RESEARCH
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EVALUATION OF THE CERAMIDE LEVELS IN SERUM AND FOLLICULAR FLUID AND THEIR CORRELATION WITH OOCYTES AND EMBRYO QUALITY IN FRESH ICSI CYCLE

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Abstract
Background: Ceramide (CER) is a form of biologically active in the mitochondrial membrane and is essential for energy processing, cell division, and reproduction.
The Aim of the study: to evaluate the relationship of Ceramide levels in follicular fluid and serum of intracytoplasmic sperm injection patients with oocyte characteristics, embryo grading, and pregnancy outcome.
Patients and Methods: The current study included 60 women. On the second day of the cycle, recombinant follicle-stimulating hormone (FSH) was administered to all of these women in preparation for ICSI. On the day of oocyte collection, samples of follicular fluid and serum were taken. These samples have their amounts of ceramide measured.
Result: The current study's pregnancy rate for all females was 30%.The mean age had a significant relationship between the two groups (P = < 0.05), and we found no difference in body mass index and the cause and type of infertility in pregnant and non-pregnant patients (all P > 0.05). There is a relationship between serum ceramide level, body mass index, ovarian reserve (AMH), follicular fluid ceramide level, and quality of eggs (P=0.009) and embryos. (p=0.049). It was revealed that serum ceramide (sCER) and follicular fluid ceramide (ffCER) levels were not related to pregnancy outcomes, (p > 0.05).
Conclusion: Serum ceramide level was positive correlation with Body mass index (BMI) for women and Anti Mollerian Hormon (AMH). follicular fluid ceramide positive correlation with oocyte and embryos charactierstics.
Keywords: ceramide, Follicular fluid, in vitro fertilization, pregnancy.

INTRODUCTION

According to the World Health Organization, infertility is a condition of the male or female reproductive system characterized by the inability to become pregnant after 12 months or more of continuous, unprotected sexual activity (Vander Borght and Wyns, 2018)[1]. Infertility affects between 10% and 15% of couples, with female infertility accounting for more than half of the cases (Nagórska et al 2019)[2]

Lifestyle changes have grown more significant in contributing to decreased fertility as assisted reproductive treatments (ART) have become more widespread (Akhondi et al. 2019)[3]. A component of ART therapies involves the extracorporeal handling of human oocytes, sperm, and embryos to conceive a female subject into a pregnant woman (Feskov et al., 2019).[4]

Evaluating the quality of eggs and selecting embryos for transfer to the mother's uterus has a significant impact on the possibility of the operation being successful, and successful implantation is considered one of the difficult tasks for embryologists (Thorat et al. 2020)[5]. Individuals with oligospermia or other problems with semen quality usually receive sperm injections into their cells. A single sperm is injected directly into the egg to enhance the fertilization process (Sharif, 2019)[6]**.** Although ICSI has contributed to the treatment of infertility, the success rate of the procedure is still low, which calls for continued research to improve the quality of eggs and embryos. Fats are considered important elements for preparing cells with energy, including ceramide (CER), which is a type of phospholipid found in cell membranes. It is necessary for cell division and reproduction. (Gomez-Larori A and colleagues, 2020)[7]. In research conducted by (Wang et al., 2015)[8] on mouse embryos, it was found that there is a relationship between assisted ovarian stimulation during reproductive technology treatments and modifications in fat metabolism in mouse eggs and embryos and the fat content in the eggs. The uterine membrane, which affects the results of artificial insemination, as in a study conducted by (Belaz et al., 2016)[9]. In cows, ovarian stimulation has been found to modify the composition of phospholipids in the fluid. Bovine follicle.

FF ceramide can both improve the understanding concerning mechanisms responsible for oocyte development and its effect on embryo implantation potential and assist in the management of IVF cycles.(Montani, D.A.,et al :2019)[10]

Fats are broken down to produce fatty acids, which have a role in egg formation. On the other hand, fatty acid oxidation is an important energy source for developing oocytes and early embryos (Prates et al., 2014).[11]There have been more statements recently that egg quality may be related to the level of ceramide (sCER) and certain phospholipids in the eggs. Cordeiro FB, et al 2018:[12] and Pascuali N, et al 2018 [13] .Although the specific roles of ceramides are uncertain, it is known that ceramides are transported into oocytes from cumulus cells through gap junctions. Ceramides are less abundant in

oocytes from aged mice compared to young mice, with effects in accelerating age-dependent oocyte apoptosis Perez G. I., et al:2005[14]. Furthermore, in older female mice, a reduced number of mitochondria appears to reduce the amounts of ceramide in oocytes Kujjo L. et al:2012 [15]. The complex microenvironments contained in ovarian follicles, which generate follicle fluid (FF), support follicle development, oocyte maturation, and somatic germ-cell interactions. The follicle fluid contains the essential metabolites of ceramide Da Broi, M. G., et al. 2018[16]Changes in lipid metabolism in rat oocytes and embryos have been linked to assisted ovarian stimulation during reproductive technology treatments Wang, Weiran, et al. [17] Although ICSI enables many individuals to contribute to the problem of infertility, the results are not always satisfactory, and this prompts finding ways to help increase the success of ICSI. The current study investigated the biomarker ceramide effect on the quality of eggs and embryos, fertilization rate, and pregnancy outcomes.

