International Journal of Theoretical and Applied Research (IJTAR)
ISSN: 2812-5878

Homepage: https://ijtar.journals.ekb.eg

Original article

Relation Between Oxidative Stress in Follicular Fluid and The Outcome of Intracytoplasmic Sperm Injection

Marwa Mahmoud abdalah Neseem[1], Nehal Ali Mostafa Abu Elnaga[2], Eman Anwar Hassan[3], and Mohamed Sobhy Nasr[*,4]

[1]*Bachelor of Science, Department of Zoology/chemistry, Zagazig University, Egypt.*
[2]*Prof. of Embryology, Faculty of Science, Al-Azhar University (girl's branch), Cairo, Egypt.*
[3]*Ass. Prof. of Embryology, International Islamic Center for Population Studies and Research, Al-Azhar University, Cairo, Egypt.*
[4]*Assistant Professor of Histology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt*

ARTICLE INFO

Received 08/10/2024
Revised 15/03/2025
Accepted 11/12/2024

Keywords

ICSI
Obesity
PCOS
ROS

ABSTRACT

Intracytoplasmic sperm injection (ICSI) technique is used to treat some causes of infertility, such as ovulatory dysfunction, reduced ovarian reserve, male factor infertility, ovarian failure, and infertility that cannot be explained. When exposed to different detrimental stimuli, the body produces too many highly oxidative molecules, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). This condition is known as oxidative stress. An abundance of ROS or RNS causes tissue damage by destroying DNA, proteins, and lipids and interfering with redox signaling. Impaired embryonic development is caused by oxidative stress. Reactive oxygen species (ROS) can come from the embryo's metabolism or environment. ICSI clinical pregnancy rates range from 30% to 40%, depending on the reason for the low success rate and the diagnosis. On the other hand, specific ROS concentrations might be necessary for the spermatozoa and oocytes to interact. The follicular fluid (FF) environment around the oocytes may be crucial for fertilizing and developing the embryo. The oocyte's environment is metabolically active and comprises leukocytes, granulosa cells, growth factors, cytokines, and steroid hormones. It is unclear how oxidative stress in follicular fluid affects oocyte maturation, fertilization, and pregnancy. It has been observed that the generation of ROS in bovine oocytes enhances their capacity for development during in vitro maturation, leading to the production of embryos.

1. Introduction

Infertility is the inability to conceive after a year of regular unprotected sexual activity (without the use of any kind of contraception) and clinically as the inability to conceive [1]. These treatments help a lot of ladies who have been diagnosed as infertile and give them a chance to become parents. These medical treatments sometimes involve many challenges and potentially stressful factors, such as financial costs, waiting for results, hormone changes, laparoscopic surgery, daily injections, blood tests, and waiting [2]. With the advancement of ICSI technology, high-pregnancy female infertility can now be achieved with the use of human-assisted reproduction technology (ART) [3].

ROS are necessary at physiological levels for oocyte maturation, ovulation, fertilization, and physiological follicular atresia; nevertheless, an excess of ROS interferes with normal reproductive function [4]. Sulfhydryl groups found in thiols, an antioxidant class, serve as ROS's electron acceptors. Disulfide molecules are created when the oxidants are changed into less harmful compounds by the thiols' neutralizing effects. Antioxidant defenses depend on intracellular redox equilibrium, which is mostly dependent on the thiol/disulfide pools [5]. The ratio of thiol to disulfide is balanced. OS is characterized by a shift in the equilibrium in favor of oxidants. An OS indication is the thiol/disulfide equilibrium [6].

Granulosa cells secrete FF, which is made up of proteins, polysaccharides, ROS, antioxidants, and metabolic products that influence folliculogenesis [7]. Enzymatic antioxidant mechanisms are required to maintain the FF's ROS levels at a suitable level. Changes in the FF's microenvironment may negatively impact oocyte quality and maturation [9].

It has been shown that certain OS indicators are present in the follicular fluid (FF) and that their concentrations change in response to ovulation. Furthermore, a potential correlation has been suggested between the

* *Corresponding author*
E-mail address: mohamednasr2394.el@azhar.edu.eg
DOI:**10.21608/IJTAR.2025.322485.1087**

variance in FF OS biomarkers and the results following assisted reproductive technology (ART). It is unclear exactly how FF OS affects oocyte maturation, fertilization, and pregnancy. ROS levels are typically measured to assess OS. By calculating ROS in the FF of patients receiving ICSI, OS was assessed. We looked at each ROS's capacity to predict pregnancy following ICSI in this study [8].

With a prevalence of 5–20% worldwide, polycystic ovarian syndrome (PCOS) is the most common endocrine disorder among reproductive-age women. Furthermore, it is thought to be the primary cause of infertility caused by anovulation. When alternative methods of ovulation induction fail, PCOS women typically add In-vitro Fertilization/Intra-Cytoplasmic Sperm Injection (IVF/ICSI) technologies to their plan therapy [9].

Since obesity is a modifiable risk factor, rising obesity rates have led to a rise in the need for ART as well as important ethical and therapeutic problems. Women who are obese or overweight typically require higher dosages of gonadotrophins to induce ovulation because they respond poorly to clomiphene stimulation. Fewer follicles are available for harvest as a result of ovarian stimulation. A fat environment has an impact on the preimplantation embryo as well. It has been demonstrated that comparing human IVF cycles with obese women increases the likelihood of producing low-quality embryos [10].

The goal of the current study is to evaluate the connection between the quality of the embryos produced during each cycle of IVF/ICSI and the ROS levels in women's follicular fluid during these operations.

2. Patients and Methods

150 couples who were referred for assisted reproduction between March 2021 and September 2022 at Al-Azhar University in Cairo, Egypt's International Islamic Centre for Population Studies and Research (IICPSR)-ART unit made up the study population. The International Islamic Centre for Population Studies and Research (IICPSR) Ethics Committee of Al-Azhar University in Cairo, Egypt, gave its approval to the study. Every participant in the study provided written informed permission, and the data were handled and processed in a private, anonymous manner. To identify the eligible individuals, a screening process was conducted on all patients who underwent ovarian stimulation using the lengthy procedure and were recommended for ICSI. The individuals that were recruited were categorized as follows: group 1 (control) and 50 cases of a normal female companion. Group 2: Partners of obese women (BMI < 35 kg/m 2; 50 instances). Group 3: 50 instances of PCO female partners.

The diagnosis of PCOS was predicated on the existence of at least two of the following three criteria, as stated by the Rotterdam Consensus Criteria: polycystic ovaries on ultrasonography, hyperandrogenemia, and oligomenorrhea/amenorrhea [11]. Every patient's medical history was obtained, including the diagnosis, length of infertility, number of prior pregnancies, related illnesses, and treatments, along with a preliminary study of the husband's semen. Every patient had a transvaginal ultrasound, hysterosalpingogram, sonohysterogram, or hysteroscopy to assess the condition of their uterus, ovaries, and tubes. [12].

