Clinical significance of serum and follicular fluid ceramide levels in women with low ovarian reserve

Serum ve foliküler sıvı seramid düzeylerinin düşük over rezervli kadınlarda klinik önemi

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Abstract

Objective: Ceramide (CER) is a bioactive component of the mitochondrial membrane. In this study, we will investigate the clinical importance of serum CER (sCER) and follicular fluid CER (fCER) levels in the lipid synthesis pathway and their effect on poor oocyte quality and in vitro fertilization (IVF) outcome.

Materials and Methods: This cross-sectional, case-control study was conducted in the IVF unit of a maternity hospital in the capital of Turkey, Ankara. A total of 88 women undergoing their first IVF cycle were included in this study patients were divided into 2 groups according to current diagnostic criteria for their ovarian reserves. Baseline sCER levels, and fCER concentrations retrieved on the oocyte pickup day were measured.

Results: The mean age, body mass index, and infertility duration of the patients was similar between the groups (all p>0.05). There was also no significant difference in the clinical pregnancy rates (38.6% vs. 47.7%, p=0.127). sCER (15.6±6.5 vs. 23.5±8.9) and fCER (82.5±34.3 vs. 116.4±46.5) levels were statistically significantly lower in the low ovarian reserve (LOR) group (both p<0.001). The performed receiver operating characteristic curve analysis revealed that sCER and fCER levels could predict both LOR and pregnancy.

Conclusion: This is the first study evaluating the sCER and fCER levels of patients undergoing IVF treatment. CER may be used as an ovarian reserve markers and a biomarker capable of predicting IVF outcomes.

Keywords: Ceramid, controlled ovarian stimulation, in vitro fertilization, ovarian reserve marker, pregnancy

ÖZ

Amaç: Seramid (SER), mitokondri zarının biyoaktif bir bileşenidir. Bu çalışmada, lipid sentez yolunun serum SER (sSER) ve foliküler sıvı SER (fSER) düzeylerinin klinik önemi ve bunların kötü oosit kalitesi ve klasik tüp bebek (in vitro fertilization - IVF) sonuçları üzerindeki etkisi araştırılacaktır.

Gereç ve Yöntemler: Bu kestisel olgu-kontrol çalışması Türkiye’nin başkenti Ankara’da bir kadın doğum hastanesinin tüp bebek ünitesinde yapıldı. İlk IVF dönüştüğine giren toplam 88 kadın bu çalışmaya dahil hastalar over rezervlerine göre üç gruba ayrıldı. Basal sSER seviyeleri ve oosit toplama gününde alınan fSER konsantrasyonları ölçüldü.

Bulgular: Hastaların yaş ortalaması, vücut kitle indeksi ve infertility süreleri gruplar arasında benzerdi (tümü p>0,05). Klinik gebelik oranlarında da anlamlı bir fark yoktu (%38,6’ya karşın %47,7; p=0,127). sSER (15,6±6,5’e karşın 23,5±8,9) ve fSER (82,5±34,3’e karşın 116,4±46,5) seviyeleri düşük yumurtalık rezervi (low ovarian reserve - LOR) grubunda istatistiksel olarak anlamal derecede düşüktü (her ikisi de p<0,001). Gerçekleştirilen alıcı işletim karakteristiği analizi, sSER ve fSER düzeylerinin hem LOR’yu hem de gebeliği öngörebileceğini ortaya koydu.

Sonuç: Bu, ülkenin ilk çalışması Gow hastaların sSER ve fSER düzeylerini değerlendirilen ilk çalışmadır. SER, yumurtalık rezerv belirtec olarak ve IVF sonuçlarının tahmin edebilen bir biyobelirteç olarak kullanılabilir.