MATERIALS AND METHODS

A study conducted on sixty infertile women who underwent ICSI at the Higher Institute for Infertility Diagnosis and Assisted Reproductive Technologies / Infertility Center / Al-Nahrain University / Baghdad / Iraq between November 2023 and March 2024, regardless of whether or not they had prior ICSI experience. The quality of the resulting embryo and the egg form were assessed in the institute's laboratory. Sixty ICSI-cycled infertile women participated in the trial. The age range covered the years from 20 to 40. Every couple underwent a basic fertility work-up, which included a history, physical examination, hormone measurements, and a semen analysis. All female infertiles followed the antagonist routine. Serum levels of Estradiol (E2), luteinizing hormone (LH), follicle-stimulating hormone (FSH), Prolactin (PRL.), and AMH were measured in all 60 infertile females on the second or third day of their cycles. Rechecking the serum Estradiol (E2) level was done on the day of the Human chorionic gonadotropin (hCG) injection. Both samples were stored in a deep freezer (-20°C) so they could be utilized later on to test the level of Ceramide. On the day of embryo transfer, the transplanted embryos' media were gathered and stored to measure the ceramide level. Basic biochemical and hormonal parameters of venous blood (2nd or 3rd day of the menstrual cycle) were examined. Samples were screened by electrochemical immunoassay (Roche Diagnostics, Mannheim, Germany). Serum and follicular fluid samples were collected on the day of egg collection and separated by centrifugation. Serum samples were centrifuged at 2000-3000 rpm for 10 minutes. The collected follicular fluid (F.F) samples were immediately centrifuged at 800 × g for 10 min to separate the fluid from the follicular cells. Serum and FF samples were kept at −20°C until CER day. Body mass index (BMI) was calculated and recorded by dividing weight in kilograms by height in meters squared (kg/m2). The frozen samples were then brought to room temperature to be thawed, and Ceramide values were measured using commercially available human ceramide ELISA kits. Test procedures were performed according to the manufacturer's instructions.

Controlled Ovarian Stimulation

Gonadotropins (r-FSH) were administered to all 60 infertile women who underwent the antagonist protocol on day two of the cycle at a dose of 75–300 IU until the leading follicle measured 12–14 mm in diameter. At that point, cetrotide (a GnRH antagonist) was administered subcutaneously at a dose of 0.25 mg every day until the day of Human chorionic gonadotropin HCG injection, at which point the ovary's response to stimulation could be tracked by measuring the serum E2 level and assessing follicular growth using ultrasound. Human chorionic gonadotropin (hCG), administered subcutaneously when at least three follicles were present, caused ovulation to occur. Reached 17–18 mm. The oocyte retrieval was carried out under transvaginal ultrasound guidance 35–36 hours after the trigger. Begueria, R., et al :2014[18]

Semen Collection, Oocyte Retrieval, and Embryo Transfer

Samples of semen were collected during masturbation following a period of two to five days without sexual activity. Following liquefaction for 30 minutes at room temperature, the obtained semen specimens were treated using the usual swim-up procedure preparation media (FertiCultTM Flushing medium, FertiPro NV). Carefully extracted, highly motile sperm in the medium were used for the intracytoplasmic sperm injection (ICSI) process. Under general anesthesia, the oocytes were collected approximately 36 hours after the trigger, using an ultrasound guide to guide the transvaginal process. A single-lumen needle was used to aspirate every follicle without flushing, passing through the posterior fornix of the vagina to reach the ovary. The needle was attached to a suction apparatus at zero pressure (120 mmHg). To gently clean the vagina, regular saline was used to flush it. The embryologist separates and measures each patient's aspirated oocytes, encircled by cumulus oophorus cells (COC). Initially, to remove any potential leftover blood from the aspiration, COCs were cleaned using flushing media. oocyte retrieval, oocyte denudation, and standard ICSI procedures were carried out on each woman. Following ICSI, oocytes were cultured individually in a unique, pre-equilibrated culture dish. In the investigation, a single-step media was employed throughout the culture time. In tabletop incubators (ESCO Medical), embryos were cultured until the fifth or sixth day at 37 °C in an atmosphere of 5% O2, 5% CO2, and 90% N2. The best-quality embryos were selected for transfer. Given the sufficient number of high-quality embryos, no more than two were transferred; the remaining embryos were cryopreserved for later use.