Venous blood was drawn from the patients for assays of (FSH, LH, PRL, TSH, and E2), liver and kidney functions (AST, ALT, and Albumin), hemoglobin level, RBC, WBC, and platelet count were all performed [13].

On day 21 of the previous cycle, all individuals underwent the GnRH long protocol, which included a depot injection of therapeutic dosage triptorelin (Decapeptyl 3.75 mg); 14 days later, the down-regulation status was evaluated. When blood E2 was less than 50 ng/ml and progesterone was less than 0.5 ng/ml, or when endometrial thickness reached 7 mm, the patient was deemed to be responding. After confirmation of down-regulation, gonadotropin was given daily at a dosage between 75 and 300 IU/ml, with adjustments made based on ovarian volume, ovarian activity, and the presence or absence of polycystic ovary syndrome (PCOS). Age, ovarian reserve, baseline blood FSH concentrations on days two or three of menstruation, and prior responsiveness to ovarian stimulation all depend on the patient. Every two to three days, vaginal ultrasonography was used to monitor the ovarian response. HCG (10,000 IU) administered intraperitoneally (IM) caused ovulation in regular responders when a minimum of four follicles measured 18 mm. [14].

Thirty-six hours after the hCG injection, oocytes coated with cumulus masses were collected using a falcon tissue culture dish and four well embryo culture dishes for oocyte retrieval utilizing a digital needle guide. Using an Olympus stereo microscope, the oocyte was discovered in a horizontal laminar flow hood; it was visible. ROS detection and preparation of follicular fluid [15].

Cumulus oophorus oocytes were denudated by subjecting them to 80 IU/ml of hyaluronidase enzyme in the HEPES-buffered solution. The corona radiata was then mechanically removed. Under an inverted microscope, the maturation and morphology of the oocytes were evaluated. About 16–18 hours after ICSI, the integrity and fertilization of the injected oocytes were assessed. When analyzing the existence of two pronuclei (2PN) and the extrusion of the second PB, oocytes were deemed to be properly fertilized [13].

After ICSI, the fertilization rate was often represented as a function of the number of injected oocytes. Ninety percent of 2-PN oocytes produced with ICSI undergo post-fertilization cleavage, giving rise to multicellular embryos. Following 48 hours, cleaved embryos to the two or four-cell stage, and 2-3 high-quality embryos per patient were inserted into the uterus. To determine if they were pregnant, patients took a serum beta HCG titer 15 ± 2 days following oocyte harvest [13].

Statistical Analysis

A statistical analysis software application (SAS, 2002) was used to examine the data. The parameters under investigation were compared in each group under study

before and after therapy using the Paired T-test and Mc Namar's test.

3. Results

As shown in Table 1 and Fig. 1, the outcomes of semen analysis of case pairs. In the thesis study, we chose mal normal so all semen parameters are in the normal range in all groups. The PCO group's mean was 42.1 ± 2.1 % sperm motility and 96.1 ± 1.0 % abnormal sperm morphology. No significant modifications (P>0.05) were observed as compared to the control group (40.3 ± 3.1% and 96.7 ± 1.1%, respectively). There was a lack of statistical significance (P>0.05) in sperm count (106/suspension) or progressive motility among these groups. However, the average percentage of abnormal sperm morphology in the obese group was 96.0 ± 1.0%, which did not exhibit a significant drop (P > 0.05) compared to the control group (96.7 ± 1.1%).

This study selected cases that did not suffer from any diseases, so all the data in the aggregate were at the expected level, like the control group. The analysis of complete blood count (CBC) in obesity and PCOS groups is shown in Table 2, Figs.2 and 3. Whereas in the PCOS and obesity groups, the mean hemoglobin values were 11.12 ±0.40 gm/dL and 11.15 ± 0.41gm/dL, respectively, and RBCs were 4.81 ± 0.40 106/µL and 4.80 ± 0.41 106/µL respectively with no significant difference (P>0.05) compared to the control group (11.13 ±0.42 gm/dL and 4.82 ± 0.41 106/µL respectively). While WBCs were 7.22 ± 1.10 103/µL and 7.23 ± 1.10 103/µL, respectively, and the platelets count (PLTs) were 300.2 ± 40.0 103/µL and 304.2 ± 40.0 103/µL respectively with no significant difference (P>0.05) compared to the control group (7.23 ± 1.11 103/µL and 302.2 ± 40.2 103/µL respectively).

Table 1: the comparison between the numerical semen assessment of case pairs for the control, PCO and obesity groups

Semen assessment	Control (n=50)	PCO (n=50)	Obesity (n=50)	P-value	
				Control &PCO	Control &Obesity
Sperm count (10^6/suspension)	21.4 ± 3.2	21.3 ± 3.5	21.4 ± 3.1	0.8818	1.0000
Sperm motility/ml (%)	40.3 ± 3.1	42.1 ± 2.1	41.1 ± 2.3	0.1000	0.1460
Progressive motility/ml (%)	19.8 ± 3.3	20.8 ± 3.2	20.1 ± 3.0	0.1272	0.6354
Abnormal sperm morphology (%)	96.7 ± 1.1	96.1 ± 1.0	96.0 ± 1.0	0.1000	0.12

Data recorded as mean± S.D; *P value<0.05 means significant; **P value<0.01 means highly significant. PCOS: polycystic ovary syndrome group.

Table 2: comparison between the complete blood count (CBC) in the control, obesity and PCOS groups

CBC	Control (n=50)	PCO (n=50)	Obesity (n=50)	P-value	
				Control &PCO	Control &Obesity
Hemoglobin (gm/dL)	11.13 ±0.42	11.12 ±0.40	11.15 ± 0.41	0.9032	0.8101
RBCs (10^6/µL)	4.82 ± 0.41	4.81 ± 0.40	4.80 ± 0.41	0.9020	0.8078
WBCS (10^3/µL)	7.23 ± 1.11	7.22 ± 1.10	7.23 ± 1.10	0.9640	1.0000
PLTs (10^3/µL)	302.2 ± 40.2	300.2 ± 40.0	304.2 ± 40.0	0.8036	0.8036

Data recorded as mean± S.D; *P value<0.05 means significant; **P value<0.01 means highly significant. PCOS: polycystic ovary syndrome group. **CBC**: complete blood count; **WBCs**: white blood cells; **RBCs**: red blood cells; **PLT**: platelet count.

