via induction of the expression and function of the enzyme 17α-hydroxylase. In return, androstenedione stimulates INSL3 synthesis in the theca cells [19]. However, the production of the main follicular steroid precursor, androstenedione, is transported to the granulosa cells to be converted to the oestrogens by aromatase activity in granulosa cells of the growing antral follicle. Moreover, estradiol, acting via its receptor results in the up-regulation of INSL3 expression in theca cell as well [19]. The second pathway is to induce the production of GDF9 by the oocyte [25]. As a result, INSL3 via its receptor acts as an autocrine/paracrine system to coordinate and amplify the effects of LH, androstenedione and oestrogens at the beginning of the menstrual cycle. Thus, it can promote and orchestrate the growth of a wave of antral follicles. A retrospective study investigated the changes in the serum INSL3 concentration during the menstrual cycle and demonstrated a cyclical pattern of INSL3. They showed that serum INSL3 concentration were consistently low during menstruation, while it showed 2- to 3-fold increase during follicular phase. Moreover, serum INSL3 concentration declined slightly during the luteal phase. It should be noted that there was substantial variability of serum INSL3 concentration during all phases of the menstrual cycle. Furthermore, circulating INSL3 levels were significantly higher in women with PCOS and lower in women with low ovarian reserve [20]. Zhu et al. evaluated the role of circulating INSL3 in different stages of POI. They demonstrated that circulating INSL3 decreased in women with POI. Their results showed that circulating INSL3 had a negative association with FSH and positive association with AMH and AFC. They did not observe a statistically significant difference between the follicular fluid INSL3 levels in women with POI and controls. They postulated that circulating INSL3 was considered as a good predictor for POI [14]. In contrast, Pelusi et al. found no correlation between serum INSL3 and AMH levels between healthy normal-weight late adolescent females with both ovulatory and anovulatory cycles [21]. In our previous experimental study, we demonstrated that INSL3 staining scores in ovaries decreased with ovarian damage induced by chemotherapeutic drugs. In addition, INSL3 staining scores were positively correlated with serum AMH level and AMH positive staining follicle count in female rats with ovarian damage [22].

As expected, positive pregnancy test and LBRs were significantly lower in women with DOR in the current study. This is in line with other studies that demonstrated significantly lower LBR in women with DOR [23]. The probability of LBR decreased with the increase in duration of infertility and basal FSH level in this group. This contrasts with the "Outcome prediction in subfertility (OPIS)" prediction models which failed to find the duration of infertility as a predictor [24]. Our current results showed no correlation between serum INSL3 level and INSL3 level in follicular fluid and positive pregnancy rates and LBRs. Hence, at this stage, INSL3 does not appear to be a predictor of ART outcome.

INSL3 could act as a coordinator during the growth of antral follicles and may be involved in their function. Based on the two-cell hypothesis, INSL3 could play a key role in the interaction between the granulosa and theca cells by stimulating thecal androgen production to drive E2 production in granulosa cell. Furthermore, it could also act to facilitate oocyte maturation by production of GDF9 or by the cAMP/protein kinase A signaling pathway [10–12, 25, 26]. It was demonstrated that INSL3 deficiency is associated with female infertility because of the reduction of ovulation, accelerated of follicular atresia and apoptosis in mice. Circulating INSL3 levels might basically reflect oocyte impairment and impaired ovarian physiology and theca and granulosa cell function. It can be hypothesized that impaired circulating INSL3 and INSL3 level in follicular fluid might contribute to lower PRS in women with DOR as supported by our result of lower circulating and follicular fluid INSL3 levels in the DOR group.

There are a few points of the current study that should be highlighted as strengths. To the best of our knowledge, our study is the first study to evaluate the circulating and follicular fluid INSL3 concentrations in women with UI and DOR. Secondly, it is a prospective study in human subjects. The majority of previous studies were retrospective or were animal studies. Moreover, we evaluated the relationship between IVF outcome and circulating and follicular fluid INSL3 and INSL3 concentrations.

There are a few points of the current study that should be highlighted as limitations. The main limitation of our study is the relatively small sample size. IVF success rates are influenced by many predictors including female age, the cause of infertility, duration of infertility, ovarian reserve, embryo quality and number of oocytes collected. Female age is the most significant predictor of IVF treatment success [27]. The highest live birth rates (LBRs) are generally observed in women aged 25–30 years, with a steady decline from 35 years onward [28] We included women between 20 and 40 years old in our study and there was a statistically significant difference between the groups in terms of female age. Although the highest quality embryos based on morphology and cleavage criteria were selected for transfer in our study, the unmatched women's age should be considered as another limitation. Another point is the differences of BMI between groups. Although the BMI levels were rather close and the differences are unlikely to be of any clinical significance, we cannot exclude a potential impact on our results. There is conflicting data on the relationship between serum AMH level and BMI. Several studies could not demonstrate a correlation between the serum AMH levels and BMI and the median, AMH levels were found to be similar between normal weight and overweight or obese women [29]. Another study suggested that the INSL3 concentration may increase with bodyweight, although this was not reported as statistically significant [30]. However, in this study, Szydlarska1 at al showed that circulating INSL3 are non-significantly higher levels in women with PCOS. Although PCOS women with high LH concentration and low BMI has elevated level of circulating INSL3, the concentration of circulating INSL3 was within the normal range in PCOS women with a normal LH level. It was considered that INSL3 is related to LH-dependent ovarian hyperandrogenism, especially in normal weight PCOS women. These findings suggest that circulating INSL3 level is related to serum LH level rather than BMI.

Both FSH and LH activity is necessary for normal follicular development and steroidogenesis in natural cycle according to the two-cell, two-gonadotropin hypothesis. Despite the potential benefits of LH activity, the definitive effect of adding LH during ovarian stimulation has been widely debated [31]. Although several studies reported that adding LH to antagonist cycles might increase IVF success especially in women with decreased ovarian reserve and inadequate response in a previous IVF cycle [32–34], other studies showed that FSH-only and FSH plus rLH stimulations resulted in similar IVF outcomes and their results failed to demonstrate that adding LH activity to stimulation was associated with better IVF outcomes [35, 36]. We use only recFSH to standardize stimulation protocol for all our patients and this could be another point of criticism. In addition, circulating INSL3 concentrations were not evaluated during oocyte retrieval although follicular fluid samples were collected during oocyte retrieval.

Conclusions

The current study presents that although the serum and follicular fluid INSL3 levels are significantly lower in women with DOR, the narrow margin between the DOR and control groups indicates that INSL3 measurement may be insufficient on its own to be of diagnostic value. In addition, higher serum levels than follicular fluid indicates there may be another source of INSL3 other than the theca interna of follicles. Further well-designed trials with larger samples size are needed to confirm our results and will open the way for other potential uses of this novel biomarker.

Author contributions

Conception and design: PO, FS; data acquisition CY, AA; data analysis: ECK, PO, OD; data interpretation: PO, ES, ECK; manuscript writing: PO, HTT, HST; critical review of the manuscript: ES; revision of manuscript: PO, HTT. All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The protocol of our study was approved by the Ethical Committee of Bezmialem University (E-112926). Written informed consent was obtained from all participants. The study was conducted in accordance with the Declaration of Helsinki.

Consent for publication
All participants provided written informed consent, permitting the authors to analyze and publish their data under the condition that any identifying information was removed.

Competing interests
The authors declare no competing interests.

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