Statistical Analyses

The following statistical tests were used:

1. The Chi-Square Test was used to evaluate the association between any two categorical variables provided that less than 20 % of cells have an expected count of less than 5. However, Fischer exact test or Yates correction for continuity was used instead when the chi-square test was not valid (in case more than 20 % of cells have an expected count of less than 5).
2. Independent Samples t-test was used to evaluate the difference in mean of numeric variables between any two groups provided that these variables we
3. Normally distributed; otherwise, Mann Whitney U test would be used instead if those variables were not normally distributed.
4. Pearson and Spearman correlation tests were used to evaluate the correlation between any 2 numeric variables and the results were expressed as correlation coefficient (*r*) and the level of significance (*P*).

The level of significance was considered at a *P*-value of equal or less than 0.05. The level of high

RESULTS

When patients enrolled in this study were followed up, the results of the pregnancy test revealed positive findings in 18 out of 60 women making the rate of biochemical pregnancy as 30 %. Therefore, the remaining result findings will be organized by comparing the pregnant and non-pregnant groups. The mean age of the pregnant group was lower than that of the non-pregnant group, 29.05 ± 5.89 years versus 32.80 ± 4.73 years, (P = 0.024) but the BMI, cause of infertility, and type of infertility of the patients were similar. . . All p > 0.05. Hormone levels were measured on the second day of the cycle.(LH, FSH, PRL, and E2)were similar between groups. All p > 0.05). AMH was measured and was similar between groups. All p > 0.05). Comparison of oocyte and embryo characteristics between pregnant and non-pregnant women was similar between groups. All p > 0.05) .The comparison of mean ceramide levels in serum and follicular fluid between pregnant and non-pregnant women was similar between the two groups. All p > 0.05) (Figure 1)The relationship between ceramide levels and egg characteristics. Follicular fluid ceramide showed a significant and positive correlation between the total oocytes (r = 0.269, p = 0.037), the number of MII oocytes (r = 0.333, p = 0.009), and the maturation rate (r = 0.327, p = 0.012) (Figure 2): Shows the association between MII oocytes and follicular fluid ceramide, which is significant and positive (P < 0.05). There was no other significant association (P < 0.05). The correlation between ceramide levels and fetal characteristics is shown in Table 2. Follicular fluid ceramide showed a significant and positive correlation with the total number of embryos (r = 0.249, p = 0.020), first-degree embryos (r = 0.0255, p = 0.049), and fertilization rate (r = 0.283, p = 0.028). There was no other significant association (P < 0.05). The association between ceramide and hormone levels and total pregnancy is shown in (Table 3). There is a significant and positive correlation between serum ceramide and follicular fluid for E2 on the day of trigger (p = 0.046, p = 0.014, respectively) and serum ceramide has a significant and positive correlation with AMH (p < 0.05) (Figure 3)

Association between ceramide levels and demographic characteristics of women participating in this study: Serum ceramide showed a significant and positive association with BMI (p = 0.021).significance was considered at a *P*-value <0.0

Table 1: The demographic characteristics of women enrolled in this study

Characteristic	Total *n* = 60	Pregnant *n* = 18	Not pregnant *n* = 42	*p*
Age (years)				
Mean ±SD	31.68±5.35	29.05 ±5.89	32.80 ±4.73	0.024 I * S
BMI (kg/m^2)				
Mean ±SD	27.26±4.58	26.45 ± 4.61	28.12 ±4.53	0.207 I NS
LH (mIU/ml)				
Mean ±SD	5.12 ±2.19	5.19 ±1.52	5.09 ±2.44	0.872 I NS
FSH (mIU/ml)				
Mean ±SD	6.55 ±2.46	6.68 ±1.34	6.49 ±2.81	0.776 I NS
Prolactin (mIU/ml)				
Mean ±SD	26.53 ±11.97	26.05 ±12.47	26.73 ±11.89	0.843 I NS
E2 at day 2 cycle (pg/ml)				
Mean ±SD	36.08 ±11.49	34.13 ±11.48	36.92 ±11.53	0.394 I NS
E2 at trigger (pg/ml)				
Mean ±SD	1272.40 ±1321.71	1573.80 ±2172.64	1143.30 ±706.87	0.251 I NS
AMH (ng/ml)				
Mean ±SD	2.43 ±1.07	2.70 ±0.86	2.29 ±1.12	0.124 I
Serum ceramide (ng/ml)				
Mean ±SD	64.97 ±30.66	62.18 ±29.05	66.17 ±31.59	0.648 I NS
Follicular fluid ceramide (ng/ml)				
Mean ±SD	89.87 ±24.79	82.37±28.20	93.09 ±22.79	0.126 I NS

n: number of cases; SD: standard deviation; BMI: body mass index; I: independent samples *t*-test; C: chi-square test; F: Fischer exact test; NS: not significant

Table 2: Correlation of ceramide levels to total pregnancy, hormone, and demographic characteristics of women enrolled in this study