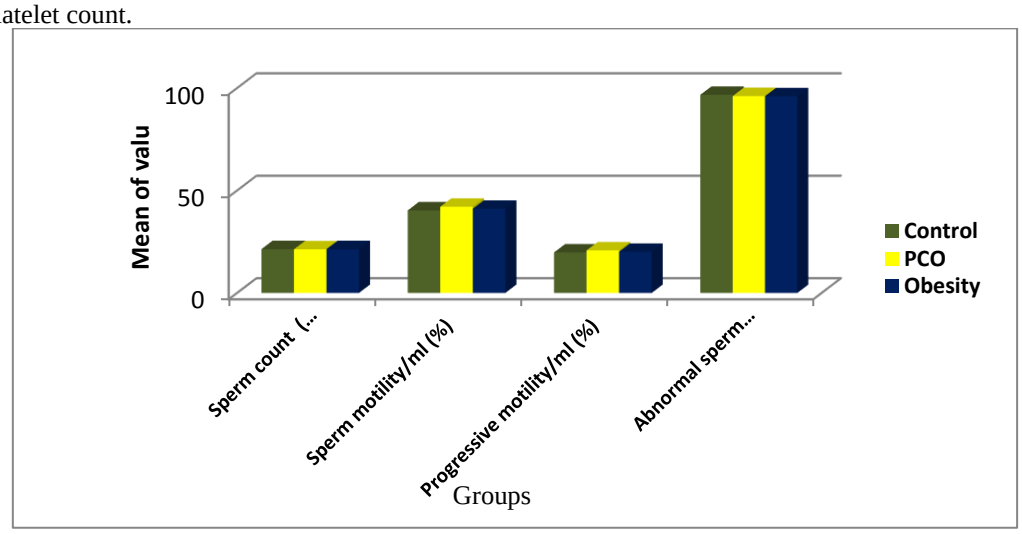

Fig.1: showing the comparison between the numerical semen assessment of case pairs for the control, PCO and obesity groups

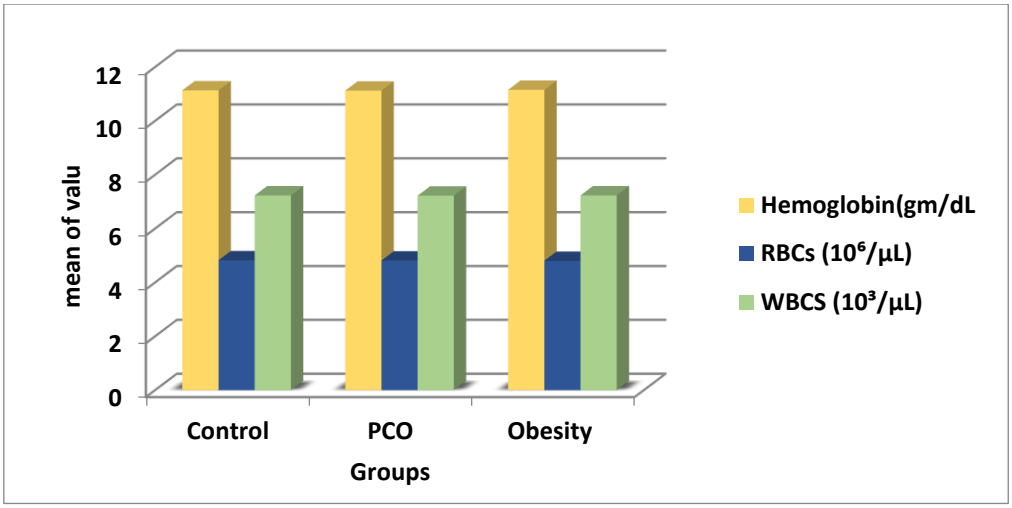

Fig.2: comparison between the control, PCOS and obesity groups

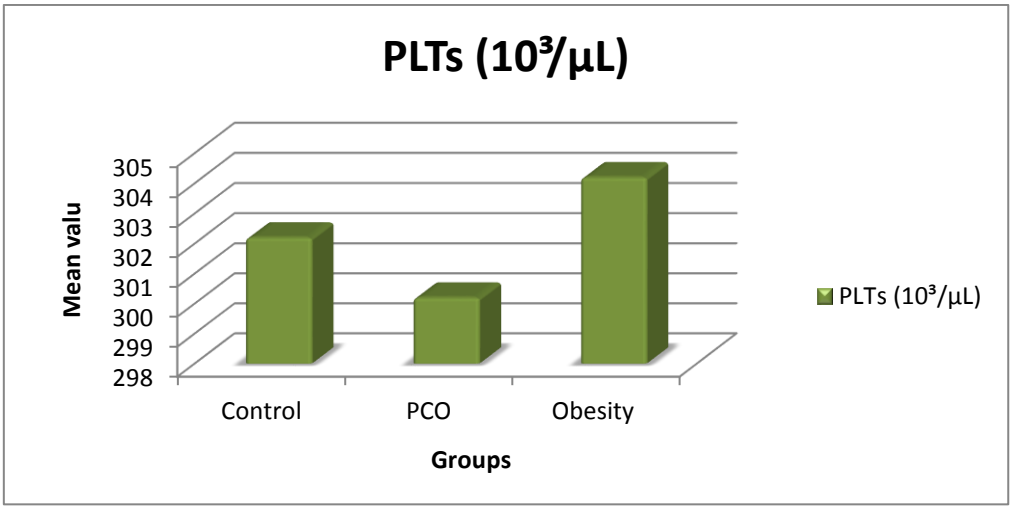

Fig.3: the platelet count. (PLTs) test in the control, PCOS, and obesity groups

Table 3, Figs.4, and 5 record the common kidney function. There were no significant differences (P >0.05) in the mean of the creatinine test between the PCOS group (0.93 ± 0.02 mg/dL), and obesity group (0.97 ± 0.03 mg/dL) than the control group (17.8±4.5 mg/dL) but in normal rang. The analysis of common liver function in PCOS and obesity groups is shown in Table 4 and Fig 6. In contrast, in the PCOS and obesity groups, the mean of the ALT test was 12.11 ± 5.30 U/L and 12.16 ± 5.33 U/L, respectively. The AST test was 14.72 ± 3.80 U/L and 14.70 ± 3.79 U/L, respectively, exhibiting no substantial change in comparison to the control group (12.12 ± 5.20 U/L and 14.70 ± 3.84 U/L, respectively). While albumin levels were 4.30 ± 0.83 U/L and 4.30 ± 0.83 U/L, respectively, showing no major change in comparison to the control group (4.32 ± 0.84 U/L)

Table 3: comparison between the common kidney function in the control, obesity and PCOS groups

Common kidney function	Control (n=50)	PCO (n=50)	Obesity (n=50)	P-value	
				Control &PCO	Control &Obesity
Urea (mg/dL)	25.4 ± 1.23	25.0 ± 1.20	25.4 ± 0.99	0.1030	1.0000
Creatinine (mg/dL)	0.91 ± 0.03	0.93 ± 0.02	0.97 ± 0.03	0.2000	0.1000

Data recorded as mean± S.D; *P value<0.05 means significant; **P value<0.01 means highly significant. PCOS: polycystic ovary syndrome group

