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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 (ffCER) 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 ffCER 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 ffCER (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 ffCER levels could predict both LOR and pregnancy.

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 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 yolundaki serum SER (sSER) ve foliküler sıvı SER (ffSER) düzeylerinin klinik önemi ve bunların kötü oosit kalitesi ve klasik tüp bebek (in vitro fertilization - IVF) sonuçları üzerindeki etkisi araştırılacaktır.

Gereç ve Yöntemler: Bu kesitsel olgu-kontrol çalışması Türkiye'nin başkenti Ankara'da bir kadın doğum hastanesinin tüp bebek ünitesinde yapıldı. İlk IVF döngüsüne giren toplam 88 kadın bu çalışmaya dahil hastalar over rezervlerine göre güncel tanı kriterlerine göre 2 gruba ayrıldı. Bazal sSER seviyeleri ve oosit toplama gününde alınan ffSER konsantrasyonları ölçüldü.

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

Sonuç: Bu, tüp bebek tedavisi gören hastaların sSER ve ffSER düzeylerini değerlendiren ilk çalışmadır. SER, yumurtalık rezerv belirteci olarak ve IVF sonuçlarını tahmin edebilen bir biyobelirteç olarak kullanılabilir.

Anahtar Kelimeler: Seramid, kontrollü ovaryan stimülasyon, tüb bebek, over rezerv markerı, gebelik

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

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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[1]. 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 Etlik 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 (ffCER) 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

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/m^2).

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 E_2 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. The TVUS-guided oocyte retrieval procedure was executed 34-36 h after hCG triggering. 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% O_2, 5% CO_2, and 90% N_2, in benchtop incubators (MIRI, ESCO Medical, Singapore). Blastocysts were scored and morphologically evaluated as previously described[13]. 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 3	37 (84.1)	29 (65.9)	0.002
Day 5	7 (15.9)	15 (34.1)	
Pregnancy	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

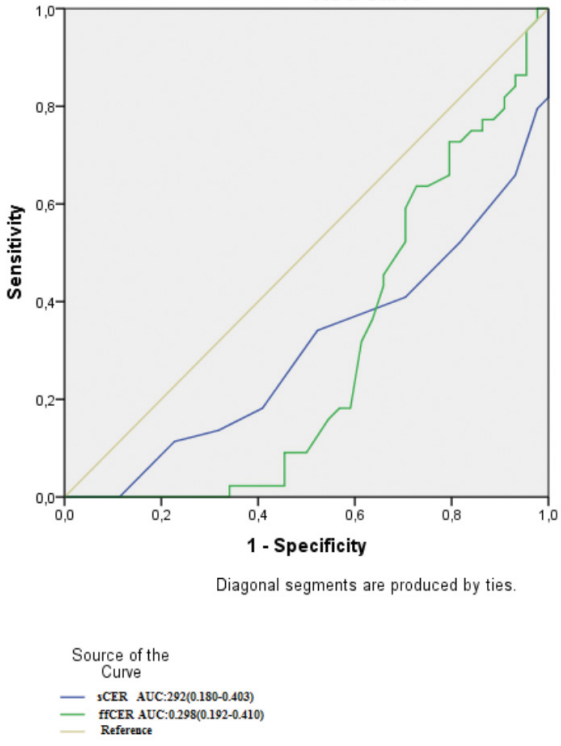

Figure 1. ROC curve analysis of sCER and ffCER level in predicting pregnancy in women undergoing IVF treatment

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

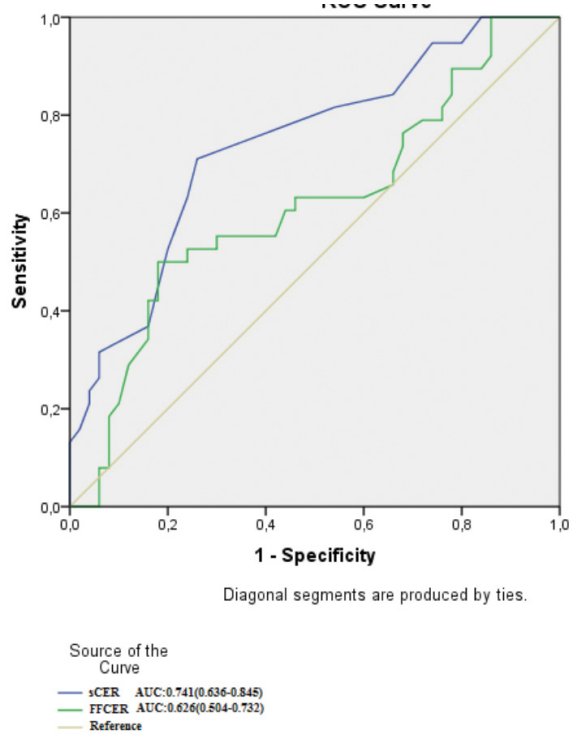

Figure 2. ROC curve analysis of sCER and ffCER level in predicting women with LOR