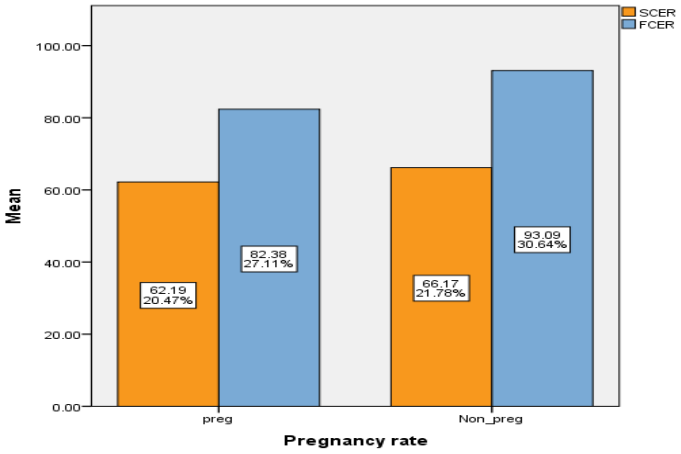
Characteristic	Serum ceramide		FF ceramide	
	r	*p*	*r*	*p*
Total pregnancy	0.060	0.684	0.200	0.125
LH (mIU/ml)	0.020	0.877	-0.006	0.963
FSH (mIU/ml)	0.043	0.742	-0.248	0.057
Prolactin (mIU/ml)	0.055	0.678	-0.081	0.539
Estradiol (pg/ml)	-0.077	0.557	0.049	0.711
E2 at trigger (pg/ml)	0.259	0.046 *	0.315	0.014 *
AMH (ng/ml)	0.296	0.022 *	0.112	0.396
Age (years)	-0.012	0.927	-0.114	0.384
BMI (kg/m^2)	0.297	0.021 *	-0.108	0.411
Causes	0.022	0.0.666	-0.086	0.0.514
Type of infertility	0.111	0.523	-0.267	0.478

*: significant at $p \leq 0.05$

Table 3: Correlation of ceramide levels to oocyte and embryo characteristics

Characteristic	Serum ceramide		FF ceramide	
	r	*p*	*r*	*p*
Total oocytes	0.048	0.717	0.269	0.037 *
MII oocytes	-0.019	0.037	0.333	0.009 **
MI oocytes	0.028	0.832	-0.152	0.247
GV oocytes	0.022	0.868	0.138	0.293
Maturation rate	-0.185	0.156	0.324	0.012 *
Total No. embryos	0.026	0.841	0.299	0.020 *
Grade I embryos	0.140	0.284	0.0.255	0.049 *
Grade II embryos	-0.116	0.379	0.187	0.152
Grade III embryos	0.152	0.245	0.228	0.079
Fertilization rate	0.021	0.873	0.283	0.028 *

*: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$



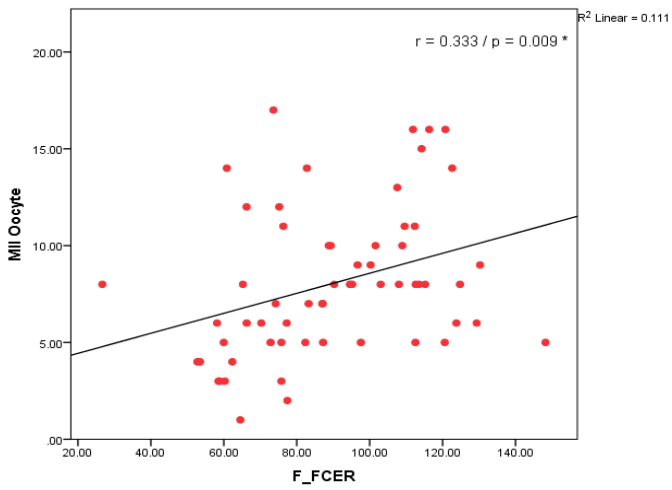


Figure 1:Comparison of mean ceramide levels in serum, follicular fluid, and between pregnant women and non-pregnant women

Figure 2: Scatter plot showing a correlation between MII oocytes and Follicular fluid ceramide