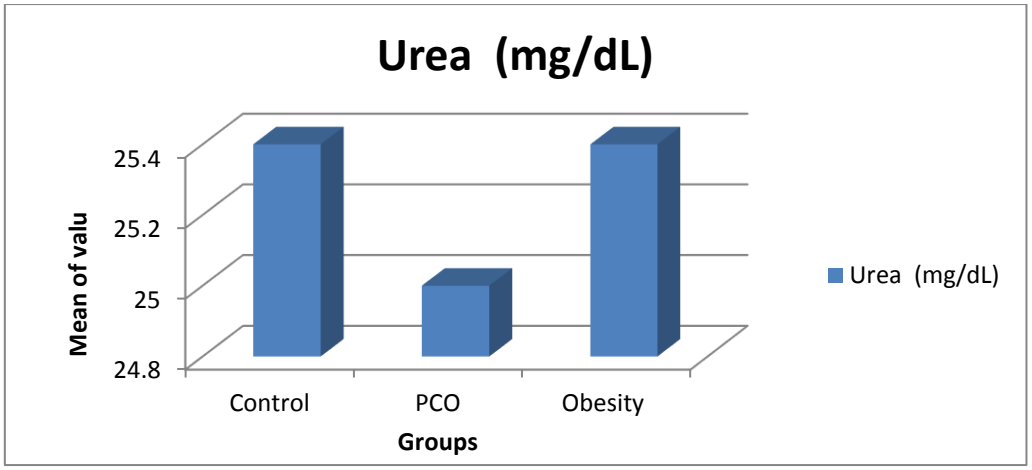

Fig.4: the urea test in the control, PCOS and obesity groups

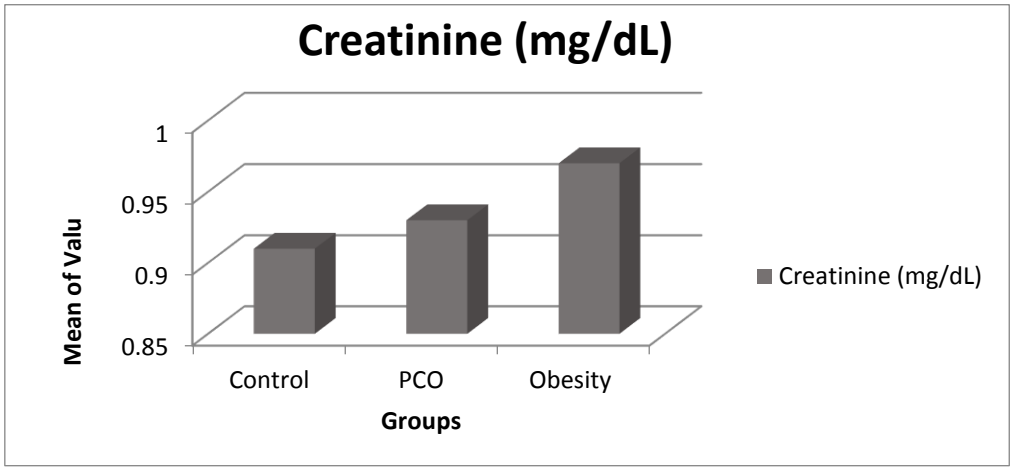

Fig.5: the creatinine test in the control, PCOS and obesity groups

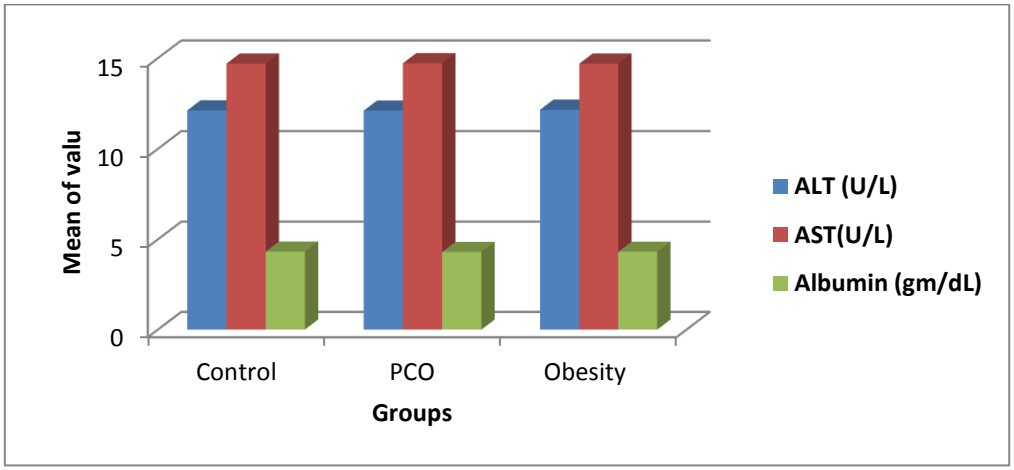

Fig.6: the common live function test in the control, PCOS and obesity groups

Table 4: comparison between the control, and PCOS obesity groups in the common live function test

Common liver function	Control (n=50)	PCO (n=50)	Obesity (n=50)	P-value	
				Control &PCO	Control &Obesity
ALT (U/L)	12.12 ± 5.20	12.11 ± 5.30	12.16 ± 5.33	0.9924	0.9697
AST(U/L)	14.70 ± 3.84	14.72 ± 3.80	14.70 ± 3.79	0.9792	1.0000
Albumin (gm/dL)	4.32 ± 0.84	4.30 ± 0.83	4.31 ± 0.73	0.9049	0.9495

Data recorded as mean± S.D; *P value<0.05 means significant; **P value<0.01 means highly significant.
PCOS: polycystic ovary syndrome group; **AST**: aspartate aminotransferase; **ALT**: alanine aminotransferase.

The mean BMI in PCOS and obesity groups were 23.7 ± 3.1 and 31.7 ± 3.2 kg/m2, which showed a significant rise (P<0.01) versus the control group (21.7 ± 3.3 kg/m2). In PCOS and obesity groups, the mean age was 33.0 ± 1.0 and 32.0 ± 1.9 years, respectively, which was insignificant compared to the control group (31.0 ± 1.9 years). There was no statistically significant difference in these groups' mean duration of infertility (table 5 and Fig. 7).

The basal hormonal profile in PCOS, obesity, and control groups was measured in the present work in Table 6 and Figure. 8. The mean basal FSH in obesity and PCOS groups were 5.2±0.9 and 5.8 ±1.1 IU/L, respectively, which recorded high significant differences (P<0.01) compared to the value of the control group (5.9±0.6 IU/L). The mean basal PRL in obesity and PCOS were 31.9 ± 2.3 and 23.9 ± 9.9 µg/L, respectively, which recorded a highly significant increase (P<0.01) compared to the value of the control group was 20.9 ± 9.6 µg/L.