Anahtar Kelimeler: Seramid, kontrollü ovarian stimülasyon, tüp bebek, over rezerv marker, gebelik

PRECIS: Reduced ceramide level is associated with low ovarian reserve and may predict pregnancy in IVF treatment.
Introduction

Women's fertility reaches its peak in the early 30s and gradually declines and disappears at menopause due to a combination of several factors\(^{(2)}\). However, decreased fertility rates in aging women are mainly due to the reduced quality of aging oocytes, which indicates chromosomal, morphological, and functional abnormalities\(^{(2)}\). The number of applications to in vitro fertilization (IVF) clinics due to low ovarian reserve (LOR) is gradually increasing mainly because of factors such as social reasons (career planning and delaying childbirth), previous ovarian surgery, exposure to radiotherapy and chemotherapy, genetic reasons [such as Fragile-X mental retardation-1 gene premutation and bone morphogenetic protein 15 (BMP-15) gene mutation], and smoking. Postponing childbearing reduces fecundity and increases the risk of infertility in women. Research has shown that lower than 5% of women with LOR can conceive\(^{(3)}\). Various adjuvant treatments are used in IVF cycles of patients with LOR. However, the place of these treatments in the perspective of evidence-based medicine is still controversial.

LOR can be described as reduced number, quality, and reproductive potential of oocytes. It is important to define LOR as part of the initial infertility assessment as women increasingly present for diagnostic infertility evaluation at a later age. Although many international guidelines suggest various definitions, there is no ideal test to evaluate ovarian reserve. Some ovarian reserve tests [such as Anti-Müllerian hormone (AMH), antral follicle count (AFC), and follicle stimulating hormone (FSH)] are used in clinical practice, but a single test that can reliably predict pregnancy potential has not yet been introduced\(^{(4)}\). Although much convincing evidence indicates that woman's chronological age is the most important determinant for IVF success, the relationship between the age and reproductive capacity can be quite variable\(^{(5)}\). Therefore, given the high cost and possible negative outcomes of IVF, investigating some parameters that can be used as predictive markers, particularly in women undergoing IVF due to LOR, is of great importance.

Ceramide (CER) is a bioactive component of the cell membrane. CER belongs to the phospholipid family and plays a key role in cell growth, differentiation, barrier function, migration, and apoptosis\(^{(6)}\). CER is formed because of the hydrolysis of sphingomyelin or the metabolism of more complex sphingolipids. It is also metabolized to form sphingosine and sphingosine 1 phosphate\(^{(7)}\). Sphingosine 1 phosphate and CER have many-opposing effects: Pro- and antiangiogenetic effects\(^{(8)}\). Recently, there have been increasing claims that the serum level of CER (sCER) and some phospholipids may be related to oocyte quality\(^{(9-11)}\). Some publications have reported a decrease in mitochondrial CER levels, especially in aging oocytes\(^{(12)}\). The synthesis and/or intracellular transport of CER, a bioactive lipid, becomes deregulated with aging. As a result, the level of CER in the mitochondria cannot reach the normal level, and this lipid imbalance decreases mitochondrial function and a negative effect on oocyte quality.

In our study, CER levels were measured for the first time in the serum and follicular fluid (FF) patients who underwent IVF treatment. In this study, we will investigate the clinical importance of CER levels in the lipid synthesis pathway and their effect on poor oocyte quality and IVF outcome.

Materials and Methods

This study was conducted in the IVF unit of the Etilik Zübeyde Hanım Training and Research Hospital Ethics Committee, between June 1, 2018, and December 31, 2018. Eighty-eight women were included in this study-half of the them were women with LOR, while the other half had mild-to-moderate male factor or tubal factor infertility. The hospital’s local ethics council approved the study protocol (date/approval number: 30.05.2018/24), and written informed consent was taken from all patients who were included in the study. All the women were on their first IVF cycle, and fresh embryo transfer was applied, when applicable, without a prenatal genetic screening test.

LOR was diagnosed when a patient below 40 years had an abnormal ovarian reserve test, which is considered AFC <5-7 follicles, AMH <1.1 ng/mL, or a day 3 FSH level of more than 10 IU/L with a simultaneous estradiol (E\(_2\)) level >80 pg/mL. Male factor infertility was defined as the presence of ≥1 abnormalities in the spermiogram, according to WHO 2010 criteria. Tubal factor infertility was diagnosed after confirmation of bilateral tubal occlusion with hysterosalpingography and/or laparoscopy.