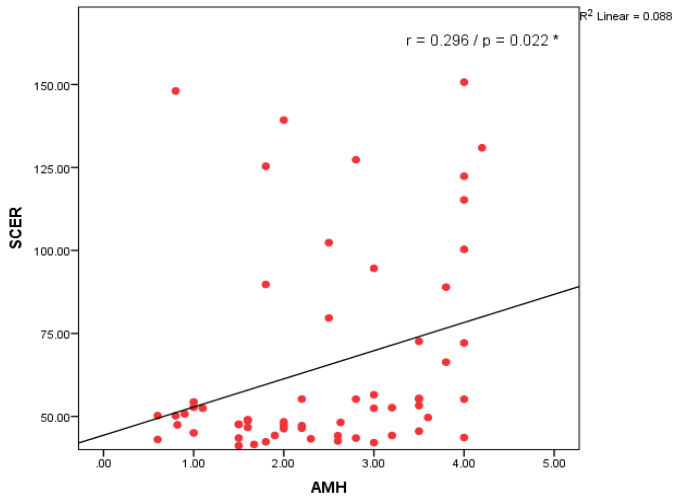


Figure 3: Scatter plot showing a correlation between Serum ceramide and AMH

DISCUSSION

Age is a major demographic component that influences ICSI outcomes and negatively impacts oocyte quality and pregnancy rate (Pierce, N. et al 2018)[19]. In the current research, the average age of patients differed significantly between pregnant and non-pregnant women, as it is shown in Table (1). This finding is consistent with a study (Al-Obaidi, M.T., et al:2018)[20] that discovered a negative relationship between maternal age and unfavorable pregnancy outcomes, as well as lower fertility. In contrast to this, a study conducted by (Crawford, N.M. et al:2015)[21] the Age is only a crude measure of ovarian reserve and does not give a very accurate estimate of a woman's ability to reproduce. The factors contributing to the age-related drop in pregnancy rates include the decrease in the number of mature eggs, the decline in the quality of the embryos created as a result of the uterine aging along with the eggs, and the potential hormonal disruption brought on by the mother's aging. The current study found no association between clinical pregnancy outcomes and BMI, which is similar to the results of other studies (Poonam Bhagwani, et al.: 2016)[22]which is similar to the results of another study (Banker, M., et al., 2017)[23]

Although another study claimed that weight loss can increase the pregnancy rate, there is an association between lower pregnancy rates in an ART program and overweight and obesity. (Luke, B., et al:2011)[24] although a study was discovered claiming that losing weight can increase the rate of pregnancy (Song, J.Y., et al., 2017),[25] Basal hormone levels (FSH, LH, and E2) were measured and E2 was remeasured on the day of operation because it is used to evaluate ovarian response in conjunction with ultrasound findings. The results of the current study, which appear in Table (1,2), indicate that basal hormone levels (FSH, LH, and E2) were not significantly different from each other, but there is a significant relationship between E2 levels during the stimulation day and trigger day with ceramide levels, and this is evident in Table(2) . Similar results were found in other investigations (Scheffer, J.A.B, et al:2018)[26] and Huang, M.C., et al:2018)[27].

Regarding the level of AMH, the results of the current study showed that there were no statistically significant differences between pregnant and non-pregnant women (P>0.05), with higher levels in pregnant women. Several studies contradict the findings of the current study (Gomez, R., et al :2016)[28]and (Mohammed, Z.I.et al :2021)[29].

The results of this study, are consistent with the results of several other studies (Pérez, P.A.S., et al :2018)[30] show that the pregnancy rate is not significantly affected by the hormone prolactin in the blood level. However, the results of an additional study conducted by(Chung, L.H., et al:2012)[31]showed that PRL regulates the function of the corpus luteum, hypothalamic-pituitary-ovarian axis, and endometrial receptivity, which in turn has a significant impact on pregnancy outcomes.The present investigation found no significant differences in serum estradiol (E2) levels ($P > 0.05$) between pregnant women on basal estradiol and those on the stimulation day).

The results of the study showed that pregnancy rates in GnRH antagonist cycles are not affected by the E2 level on the second day and the day of hCG injection (Pinheiro, L.M.A., et al:2017)[32] - according to a study conducted by (Siddhartha, N., et al:2016)[33] Estradiol levels are upregulated on day 2 of the menstrual cycle and the day of hCG administration has a significant impact on the success of IVF/ICSI outcomes or perhaps a more accurate indicator of pregnancy outcomes in women treated with ART (Prasad, S., et al., 2014) [34]

These results contradict the results of the current study. The current study did not find statistically significant differences between pregnant and non-pregnant women in serum ceramide levels and follicular fluid according to Table (1).

Ceramides appear to be important during pregnancy and in the event of unfavorable obstetric outcomes. Biomarkers in the first trimester of pregnancy, but there is a paucity of research on ceramide modulation. To determine their function in metabolic pathways and for clinical application as indicators of pregnancy (Lanzanacci, Maria, et al.: 2023)[35]

In the current study, we did not find a relationship between ceramide levels and pregnancy outcomes, and this is clear in (Table 2), and this is consistent with the research results. According to a study (Juchtnicka, I. et al., 2022)[36]. Many pregnancies with different levels of sphingolipids have been examined, but no clear indication has been observed. Fats serve as markers for outcomes other than pregnancy.

In a study conducted by researchers (Timur, B. et al., 2022) [37] there is an important relationship between ceramide levels and pregnancy outcomes. This is in contrast to the current study.

In this study, we evaluated baseline sCER and ffCER levels in infertile women who underwent IVF cycles. It was found that there is a significant positive relationship between the hormone AMH and the level of ceramide in the serum.

As shown in Table (2) and Figer (3), this is consistent with the study conducted by (Simsir C. et al. (2019)[38]. It has been found that patients with low AMH have lower levels of ceramide in the blood. Since E2 is used to evaluate ovarian response in conjunction with ultrasound findings, remeasurement was performed on the trigger day. It turns out that there is a significant relationship between E2 on the trigger day and serum ceramide level, as in Table (3)

As in the results of the current research shown in Table (3), there is no significant difference between basal hormone (FSH) levels. and LH and E2 levels). Other experiments (Kamel et al., 2018;)[39] yielded similar results.