On the other hand, the mean basal LH in PCOS and obesity groups were 5.2±0.9 and 5.8 ±1.1 IU/L, respectively, Significant increases (P<0.01) were seen compared to the control group (5.9±0.6 IU/L). The mean basal E2 and the importance of TSH in the PCOS and obesity were 39.0 ±10.2 pg/ml, 3.1 ± 1.20 IU/L, 44.9 ±12.3 pg/ml, and 3.1 ± 1.22 IU/L no significant (P>0.05) difference from the control group.

Table 5: comparison between the control, obesity and PCOS groups as regards the general observations of Female characters

Female characters	Control (n=50)	PCO (n=50)	Obesity (n=50)	P-value	
				Control &PCO	Control &Obesity
Age (year)	31.0 ± 1.9	33.0 ± 1.0	32.0 ± 1.9	0. 1000	0. 8100
BMI (kg/m^2)	21.7 ± 3.3	23.7 ± 3.1**	31.7 ± 3.2**	0.0024	< 0.0001
Duration of infertility (year)	5.1 ± 3.3	6.1 ± 3.2	6.2 ± 3.0	0.1272	0.0843

Data recorded as mean± S.D; *P value<0.05 means significant; **P value<0.01 means highly significant. PCOS: polycystic ovary syndrome group and **BMI**: body mass index.

Table 6: The basal hormonal profile in obesity, PCOS and control groups

Basal hormones levels	Control (n=50)	PCO (n=50)	Obesity (n=50)	P-value	
				Control &PCO	Control &Obesity
FSH (IU/L)	5.1 ± 1.6	4.9 ± 1.5	5.9 ± 1.2**	0.5205	0.0057
LH (IU/L)	4.1 ± 1.3	5.3 ± 2.3**	5.1 ± 2.2**	0.0018	0.0068
PRL (µg/L)	20.9 ± 9.6	23.9 ± 9.9	31.9 ± 2.3**	0.1272	< 0.0001
E2 (pg/ml)	39.3 ± 16.3	39.0 ±10.2	44.9 ±12.3	0.9124	0.0554
TSH (mIU/L)	3.1 ± 1.21	3.1 ± 1.20	3.1 ± 1.22	1.0000	1.0000

Data recorded as mean± S.D; *P value<0.05 means significant; **P value<0.01 means highly significant. PCOS: polycystic ovary syndrome group **LH**: luteinizing hormone; **FSH**: follicle stimulating hormone; **E2**: estradiol hormone; **PRL**: prolactin hormone; **TSH**: thyroid–stimulating hormone

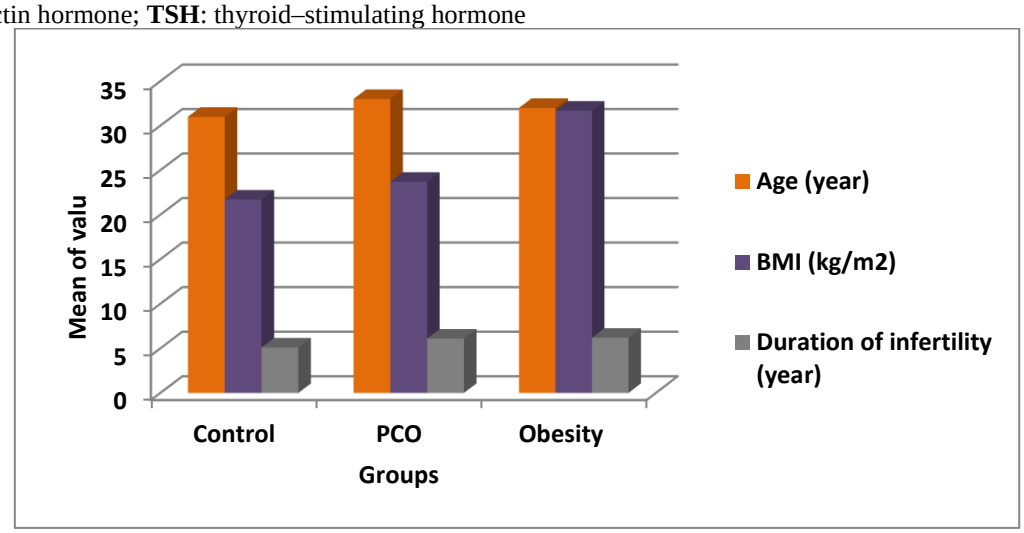

Fig.7: the general observations of female characters in the control, PCOS and obesity groups

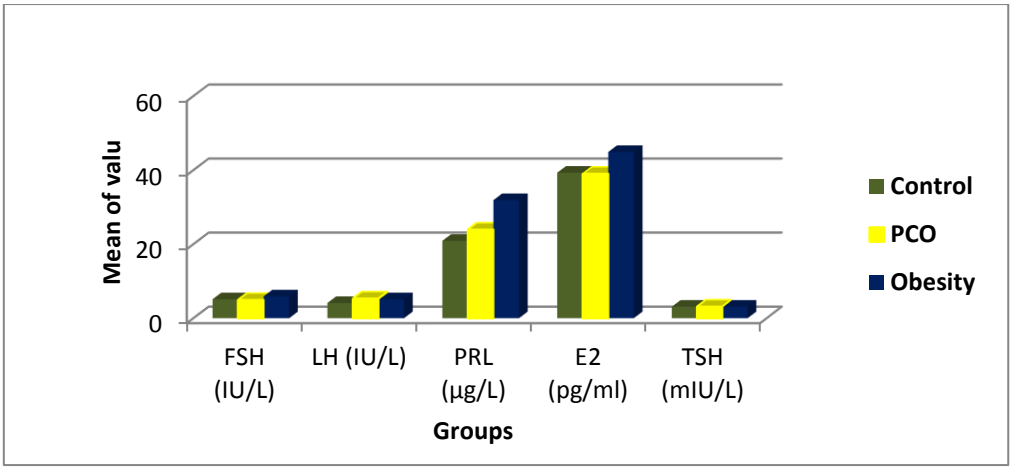

Fig 8: The basal hormonal profile in the PCOS, obesity and control groups

Table 7: out com of ICSI procedure in all groups

ICSI outcome	Control (n=50)	PCO (n=50)	Obesity (n=50)	P-value	
				Control &PCO	Control &Obesity
Oocyte collected	8.4 ± 2.9	12.4 ± 2.1**	9.4 ± 1.9*	< 0.0001	0.0441
Mature oocyte (MII)	6.9 ± 2.2	8.9 ± 1.2**	6.1 ± 2.0**	< 0.0001	0.0600
Fertilization rate (%)	79.7 ± 4.1	71.1 ± 4.9**	69.6 ± 3.1**	< 0.0001	< 0.0001
Cleavage rate (%)	88.9 ± 3.1	78.2 ± 3.6**	80.9 ± 2.9**	< 0.0001	< 0.0001
Top quality embryos	78.1 ± 2.1	68.1 ± 3.1**	65.2 ± 2.9**	< 0.0001	< 0.0001
Sub quality embryos	21.8 ± 3.1	31.6 ± 2.1**	34.8 ± 2.8**	< 0.0001	< 0.0001
No of embryos transferred	1.8 ± 0.7	2.2 ± 0.6**	2.8 ± 0.3**	0.0028	< 0.0001

Data recorded as mean± S.D; *P value<0.05 means significant; **P value<0.01 means highly significant. PCOS: polycystic ovary syndrome group and **MII**: oocytes progressed to metaphase II.