Women aged 23-39 years who were scheduled for infertility evaluation required for IVF (routine clinical examination, hormonal panel, and ultrasonographic evaluation), diagnosed with LOR, had mild/moderate male or tubal factor infertility, and had nonhemorrhagic FF were included in the study. Women or husbands who had endocrine [e.g., polycystic ovary syndrome (PCOS), diabetes, hypothalamic dysfunction, and thyroidal disorders], cardiovascular (hypertension and coronary artery disease), renal, hepatic, or immunologic diseases; had undergone pelvic surgery including the uterus or ovaries; had congenital or acquired uterine abnormalities (e.g., submucosal myoma, polyp, uterine septum, and intrauterine adhesion) diagnosed by hysteroscopy, or had severe male factor (azoospermia, severe oligoastenoteratospermia, etc.) were excluded from this study.

Biochemical parameters and baseline hormonal parameters were investigated after at least 8 h of fasting, and venous blood samples were taken by electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany). All laboratory parameters, except for sCER and follicular fluid CER (fCER) measurements, were studied on the day the blood sample was drawn. Basal (second or third menstrual day) venous blood samples for CER were separated by centrifugation.
The TVUS-guided oocyte retrieval procedure was executed for the intracytoplasmic sperm injection (ICSI) procedure. The TVUS was used for the intracytoplasmic sperm injection (ICSI) procedure. NV) after liquefaction for 30 min at room temperature. Highly motile sperm in the medium was carefully removed and used for the intracytoplasmic sperm injection (ICSI) procedure. The TVUS-guided oocyte retrieval procedure was executed at 2,400 g for 10 min. FF samples were drawn on the day of oocyte pick up (OPU) from the single mature follicle. Collected FF samples were immediately centrifuged at 800x g for 10 min to separate the fluid from follicular cells. Serum and FF samples were kept at -80 °C until the working day of CER. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m²).

Frozen samples were subsequently brought to room temperature to be dissolved, and CER values were measured using commercially available human ceramide ELISA kits (Eastbiopharm Co., Ltd., Hangzhou, PRC). The testing procedures were performed as per manufacturer's instructions. sCER and ffCER levels were calculated from a standard curve expressed as nanograms per milliliter. The intra- and interassay coefficients of variance were <10%, and the minimum detection rate was 1 ng/mL.

**Controlled Ovarian Stimulation**

The women were managed and monitored according to the unit’s clinical protocols using 150-450 IU/day of recombinant FSH (Gonal F, Merck Serono) or purified human menopausal gonadotropin (HMG) (Merional, IBSA). Controlled ovarian stimulation (COS) was initiated on the second or third day of menstruation, and the gonadotropin doses were adjusted according to the patients’ age, AFC, and BMI. The follicle monitoring was done by serum E₂ level and transvaginal ultrasound (TVUS) from the 5th day of COS, and every 1-3 days after that. An antagonist protocol was used to provide pituitary down-regulation with daily use of gonadotropin releasing hormone antagonist (Cetrotide, Merck Serono) that was initiated from the 5th or 6th day of COS when the leading follicle arrived at 14 mm. The doses of recombinant FSH/highly purified HMG were arranged according to the patients’ ovarian response. Standard final oocyte maturation with 250 mcg of recombinant human chorionic gonadotropin (hCG) (Ovitrelle, Merck Serono) was triggered when two follicles reached above 18 mm or three ovarian follicles of greater than or equal to 17 mm were visible by TVUS. OPU was performed by transvaginal aspiration 34-36 h later under ultrasound guidance. For luteal phase support, vaginal progesterone (Crinone 8%, vaginal gel, Merck Serono) was applied twice daily starting from the day of OPU until pregnancy testing. Progesterone supplementation in all transfer cycles was continued until the 12th week of pregnancy for patients who conceived.