The current study found a positive significant correlation between body mass index (BMI) and serum ceramide levels, as shown in Table (2). In a related study conducted by (Wigger, L. et al. 2017)[40] it was shown that obesity and a high-calorie diet lead to an increase and accumulation of ceramides. This is consistent with the current study. In a related study, women with

a BMI associated with obesity showed greater levels of ceramides compared to women with a moderate BMI (García-Ferreyra et al., 2021)[41]

In the current study, there is a significant relationship between the level of ceramide in the follicular fluid and the number and maturation of eggs. This is clear in the table (3) and figure (2), and this is consistent with a study conducted by ((Babayev and Seli, 2015)[42]. Since ceramide is a crucial energy source for the cell - during egg maturation, mitochondria multiply and increase in quantity to prepare for mitotic divisions, which increases... Ceramide production - it is an essential source for these processes and this is consistent with the current study.

Based on studies conducted by(Hendricks et al. (2019)[43]. It should be noted that in horse eggs, mitochondria multiply and the rest of the egg cell organelles stop in the early stages of mitosis and continue until the formation of the blastocyst. Ceramide production increases to prepare energy for healthy embryonic growth. This is consistent with the findings of the current study

In the current study, it was found that ceramide concentrations in follicular fluid have a significant positive relationship with fetal quality, as shown in Table (3). In a related study, it was reported that lipid receptors in the follicular fluid could help manage the IVF cycle and enhance knowledge of the processes governing egg development and their impact on the possibility of embryo implantation. (Da Montagnian et al., 2019)[44] Sphingolipids were found to have important structural functions and are involved in cellular signaling for embryonic division in another study by (Ginkel, C., et al. 2012)[45].

STUDY LIMITATIONS

The primary limitations of this study were the small sample size, the somewhat diverse control group, and the FF analysis of a small number of follicles.

Furthermore, hepatic secretions could be the cause of high CER levels.

Another potential source of serum CER is the endothelium. It should be taken into account that proinflammatory cytokines and oxidative stress may promote endothelial CER synthesis via sphingomyelinase activation

CONCLUSIONS

The current study concluded the following:

Serum ceramide level was positive correlation with Body mass index (BMI) for women and Anti Mollerian Hormon (AMH). follicular fluid ceramide positive correlation with oocyte and embryos charactierstics .

- Follicular fluid ceramide positive correlation with oocyte and embryos charactierstics .
- Serum ceramide level was positive correlation with Body mass index (BMI) for women and Anti Mollerian Hormon (AMH).
- Evaluation of ceramide levels in serum and follicular fluid may not be a promising factor for predicting pregnancy rate in ICSI cycles

References

1. *Vander Borght, M. and Wyns, C., 2018. Fertility and infertility: Definition and epidemiology. Clinical biochemistry, 62, pp.2-10.*
2. *Nagórska M, Bartosiewicz A, Obrzut B, Darmochwał-Kolarz D. Gender Differences in the Experience of Infertility Concerning Polish Couples: Preliminary Research. Int J Environ Res Public Health. 2019;16(13):2337. [DOI:10.3390/ijerph16132337] [PMID] [PMCID]*
3. *Akhondi, M.M., Ranjbar, F., Shirzad, M., Ardakani, Z.B., Kamali, K. and Mohammad, K., 2019. Practical difficulties in estimating the prevalence of primary infertility in Iran. International journal of fertility & sterility, 13(2), p.113.*
4. *Feskov, A., Feskova, I., Zhylkova, I. and Zhilkov, S., MEZADATA MEDICAL IP HOLDING LLC, 2019. Method of in vitro fertilization with delay of embryo transfer and use of peripheral blood mononuclear cells. U.S. Patent 10,271,876.*
5. *Thorat, R.S., More, A., Salve, M. and Shrivastava, D., 2020. To Study the Correlation of Embryo and Oocyte Quality with Clinical Pregnancy Rate. International J Cur Res Rev, 12, pp.150-3.*
6. *Sheriff, D.S., 2019. Infertility, Assisted Methods of Reproduction, and Hormonal Assays. In Infertility, Assisted Reproductive Technologies and Hormone Assays. IntechOpen.*
7. *Gomez-Larrauri A, Presa N, Dominguez-Herrera A, Ouro A, Trueba M, Gomez-Muñoz A. Role of bioactive sphingolipids in physiology and pathology. Essays Biochem 2020;64:579-89.*
8. *Wang M, Jiang J, Xi Q, Li D, Ren X, Li Z, et al. Repeated cryopreservation process impairs embryo implantation potential but does not affect neonatal outcomes. Reprod BioMed Online (2021) 42:75–82. doi: 10.1016/j.rbmo.2020.11.007*
9. *Belaz, K.R.A., Tata, A., França, M.R., Santos da Silva, M.I., Vendramini, P.H., Fernandes, A.M.A., D'Alexandri, F.L., Eberlin, M.N. and Binelli, M., 2016. Phospholipid profile and distribution in the receptive oviduct and uterus during early diestrus in cattle. Biology of Reproduction, 95(6), pp.127-1.*
10. *Montani, D.A., Braga, D.P.d.F., Borges, E. et al. Understanding mechanisms of oocyte development by follicular fluid lipidomics. J Assist Reprod Genet 36, 1003–1011 (2019).*
11. *Prates, E.G., Nunes, J.T. and Pereira, R.M., 2014. A role of lipid metabolism during cumulus-oocyte complex maturation: impact of lipid modulators to improve embryo production. Mediators of inflammation, 2014.*
12. *Cordeiro FB, Cataldi TR, de Souza BZ, Rochetti RC, Fraietta R, Labate CA, et al. Hyperresponse to ovarian stimulation affects the follicular fluid metabolomic profile of women undergoing IVF similar to polycystic ovary syndrome. Metabolomics 2018;14:51*
13. *Pascuali N, Scotti L, Di Pietro M, Oubiña G, Bas D, May M, et al. Ceramide1-phosphate has protective properties against cyclophosphamide-induced ovarian damage in a mice model of premature ovarian failure. Hum Reprod 2018;33:844-59.*
14. *Perez G. I., Jurisicova A., Matikainen T., Moriyama T., Kim M.-R., Takai Y., et al. (2005). A central role for ceramide in the age-related acceleration of apoptosis in the female germline. FASEB J. 19, 860–862. 10.1096/fj.04-2903fje*
15. *Kujjo L. L., Perez G. I. (2012). Ceramide and mitochondrial function in aging oocytes: joggling a new hypothesis and old players. Reproduction 143, 1–10. 10.1530/REP-11-0350*
16. *Da Broi, M. G., et al. "Influence of follicular fluid and cumulus cells on oocyte quality: clinical implications."*