The information in Table 7 and Figure 9 showed how the ICSI process went for all three groups. The PCOS group had 12.4% more oocytes collected than the control group, and the fat group had 9.4% more eggs collected than the control group. Also, the number of mature oocytes (MII) dropped in the obese group compared to the control group, going from 6.9 ± 2.2% to 6.1 ± 2.0%. The number of developed eggs rose to 8.9 ± 1.2% in the PCOS group.

The percentage of mature eggs that were fertilized was 71.1% ± 4.9% in the PCOS group and 69.6 ± 3.1% in the fat group. This was significantly lower (P<0.01) than the control group 79.7 ± 4.1%. The zygotes were successfully cut in the PCOS and fat groups (78.2 ± 3.6% and 80.9 ± 2.9%, respectively) after fertilization. This was significantly less than the control group (88.9 ± 3.1%) (P<0.01).

The number of high-quality embryos in mothers with PCOS and obesity was 68.1% ± 3.1% and 65.2% ± 2.9%, respectively. This was significantly lower (P<0.01) than the control group, which had 78.1 ± 2.1%. In the PCOS and obesity groups, there were significantly more embryos that were not of good quality (31.6 ± 2.1 and 34.8 ± 2.871.2%, respectively) than in the normal groups (21.8 ± 3.1). Figs 10 and 11 show the picture of the types of embryos in the ART unit at Al-Azhar University

The results demonstrated in Table 8 and illustrated in Fig. 12 showed OS in FF of the PCOS and obesity than the control groups. The mean values of oxidative stress were in the PCOS group and the obesity group, 242.15 ± 34.4 nmol/mL and 340.05 ± 14.4 nmol/mL, respectively, which recorded a significant rise (P<0.01) relative to the control group 184.14 ± 16.22 nmol/mL.

Table 8: detection of ROS in FF preparation for the PCOS, Obesity, and control groups

Oxidative stress	Control (n=50)	PCO (n=50)	Obesity (n=50)	P-value	
				Control &PCO	Control &Obesity
ROS (nmol/mL)	184.14 ± 16.22	242.15 ± 34.4**	340.05 ± 14.4 **	P < 0.0001	P < 0.0001

Data recorded as mean± S.D; *P value<0.05 means significant; **P value<0.01 means highly significant. PCOS: polycystic ovary syndrome group and **ROS:** reactive oxygen species.