**Semen Collection, Oocyte Retrieval, and Embryo Transfer**

Semen samples were obtained from masturbation after a period of 2-5 days sexual abstinence. The collected semen specimens were processed using the standard swim-up technique preparation media (FertiCult™ Flushing medium, FertiPro NV) after liquefaction for 30 min at room temperature. Highly active motile sperm in the medium was carefully removed and used for the intracytoplasmic sperm injection (ICSI) procedure. Sonographic examinations and OPU procedures were carried out on all the women by the same senior clinician with has significant expertise in reproductive endocrinology. Oocyte retrieval, oocyte denudation and conventional ICSI procedures were performed in all women to rule out fertility problems. Oocytes were cultured separately in a special preequilibrated culture dish after the ICSI procedure. Throughout the culture period, a single-step medium enriched with human serum albumin (Continuous Single Culture™, Irvine Scientific, CA, USA), was used in the study. Embryo culture was performed until the 5th or 6th day at 37 °C in an air of 5% O₂, 5% CO₂, and 90% N₂, in benchtop incubators (MIRI, ESCO Medical, Singapore). Blastocysts were scored and morphologically evaluated as previously described[30]. Embryos with the best quality were chosen for transfer. A maximum of 2 embryos were transferred, and the rest were cryopreserved for future use, as there were enough good quality embryos.

**Statistical Analyses**

The normality distribution of the continuous variables and were tested by Kolmogorov-Smirnov test. Differences between categorical data were evaluated using the chi-square test. Student's t-test or Mann-Whitney U test was performed to compare the two independent groups. Data are shown as mean ± standard deviation, number (percentage), and median (minimum-maximum) where appropriate. Receiver operating characteristic (ROC) analysis of the area under the curve was used to determine the predictive values of CER. Spearman’s correlation analysis was used to measure the strength and direction of associations between body fluids CER levels and other variables. The data were analyzed with SPSS 21.0 software (IBM Corporation, Armonk, NY, USA). A p-value <0.05 was considered as statistically significant.

**Results**

A total of 88 patients participated in this cross-sectional study-44 patients each in the LOR and the control groups. In the control group, 32 patients had male factor-induced infertility, whereas 12 patients had a tubal factor. All the patients included in the study underwent the IVF cycle for the first time. The mean age, BMI, and infertility duration of the patients were similar (all p>0.05). The basal FSH level was statistically significantly higher in the LOR group than in controls (9.4±1.8 vs. 6.5±1.0, p<0.001). Other baseline hormone levels, including estradiol, progesterone, luteinizing hormone, thyroid-stimulating hormone and prolactin, were similar among the groups. AFC (6.2±2.4 vs. 13.7±4.8) and serum AMH (0.7±0.4 vs. 3.0±1.2) levels, which are ovarian reserve markers, were low in the LOR group (both p<0.001). Considering the cycle characteristics of the patients, the gonadotropin dose used (2843.6±760.9 vs. 1979.1±691.2, p<0.001) was higher in the LOR group, while the peak estrogen level (1263.3±6373.8 vs. 1776.0±859.0, p<0.001) was lower, as expected. However, no statistically significant
difference was observed in endometrial thickness (9.5±3.0 vs. 10.7±3.6, p=0.426) and stimulation length (11.3±1.8 vs. 11.1±1.8, p=0.517). The number of oocytes collected and embryos obtained were higher in the control group. While fertilization rates and the number of transferred embryos were similar between the two groups, embryo quality was worse in the LOR group, and the embryos transferred on the third day were more common. A comparison of demographics, baseline hormone levels, and cycle characteristics between the LOR and control groups are provided in Table 1. Markers of lipid (low density lipoprotein cholesterol, high density lipoprotein cholesterol, very low-density lipoprotein, triglyceride, and total cholesterol) and glucose metabolism (glucose, insulin, and homeostatic model assessment insulin resistance) were similar between the two groups (Table 2). There was no significant difference in the clinical pregnancy rates (38.6% vs. 47.7%, p=0.127). sCER (15.6±6.5 vs. 23.5±8.9) and ffCER (82.5±34.3 vs. 116.4±46.5) levels were statistically significantly lower in the LOR group (both p<0.001). When the patients were categorized according to their pregnancy status, both serum and FF CER levels were found to be statistically significantly higher in the pregnant group (p<0.001, p=0.036, respectively) (Table 3). A statistically significant positive correlation was observed between basal sCER and ffCER levels both between the groups and in the whole cohort (r=0.056, p<0.001). The performed ROC curve analysis revealed that sCER and ffCER levels could predict LOR and pregnancy. A sCER level lower than 16.5 ng/mL may predict women with LOR with sensitivity of 40.9% and specificity of 70.5%, whereas ffCER level lower than 98.5% may predict the same patients with a sensitivity of 76.5% and specificity of 45.5% (Figure 1). In contrast, a sCER level higher than 18.5 ng/mL may predict pregnancy in women undergoing IVF treatment with a sensitivity of 71.1% and specificity of 74%, whereas an ffCER level higher than 121