Journal of assisted reproduction and genetics 35 (2018): 735-751.
17. *Wang, Weiran, et al. "On deep multi-view representation learning." International conference on machine learning. PMLR, 2015.*
18. *Begueria, R., Garcia, D., Obradors, A., Poisot, F., Vassena, R. and Vernaeve, V., 2014. Paternal age and assisted reproductive outcomes in ICSI donor oocytes: is there an effect of older fathers? Human Reproduction, 29(10), pp.2114-2122.*
19. *Pierce, N. and Mocanu, E., 2018. Female age and assisted reproductive technology. Global Reproductive Health, 3(2), p.e9.*
20. *Al-Obaidi, M.T., Mahdi, H.B. and Alwasiti, E., 2018. The impact of age and BMI on oocyte maturation and embryo development. Biomedical Research, 29(9), pp.1920-1924.*
21. *Crawford, N.M. and Steiner, A.Z., 2015. Age-related infertility. Obstetrics and Gynecology Clinics, 42(1), pp.15-25.*
22. *Poonam Bhojwani, Raksha Sharma, Pragati Meena, Sadhana Mathur., (2016). Does maternal BMI affect pregnancy outcomes after IVF/ICSI? A prospective observational study. International Journal of Contemporary Medical Research.; 3(12), pp.3464-3466.*
23. *Banker, M., Sorathiya, D. and Shah, S., 2017. Effect of body mass index on the outcome of in-vitro fertilization/intracytoplasmic sperm injection in women. Journal of Human Reproductive Sciences, 10(1), pp.37-43.*
24. *Luke, B., Brown, M.B., Stern, J.E., Missmer, S.A., Fujimoto, V.Y., Leach, R. and A SART Writing Group, 2011. Female obesity adversely affects assisted reproductive technology (ART) pregnancy and live birth rates. Human Reproduction, 26(1), pp.245-252.*
25. *Song, J.Y., Xiang, S. and Sun, Z.G., 2017. Effect of weight loss on In Vitro fertilization treatment outcome. Reproductive and Developmental Medicine, 1(04), pp.210-215.*
26. *Scheffer, J.A.B., Scheffer, B., Scheffer, R., Florencio, F., Grynberg, M. and Lozano, D.M., 2018. Are age and anti-Müllerian hormone good predictors of ovarian reserve and response in women undergoing IVF? JBRA assisted reproduction, 22(3), p.215.*
27. *Huang, M.C., Tzeng, S.L., Lee, C.I., Chen, H.H., Huang, C.C., Lee, T.H. and Lee, M.S., 2018. GnRH agonist long protocol versus GnRH antagonist protocol for various aged patients with diminished ovarian reserve: A retrospective study. PLoS One, 13(11), p.e0207081*
28. *Gomez, R., Schorsch, M., Hahn, T., Henke, A., Hoffmann, I., Seufert, R. and Skala, C., 2016. The influence of AMH on IVF success. Archives of gynecology and obstetrics, 293, pp.667-673.*
29. *Mohammed, Z.I. and Qasim, M.T., 2021. Correlation of AMH and LH levels in PCOS patients with pregnancy rate. Annals of the Romanian Society for Cell Biology, pp.945-951.*
30. *Pérez, P.A.S., Ceschin, Á.P., de Moraes, D.M., de Oliveira, L.K., Ceschin, I.I. and Ceschin, N.I., 2018. Early serum progesterone and prolactin analysis at day 9 of oocyte retrieval as a predictor of success in fresh ICSI cycles. JBRA Assisted Reproduction, 22(2), p.95.*
31. *Chung, L.H., Liu, D., Liu, X.T. and Qi, Y., 2021. Ceramide transfer protein (CERT): An overlooked molecular player in cancer. International journal of molecular sciences, 22(24), p.13184.*
32. *Pinheiro, L.M.A., da Silva Cândido, P., Moreto, T.C., Di Almeida, W.G. and de Castro, E.C., 2017. Estradiol use in the luteal phase and its effects on pregnancy rates in IVF cycles with GnRH antagonist: a systematic review. JBRA assisted reproduction, 21(3), p.247.*