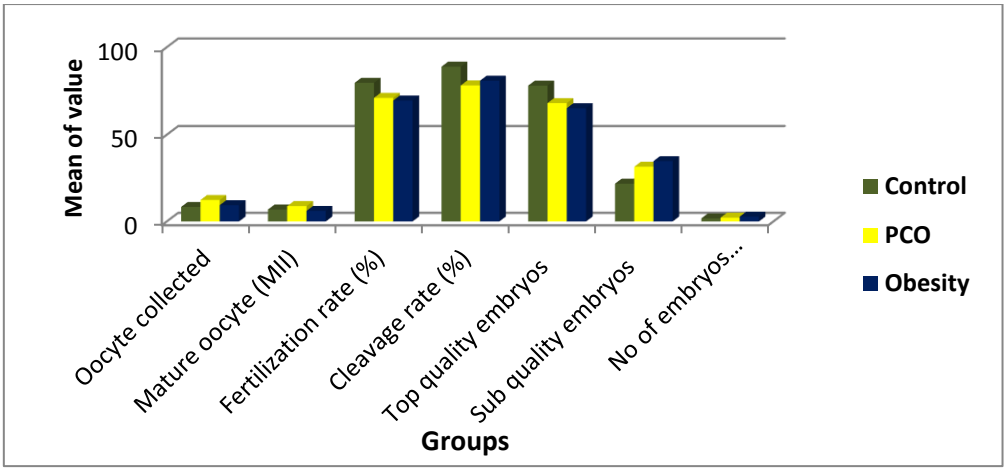

Fig.9: out com of ICSI procedure in the all groups

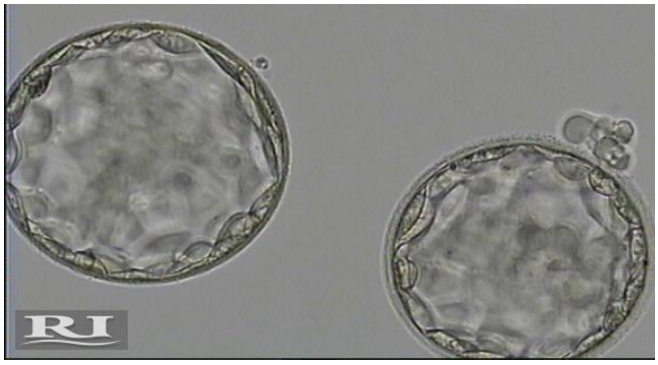

Fig.10: top quality embryo

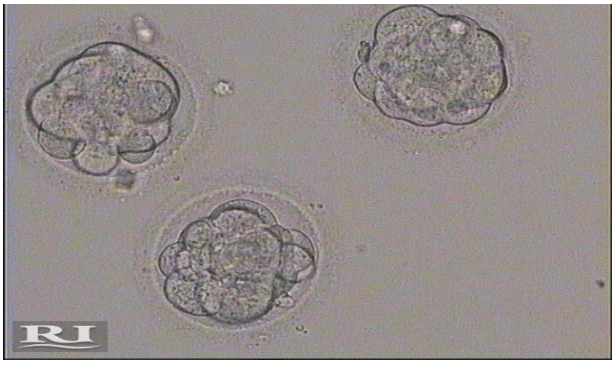

Fig.11: sub quality embryos

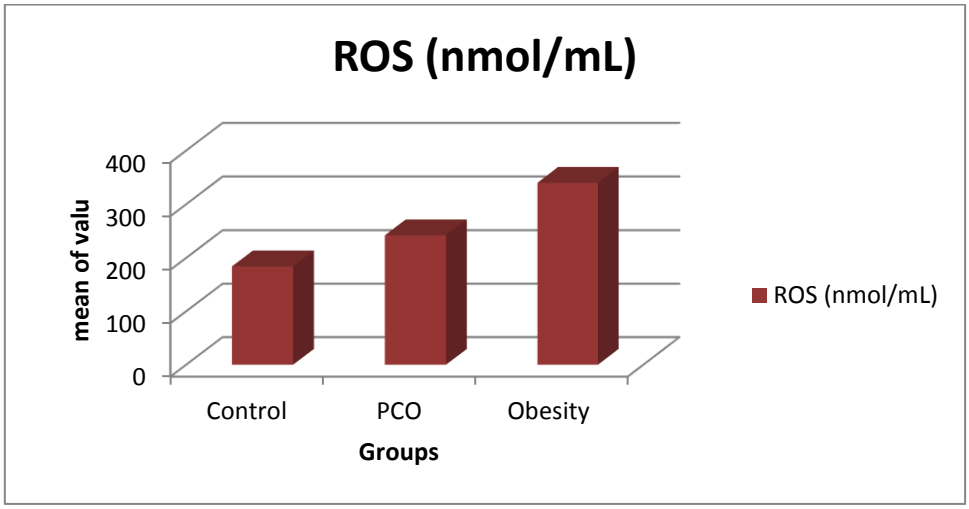

Fig12: reactive oxygen species in follicular fluid preparation for the PCOS, Obesity, and control groups

4. Discussion

Oxidative stress impacts both local and systemic levels. Follicular fluid provides a localized, direct milieu for oocyte development, while serum serves only a limited, indirect role. Numerous recent researches have been undertaken on PCOS and OS. Their primary focus was on serum [8]. The present study indicates that heightened oxidative stress response in follicular fluid correlates with female obesity and PCOS in ICSI patients. Healthy males and females without any medical issues were chosen for this study. Consequently, akin to the control group, all aggregate data were at the anticipated level. These findings align with the 2019 research conducted by Abu-Elnaga et al. [13].

The study revealed substantial variations ($P<0.01$) in the mean basal FSH levels across the PCOS, obese, and control groups. In contrast to the control group, the mean basal PRL in obesity and PCOS exhibited a statis-

tically significant increase (P<0.01). In contrast, the groups with PCOS and obesity had significantly elevated mean baseline LH levels (P<0.01) compared to the control group. Obese women with polycystic ovarian syndrome have metabolic anomalies in androgen and estrogen levels. A study indicates that dysfunctional hypothalamic-pituitary-ovarian (HPO) axis activity may be the cause of PCOS. LH has been found to activate theca interna cells to synthesize androgens in mammalian ovaries. It has been determined that granulosa cells activate aromatase in response to FSH. The bicellular gonadotropin theory of estrogen synthesis is founded on the coordinated activity of these two cell types and pituitary hormones [16]. LH hypersecretion, both at baseline and in response to GnRH, is a hallmark of PCOS. Thus, elevated androgen synthesis may ensue from LH hypersecretion. The primary aberrations in conventional PCOS occur. In people with PCOS, LH/GnRH pulses result in hyperandrogenism and inadequate follicular maturation. Consequently, it becomes unresponsive to the inhibition of hypothalamic GnRH production by estrogen and progesterone. Premenarchal irregularities in females with hyperandrogenemia Polycystic ovary syndrome (PCOS) may serve as a possible mechanism due to this insensitivity [17]. The cellular antioxidant defense system is very efficient and primarily comprises enzymes such as glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD). Under physiological circumstances, it can modulate the amounts of reactive oxygen species (ROS) and reactivate those generated by cellular metabolism. [18]. The present study revealed that oocytes retrieved from obese female patients with PCOS adversely affected ICSI outcomes. Elevated concentrations of follicular fluid C-reactive protein, an inflammatory marker often associated with oxidative stress, have been correlated with obesity and polycystic ovary syndrome in women. Additionally, a high-fat diet was associated with a reduced blastocyst formation rate and glutathione depletion in pre-ovulatory oocytes and zygotes of female mice, along with an elevation in ROS levels within the cumulus-oocyte complex [19].

We established that it is difficult to ascertain the impact of OS on oocytes, as they partially rectify DNA damage. Consequently, one approach to identify this damage may include monitoring the reactive oxygen species in follicular fluid. Furthermore, it has been observed that the stage of oocyte development influences oocyte defense against oxidative stress. Consequently, OS may be essential during some stages of oocyte maturation in the surrounding milieu [20]. In follicular fluid (FF), increased reactive oxygen species (ROS) activity might adversely affect embryonic development. An imbalance between reactive oxygen species and antioxidants in the oocyte microenvironment might adversely affect the results of assisted reproductive technologies [21]. In contrast, the PCOS and obese groups exhibited a markedly significant reduction in the proportion of fertilized mature oocytes relative to the control group. In obese and PCOS women undergoing IVF and embryo transfer, OS-mediated alterations in granulosa cells adversely affect fertilization, embryo quality, and pregnancy rates [22].

These findings showed that parents with PCOS and obesity had significantly fewer high-quality embryos than the control group. Compared to the control group, PCOS and obese groups had significantly higher mean oxidative stress values (P<0.01). Our findings are similar to an earlier study that demonstrated higher serum and follicular fluid oxidative stress in obese and PCOS individuals [23].

Melatonin was lower in FF in PCOS subjects, although it was unclear how this related to the quality of the embryo. Furthermore, the quality of the embryo was similarly connected to the copy number of mitochondrial DNA in Cumulus granulosa cells [24]. It's also possible that some cumulus cell genes will serve as biomarkers for the quality of embryos. However, this necessitated the concurrent testing of several genes and conjoint analysis. Furthermore, although the cost of detection is somewhat expensive, metabolomics and proteomics have also been applied to FF or embryo culture media. OS markers in FF are powerful additions to the existing predictors of embryo quality since they are simple to test and have an outstanding ability to predict embryo quality [25].

5. Conclusion

It is possible to conclude that, in comparison to the control group, the PCOS and obese groups had much lower ICSI outcomes. In comparison to the control group, the follicular fluid of the women with PCOS and obesity showed a higher value of oxidative stress.

Acknowledgments

Gratitude is extended to all staff members and patients of the Assisted Reproductive Unit of the IICPSR, Al-Azhar University.