Table 1. Comparison of demographics, baseline hormone levels, and cycle characteristics between the LOR and control groups

| Variables                      | LOR (n=44) | Control (n=44) | p    |
|--------------------------------|------------|----------------|------|
| Age (years)                   | 30.2±4.4   | 30.1±5.4       | 0.148|
| BMI (kg/m²)                   | 24.5±4.6   | 25.0±4.2       | 0.834|
| Infertility duration (years)  | 4.5±2.6    | 4.3±2.0        | 0.218|
| FSH (mIU/mL)                  | 9.4±1.8    | 6.5±1.0        | 0.000|
| TPMSC (mil)                   | 28.8±25.7  | 13.6±15.8      | 0.000|
| AFC                            | 6.2±2.4    | 13.7±4.8       | 0.000|
| AMH (ng/mL)                   | 0.7±0.4    | 3.0±1.2        | 0.000|
| sCER (ng/mL)                  | 15.6±6.5   | 23.5±8.9       | 0.000|
| ffCER (ng/mL)                 | 82.5±34.3  | 116.4±46.5     | 0.000|
| Number of oocytes retrieved   | 3.6±1.3    | 13.3±5.2       | 0.000|
| Number of M2 oocytes          | 3.2±1.3    | 10.2±4.1       | 0.000|
| Fertilization rate (years)    | 69.3±25.5  | 79.0±20.1      | 0.743|
| Number of 2PN embryos         | 1.9±1.0    | 7.8±2.8        | 0.000|
| Number of embryos             | 1.7±1.0    | 7.5±2.7        | 0.000|
| ET                            | 1 (0-2)    | 1 (1-2)        | 0.564|

Embryo quality

| FF                | 3 (6.8) | 1 (2.3) | 0.001 |
|-------------------|---------|---------|-------|
| Grade 1           | 15 (34.1) | 24 (54.5) |       |
| Grade 2-3         | 26 (59.1) | 19 (43.2) |       |

Transfer day

| Day            | ET (n=4) | ET (n=4) | p     |
|----------------|----------|----------|-------|
| Day 3          | 37 (84.1)| 29 (65.9)| 0.002 |
| Day 5          | 7 (15.9)  | 15 (34.1) |       |

Pregnancy

| Day            | ET (n=4) | ET (n=4) | p     |
|----------------|----------|----------|-------|
| Day 3          | 17 (38.6)| 21 (47.7)| 0.127 |

LOR: Low ovarian reserve, BMI: Body mass index, FSH: Follicle stimulating hormone, TPMSC: Total progressively motile sperm count, AFC: Antral follicle count, AMH: Anti-Müllerian hormone, sCER: Serum ceramide, ffCER: Follicular fluid ceramide, ET: Embryo transfer, FF: Fertilization failure. Data were shown as mean ± standard deviation, number (percentage), and median (minimum-maximum)
may predict pregnancy with sensitivity of 50% and specificity of 80% (Figure 2).

Discussion

In this study, we assessed the baseline sCER and ffCER levels in infertile women undergoing IVF cycles due to LOR for the first time and compared them with women with normal ovarian reserve markers. CER is an important bioactive molecule located in the cytoplasm and mitochondria with different functions. We found that sCER and ffCER levels are lower in patients with LOR, and their serum and FF levels may predict IVF outcomes. Embryo quality, which is primarily determined by oocyte quality, is the most important determinant of IVF outcomes\(^{(14)}\). We know that AMH is closely associated with the existing

![Figure 1. ROC curve analysis of sCER and ffCER level in predicting pregnancy in women undergoing IVF treatment](image1)