33. *Siddhartha, N., Reddy, N.S., Pandurangi, M., Tamizharasi, M., Radha, V. and Kanimozhi, K., 2016. Correlation of serum estradiol level on the day of ovulation triggers with the reproductive outcome of intracytoplasmic sperm injection. Journal of Human Reproductive Sciences, 9(1), pp.23-27*
34. *Prasad, S., Kumar, Y., Singhal, M. and Sharma, S., 2014. Estradiol level on day 2 and day of trigger: a potential predictor of the IVF-ET success. The Journal of Obstetrics and Gynecology of India, 64, pp.202-207.*
35. *Lantzanaki, Maria, et al. "Ceramides during Pregnancy and Obstetrical Adverse Outcomes." Metabolites 13.11 (2023): 1136.*
36. *Juchnicka, I., Kuźmicki, M., Zabielski, P., Krętowski, A., Błachnio-Zabielska, A. and Szamatowicz, J., 2022. Serum C18: 1-Cer as a potential biomarker for early detection of gestational diabetes. Journal of Clinical Medicine, 11(2), p.384.*
37. *Timur, B., Aldemir, O., İnan, N., Kaplanoğlu, İ. and Dilbaz, S., 2022. Clinical significance of serum and follicular fluid ceramide levels in women with low ovarian reserve. Turkish Journal of Obstetrics and Gynecology, 19(3), pp.207-214*
38. *Şimşir, C., Ecemiş, T., Erşahin, A.A., Güney, G., Coşkun, B., Çoşkun, B. and Kılıç, S.H., 2019. Serum AMH levels are not associated with adverse perinatal outcomes in women undergoing IVF treatment due to diminished ovarian reserve: Association between AMH and perinatal outcomes. Medical Science and Discovery, 6(9), pp.160-165.*
39. *Kamel, A., Halim, A.A., Shehata, M., AlFarra, S., El-Faissal, Y., Ramadan, W. and Hussein, A.M., 2018. Changes in serum prolactin level during intracytoplasmic sperm injection, and effect on clinical pregnancy rate: a prospective observational study. BMC Pregnancy and Childbirth, 18, pp.1-7.*
40. *Wigger, L., Cruciani-Guglielmacci, C., Nicolas, A., Denom, J., Fernandez, N., Fumeron, F., Marques-Vidal, P., Ktorza, A., Kramer, W., Schulte, A. and Le Stunff, H., 2017. Plasma dihydroceramides are diabetes susceptibility biomarker candidates in mice and humans. Cell Reports, 18(9), pp.2269-2279.*
41. *García-Ferreyra, J., Carpio, J., Zambrano, M., Valdivieso-Mejía, P. and Valdivieso-Rivera, P., 2021. Overweight and obesity significantly reduce pregnancy, implantation, and live birth rates in women undergoing in vitro fertilization procedures. JBRA assisted reproduction, 25(3), p.394.*
42. *Babayev, E. and Seli, E., 2015. Oocyte mitochondrial function and reproduction. Current Opinion in Obstetrics and Gynecology, 27(3), pp.175-181.*
43. *Hendriks, T., Warren, M.A., Schotanus-Dijkstra, M., Hassankhan, A., Graafsma, T., Bohlmeijer, E. and de Jong, J., 2019. How WEIRD are positive psychology interventions? A bibliometric analysis of randomized controlled trials on the science of well-being. The Journal of Positive Psychology, 14(4), pp.489-501.*
44. *Montani, Daniela Antunes, et al. "Understanding mechanisms of oocyte development by follicular fluid lipidomics." Journal of Assisted Reproduction and Genetics 36 (2019): 1003-1011.*

45. *Ginkel, C., Hartmann, D., vom Dorp, K., Zlomuzica, A., Farwanah, H., Eckhardt, M., Sandhoff, R., Degen, J., Rabionet, M., Dere, E. and Dörmann, P., 2012. Ablation of neuronal ceramide synthase 1 in mice decreases ganglioside levels and expression of myelin-associated glycoprotein in oligodendrocytes. Journal of Biological Chemistry, 287(50), pp.41888-41902.*