Funding

No Funding**.**

Reference

1. G. Demirel, F. Taskin, A.Yenicesu, *The relation between religious copings, adjustment to fertility, being affected by infertility, and satisfaction with life in Turkish women with infertility*. J. of Rel. and Heal.60(6) (2021) 4264-4277. https://doi.org/10.1007/s10943-021-01319-1
2. T. Wischmann, H. Scherg, T. Strowitzki, R. Verres,: *Psychosocial characteristics of women and men attending infertility counselling.* Hum. Rep., 24, (2019) 378-385. doi: 10.1093/humrep/den401
3. H. Hanevik, D. Hessen,. IVF and human evolution. *Hum. Rep. 28*(4) (2022)457-479. https://doi.org/10.1093/humupd/dmac014
4. M. Jamil, H. Debbarh, S. Aboulmaouahib, Reactive oxygen species in reproduction: harmful, essential or both?. Zygote. 28(2020):255-269. doi: 10.1017/S0967199420000179
5. Ö. Erel, S. Erdoğan, Thiol-disulfide homeostasis: an integrated approach with biochemical and clinical

aspects. Turk. J. Med. Sci. 50(2020):1728-1738. doi: 10.3906/sag-2003-64

6. U. Micoogullari, M. Cakici, F. Kilic, *Evaluation of the role of thiol / disulfide homeostasis in the etiology of idiopathic male infertility with a novel and automated assay*. Syst. Biol. Rep. Med. 68(2022):162-168. doi: 10.1080/19396368.2021.2003481
7. D. Yuan, J. Luo, Y. Sun, L. Hao, J. Zheng, Z. Yang, *PCOS follicular fluid derived exosomal miR-424-5p induces granulosa cells senescence by targeting CDCA4 expression.* Cell Sig. 85(2021):110030. doi: 10.1016/j.cellsig.2021.110030
8. A. Brinca, A. Ramalhinho, Â. Sousa, Oliani, A. Breitenfeld, L., Passarinha, L. E. Gallardo, Follicular fluid: a powerful tool for the understanding and diagnosis of polycystic ovary syndrome. *Bio.*, *10*(6) (2022) 1254. https://doi.org/10.3390/biomedicines10061254
9. A. Moini, T. Rezaee, A. Aleyasin, A. Arabipoor, M. Moayed, The effect of metabolic syndrome on controlled ovarian stimulation outcome in infertile women with polycystic ovary syndrome undergoing assisted reproductive technology cycles. Arch. of Endo. and Meta. 27 (2022) 1-12. DOI: 10.20945/2359-3997000000518
10. S. Zia, *Obesity: Impact and Outcome on Infertility—A Literature Review*. O J. of Obs. and Gyn.13(2) (2023) 214-240. DOI: 10.4236/ojog.2023.132023
11. H. Dema, A. Videtič Paska, K. Kouter, M. Katrašnik, M. Jensterle, A. Janež, J. Bon, *Effects of Mindfulness-Based Therapy on Clinical Symptoms and DNA Methylation in Patients with Polycystic Ovary Syndrome and High Metabolic Risk*. Mol. Bio.45 (4) (2023) 2717-2737. https://doi.org/10.3390/cimb45040178
12. H. Toufig, T. Benameur, M. Twfieg, H. Omer, T. El-Musharaf, *Evaluation of hysterosalpingographic findings among patients presenting with infertility*. Sau. J. of Bio. Sci., 27(11) (2020) 2876-2882. https://doi.org/10.1016/j.sjbs.2020.08.041
13. N. Abu-Elnaga, A. Abdel Ghany, H. Youssef, S. Mahmoud, A. Salim, *Impact of oocyte morphology on fertilization, embryo quality and pregnancy after ICSI: Evaluation with Diabetes and PCOS*. *Arab J.of Nuc. Sci. and App. 52*(4) (2019) 110-118. DOI: 10.21608/ajnsa.2019.10225.1184
14. H. Wu, F. Wei, W. Tan, M. Dong, Y. Tan, X. Zhang, L. Liu, *Administration of depot GnRH agonist prior to programmed frozen-thawed embryo transfer does not improve the live birth rate in ovulatory women: A large, multi-center retrospective study*. *Med.*, *101*(40) (2022)30991. *DOI:* 10.1097/MD.0000000000030991
15. H. Guo, Q. Zhu, H. Gao, Q. Lyu, W. Chai, L. Wu, B. Li,. *Metabolomics analysis of follicular fluid in ovarian endometriosis women receiving progestin-primed ovary stimulation protocol for in vitro fertilization*. *Sci. Rep.*, *13*(1) (2023), 5747. https://doi.org/10.1038/s41598-023-32797-w
16. K. Uçkan, H. Demir, K. Turan, E. Sarıkaya, C. Demir, *Role of oxidative stress in obese and nonobese PCOS patients*. Int. J. of Cl. Pr.,9 (2022). https://doi.org/10.1155/2022/4579831
17. S. Oberfield, *005 New insights into polycystic ovary syndrome (PCOS) in adolescents*. Eur. J. of Obs. and Gy. and Rep. Bio., 273, (2022) 3. DOI:https://doi.org/10.1016/j.ejogrb.2022.02.034
18. J. Gaspar, *Is polycystic ovary syndrome a hypothalamic disease?*. *E. Bio. Med.*, 92 (2023). DOI:https://doi.org/10.1016/j.ebiom.2023.104598
19. N., Forghani, Z., Karimi, M., Mokhtari, M., Shariati, F.Masjedi, *Association of oxidative stress with kidney injury in a hyperandrogenemic female rat model. Ira. J.of Med. Sc.* 28 (2)(2023)5-15. https://doi.org/10.30476/ijms.2022.93594.2497
20. S. Karabulut, O. Korkmaz, P. Kutlu, H. Gozel, I. Keskin, *Effects of follicular fluid oxidative status on human mural granulosa cells, oocyte competency and ICSI parameters*. Eur. J. of Ob. Gy. and Rep. Bio, 252(2020), 127-136. https://doi.org/10.1016/j.ejogrb.2020.06.021
21. R., Rodríguez-Díaz, R., Blanes-Zamora, S., Paz-Montelongo, J., Gómez-Rodríguez, S. Fiestas, D. González-Weller, E. González-Dávila,. *The influence of follicular fluid metals on assisted reproduction outcome*. *Bio. Trace El. Re.*, (2023)1-14. https://doi.org/10.1007/s12011-023-03578-3
22. L. Wu, Q. Fang, M. Wang, Y. Wang, X. Zhu, Z. Fang, X. Tong, *Effect of weight loss on pregnancy outcomes, neuronal-reproductive-metabolic hormones and gene expression profiles in granulosa cells in obese infertile PCOS patients undergoing IVF-ET*. *Fro. End. 13*(2022). doi: 10.3389/fendo.2022.954428
23. I. Zec, M. Goldštajn, K. Kuna, M. Mikuš, G. Stabile, B. Bianco, A. Laganà, *Oxidative homeostasis in follicular fluid and reproductive outcomes–from bench to bedside*. *Men. Re. Prz. Men.*, *21*(4)(2022) 276-284. DOI: https://doi.org/10.5114/pm.2022.124019
24. C. Butts, *Toxic Trace Elements in Follicular Fluid, Oxidative Stress, and In Vitro Fertilization (IVF) Outcomes*. St. Un. of Ne.Yo.at Alb. (2020). https://www.proquest.com/openview
25. Y. Liu, Z. Yu, S. Zhao, L. Cheng, Y.Man, X. Gao, H. Zhao, *Oxidative stress markers in the follicular fluid of patients with polycystic ovary syndrome correlate with a decrease in embryo quality*. *J. of As. Rep. and Gen. 38*(2021), 471-477. https://doi.org/10.1007/s10815-020-02014-y