**Figure 1.** ROC curve analysis of sCER and ffCER level in predicting pregnancy in women undergoing IVF treatment

**ROC:** Receiver operating characteristic, sCER: Serum CER, ffCER: Follicular fluid CER, IVF: In vitro fertilization

![Figure 2. ROC curve analysis of sCER and ffCER level in predicting women with LOR](image2)

**Figure 2.** ROC curve analysis of sCER and ffCER level in predicting women with LOR

**ROC:** Receiver operating characteristic, sCER: Serum CER, ffCER: Follicular fluid CER, LOR: Low ovarian reserve

| Table 2. Comparison of two groups for markers of lipid and glucose metabolism |
|-----------------------------|-----------------------------|-----------------------------|
| Variables                  | LOR (n=44)                  | Control (n=44)               | P     |
| Glucose (mg/dL)            | 90.9±8.7                    | 89.6±9.5                    | 0.231 |
| Insulin (mIU/L)            | 15.7±9.5                    | 11.5±5.9                    | 0.096 |
| HOMA-IR                    | 2.6±1.8                     | 2.6±1.4                     | 0.455 |
| T. cholesterol (mg/dL)     | 171.8±30.6                  | 169.8±29.4                  | 0.745 |
| LDL-C (mg/dL)              | 97.0±26.8                   | 95.3±31.6                   | 0.532 |
| HDL-C (mg/dL)              | 51.3±14.9                   | 54.5±13.2                   | 0.367 |
| VLDL (mg/dL)               | 20.2±8.6                    | 20.0±9.1                    | 0.889 |
| TG (mg/dL)                 | 99.3±46.2                   | 101.2±42.8                  | 0.712 |
| T. chol/HDL                | 3.3±1.0                     | 3.3±1.0                     | 0.871 |

LOR: Low ovarian reserve, HOMA-IR: Homeostatic Model Assessment Insulin Resistance, LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol, VLDL: Very low density lipoprotein, TG: Triglyceride, T. chol: Total cholesterol. Data were expressed as mean ± standard deviation
Mitochondria are essential organelles in sphingolipid metabolism, and many sphingolipid metabolizing enzymes are located in the mitochondria. The presence of these pathways is an indirect indicator that lipid products also have specific functions. CER signaling, which is one of these functions, involves a complex molecular and subcellular network, all implicated in various cellular processes such as proliferation, differentiation, survival, necrosis and aging. An experimental study showed that after the addition of a CER metabolizing enzyme, called acid ceraminidase, which is expressed in human cumulus cells and FF, to the culture medium, embryo morphology significantly improved and healthy births were achieved five-fold higher. Histologically and hormonally, an experimental study showed that local ovarian CER 1 phosphate injections reduced cyclophosphamide-induced ovarian damage by protecting the ovarian reserve, restoring hormonal secretions, inhibiting apoptosis, and improving stromal vascularity. Thus, fertility, oocyte quality, and uterine morphology are protected by CER 1 phosphate.

Available data suggest that plasma lipoproteins, particularly high-density lipoprotein cholesterol, contain notable sphingolipids such as CER and sphingosine-1-phosphate, and they can mediate cardiovascular protection in healthy pregnancy. However, we could not find any significant differences in lipid profiles of the groups. Recently, different subclasses of CER have been suggested as novel lipidomic markers for diagnosing PCOS. Similarly, some FF metabolomics, including fatty acid, di/triacylglycerol, CER, and many sphingolipid metabolizing enzymes are located in the mitochondria. The presence of these pathways is an indirect indicator that lipid products also have specific functions. CER signaling, which is one of these functions, involves a complex molecular and subcellular network, all implicated in various cellular processes such as proliferation, differentiation, survival, necrosis and aging. An experimental study showed that after the addition of a CER metabolizing enzyme, called acid ceraminidase, which is expressed in human cumulus cells and FF, to the culture medium, embryo morphology significantly improved and healthy births were achieved five-fold higher. Histologically and hormonally, an experimental study showed that local ovarian CER 1 phosphate injections reduced cyclophosphamide-induced ovarian damage by protecting the ovarian reserve, restoring hormonal secretions, inhibiting apoptosis, and improving stromal vascularity. Thus, fertility, oocyte quality, and uterine morphology are protected by CER 1 phosphate.