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

Table 3. sCER, ffCER and other ovarian reserve parameters in pregnant and non-pregnant cases

Variables	Pregnant (n=38)	Non-pregnant (n=50)	p
AMH (ng/mL)	2.0±0.9	1.8±0.7	0.223
Estradiol (pg/mL)	38.5±16.5	39.0±12.8	0.523
FSH (mIU/mL)	7.3±1.8	7.7±2	0.294
AFC	10.6±5.6	11.8±6.7	0.429
sCER (ng/mL)	21.5±5.8	16.3±6.9	0.001
ffCER	108.5±41.2	92.1±39.2	0.036

AMH: Anti-Müllerian hormone, AFC: Antral follicle count, FSH: Follicle stimulating hormone, sCER: Serum ceramide, ffCER: Follicular fluid ceramide. Data were expressed as mean ± standard deviation

ovarian reserve. The decrease in serum AMH levels due to aging is accompanied by a decline in the number of the primordial follicles, as well as increased apoptosis in the granulosa cell which indicates diminished oocyte quality. Although low-pre-treatment AMH levels in women undergoing IVF cycles indicate that the number of oocytes retrieved and oocyte quality is low, it cannot predict pregnancy. Even poor-quality embryos derived from poor-quality oocytes may result in a live birth[15].

Mitochondria are the most important energy-producing organelles of the cell and are separated from the cytoplasm by a double-layered membrane. It produces ATP, which is vital for several cellular activities. Apart from energy production, it also plays a role in the oxidation of fatty acids, calcium homeostasis, and apoptosis[16]. Poor oocyte quality and associated embryo quality may be associated with impaired energy production in the oocyte cytoplasm, although it results in live birth[17]. This energy production impairment may have different and wide range of effects from implantation to after birth.

From an evolutionary perspective, mitochondria are thought to be the remnants of bacteria that have invaded eukaryotic cells. Although many proteins necessary for its function are encoded by the nucleolus genome, mitochondria are the only animal organelles that include DNA outside the nucleus[18]. Human mitochondrial DNA (mtDNA) is a circular structure and contains 37 genes. Since it does not contain protective proteins such as histones, mtDNA is susceptible to mutations. Mitochondria play an important role in human reproduction[19]. Fertility has been shown to be severely reduced in transgenic mice with induced mtDNA mutations[20]. MtDNA is maternally inherited because sperm mitochondria are degraded after they enter the oocyte. Although the exact reason for this is unknown, it may be to protect the embryo from dangerous mutations that may occur in the mtDNA of the sperm exposed to high oxygen radicals during spermatogenesis. A fully grown oocyte has approximately 100,000 mitochondria. Since the need for ATP is also low in immature oocytes, the mitochondria that are waiting silently replicate in the late folliculogenesis stage and after fertilization. The oocytes in the mitochondria are vital for early embryonic development[21,22].

Mitochondria are essential organelles in sphingolipid metabolism, and many sphingolipid metabolizing enzymes are located in the mitochondria[12]. 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[23-26]. 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[9]. 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[10].

Available data suggest that plasma lipoproteins, particularly high-density lipoprotein cholesterol, contain notable sfingolipids such as CER and sphingosine-1-phosphate, and they can mediate cardiovascular protection in healthy pregnancy[27]. 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, CER-phosphate, phosphatidylcholine, and sphingomyelin, have been shown to be elevated in hyper-responder women with or without PCOS undergoing IVF treatment[11]. Additionally, various growth factors in the FF have been reported to be altered according to the different ovarian responses. Vascular endothelial growth factor (VEGF) has increased in the FF of women with poor response[28]. It has also been reported that serum VEGF levels did not differ in poor responders compared to normo-responders and did not foresee ovarian response[29]. In another study, serum insulin-like growth factor-1 levels also did not differ between the poor and normo-responders[30], but a minor polymorphism

in BMP-15 that also has growth factor properties, has been shown to be related to the high response to COS[31]. 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.İ., İ.K., Analysis or Interpretation: O.A., Literature Search: N.İ., İ.K., S.D., Writing: B.T.

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

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