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in BMP-15 that also has growth factor properties, has been shown to be related to the high response to COS\(^3\). We did not classify study groups based on ovarian response, but as expected, women diagnosed with LOR poorly responded to COS and had lower sCER and fFCER levels. Therefore, we may speculate that as a growth factor, CER is associated with a poor ovarian response to COS. We showed that CER is markedly lower in patients with LOR but significantly higher in women who could conceive, unlike other ovarian reserve markers. CER also was not correlated with the other ovarian reserve markers. However, sCER and fFCER levels were well correlated. CER may play a crucial role in both the physiological and pathological processes of the ovarian folliculogenesis and may be used independently to predict pregnancy in women undergoing IVF treatment.

**Study Limitations**

The main drawbacks of this work are the limited sample size, the partially heterogeneous control group, the analysis of FF from only one follicle, the analysis being based on only one cycle, and the lack of cumulative pregnancy rates. Additionally, increased CER levels may be due to secretions from the liver. The endothelium may be another source of serum CER. It should be considered that oxidative stress and proinflammatory cytokines may increase endothelial CER production by activating sphingomyelinase.

**Conclusion**

In conclusion, this is the first study evaluating the sCER and fFCER levels of patients undergoing IVF treatment. CER may be used as an ovarian reserve marker and a biomarker capable of predicting IVF outcomes. It may also be used as a therapeutic agent in patients with LOR or poor quality of oocyte. There is a need for further investigations to reveal the involvement of CER and other sphingolipids with female reproductive functions.

**Ethics**

**Ethics Committee Approval:** The hospital’s local ethics council approved the study protocol (date/approval number: 30.05.2018/24).

**Informed Consent:** Written informed consent was taken from all patients who were included in the study.

**Peer-review:** Externally peer-reviewed.

**Authorship Contributions**

Concept: B.T., Design: B.T., Data Collection or Processing: B.T., N.I., I.K., Analysis or Interpretation: O.A., Literature Search: N.I., I.K., S.D., Writing: B.T.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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**References**

1. Jerge PR. Poor ovarian reserve. J Hum Reprod Sci 2016;9:63-9.
2. Perheentupa A, Huhtaniemi I. Aging of the human ovary and testis. Mol Cell Endocrinol 2009;299:2-13.
3. Levi AJ, Raynault MF, Bergh PA, Drews MR, Miller BT, Scott RT Jr. Reproductive outcome in patients with diminished ovarian reserve. Fertil Steril 2001;76:666-9.
4. Practice Committee of the American Society for Reproductive Medicine. Testing and interpreting measures of ovarian reserve: a committee opinion. Fertil Steril 2020;114:1151-7.
5. Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. Hum Reprod Update 2006;12:685-718.
6. Gomez-Larrauri A, Presa N, Dominguez-Herrera A, Outo A, Trueba M, Gomez-Munoz A. Role of bioactive sphingolipids in physiology and pathology. Essays Biochem 2020;64:579-89.
7. Hannun YA, Obeid LM. Many ceramides. J Biol Chem 2011;286:27855-62.
8. Huang YL, Huang WP, Lee H. Roles of sphingosine 1-phosphate on tumorigenesis. World J Biol Chem 2011;12:25-34.
9. Eliyahu E, Shtraizent N, Martinuzzi K, Barritt J, He X, Wei H, et al. Acid ceramidase improves the quality of oocytes and embryos and the outcome of in vitro fertilization. FASEB J 2010;24:1229-38.
10. Pasquali N, Scotti L, Di Pietro M, Oubinta G, Bas D, May M, et al. Ceramide-1-phosphate has protective properties against cyclophosphamide-induced ovarian damage in a mice model of premature ovarian failure. Hum Reprod 2018;33:844-59.
11. Cordeiro FB, Cataldi TR, de Souza BZ, Rochetti RC, Fraieta R, Labate CA, et al. Hyper response to ovarian stimulation affects the follicular fluid metabolomic profile of women undergoing IVF similarly to polycystic ovary syndrome. Metabolomics 2018;14:51.
12. Kuijio LL, Perez GI. Ceramide and mitochondrial function in aging oocytes: juggling a new hypothesis and old players. Reproduction 2012;143:1-10.
13. Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertil Steril 2000;73:1155-8.
14. Şimşir C, Ecemis T, Erşahin AA, Güney G, Coşkun B, Coşkun B, et al. Serum AMH levels are not associated with adverse perinatal outcomes in women undergoing IVF treatment due to diminished ovarian reserve. Medical Science and Discovery 2019;6:160-5.
15. Iliodromiti S, Relsey TW, Wu O, Anderson RA, Nelson SM. The predictive accuracy of anti-Müllerian hormone for live birth after assisted conception: a systematic review and meta-analysis of the literature. Hum Reprod Update 2014;20:560-70.
16. Osellame LD, Blacker TS, Duchen MR. Cellular and molecular mechanisms of mitochondrial function. Best Prac Res Clin Endocrinol Metab 2012;26:711-23.
17. Babayev E, Seli E. Oocyte mitochondrial function and reproduction. Curr Opin Obstet Gynecol 2015;27:175-81.
18. Benkhalifa M, Ferreira YJ, Chahine H, Louanjli N, Miron P, Merviel P, et al. Mitochondria: participation to infertility as source of energy and cause of senescence. Int J Biochem Cell Biol 2014;55:60-4.
19. Van Blerkom J. Mitochondrial function in the human oocyte and embryo and their role in developmental competence. Mitochondrion 2011;11:797-813.
20. Trifunovic A, Wredenberg A, Falkenberg M, Spellbrink JN, Rovio AT, Bruder CE, et al. Premature ageing in mice expressing defective mitochondrial DNA polymerase. Nature 2004;429:417-23.
21. Cummins JM, Wakayama T, Yanagimachi R. Fate of microinjected spermatid mitochondria in the mouse oocyte and embryo. Zygote 1998;6:213-22.
22. Aitken RJ. Free radicals, lipid peroxidation and sperm function. Reprod Fertil Dev 1995;7:659-68.
23. Okazaki T, Bielewaska A, Bell RM, Hannun YA. Role of ceramide as a lipid mediator of 1 alpha,25-dihydroxyvitamin D3-induced HL-60 cell differentiation. J Biol Chem 1990;265:15823-31.
24. Adam D, Heinrich M, Kabelitz D, Schütze S. Ceramide: does it matter for T cells? Trends Immunol 2002;23:1-4.
25. Hetz CA, Hunn M, Rojas P, Torres V, Leyton L, Quest AF. Caspase-dependent initiation of apoptosis and necrosis by the Fas receptor in lymphoid cells: onset of necrosis is associated with delayed ceramide increase. J Cell Sci 2002;115:4671-83.
26. Venable ME, Webb-Froehlich LM, Sloan EF, Thomley JE. Shift in sphingolipid metabolism leads to an accumulation of ceramide in senescence. Mech Ageing Dev 2006;127:473-80.
27. Patanapirunhakit P, Karlsson H, Mulder M, Ljunggren S, Graham D, Freeman D. Sphingolipids in HDL - Potential markers for adaptation to pregnancy? Biochim Biophys Acta Mol Cell Biol Lipids 2021;1866:158955.
28. Neulen J, Wenzel D, Hornig C, Wünsch E, Weissenborn U, Grunwald K, et al. Poor responder-high responder: the importance of soluble vascular endothelial growth factor receptor 1 in ovarian stimulation protocols. Hum Reprod 2001;16:621-6.
29. Battaglia C, Genazzani AD, Regnani G, Primavera MR, Petraglia F, Volpe A. Perifollicular Doppler flow and follicular fluid vascular endothelial growth factor concentrations in poor responders. Fertil Steril 2000;74:809-12.
30. Keay SD, Liversedge NH, Akande VA, Mathur RS, Jenkins JM. Serum IGF-1 concentrations following pituitary desensitization do not predict the ovarian response to gonadotrophin stimulation prior to IVF. Hum Reprod 2003;18:1797-801.
31. Hanevik HI, Hilmarsen HT, Skjelbred CF, Tanbo T, Kahn JA. A single nucleotide polymorphism in BMP15 is associated with high response to ovarian stimulation. Reprod Biomed Online 2011;23:97-104.