RESEARCH ARTICLE

Physiological and Immunological Assessment in Infertile Women Undergoing *In-vitro* Fertilization

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ABSTRACT

All the available reports on the issue of infertility confirmed the increase in this population problem worldwide. Although the accurate estimate of the number of infertile people is due to several reasons, including the discrepancy in the true definition of infertility (whether it extends for one, two or five years of failed pregnancy attempts), as well as the great discrepancy in the size of the selected population groups (large population sample size versus epidemiological studies) and defining the category that diagnosed included (individuals, women, or couples).

The goal of today's IVF program is to obtain high-quality embryos with high efficiency in development, which leads to an increase in live birth rates. Apoptosis of the ovarian follicles of infertile women is one of the factors that can determine the outcome of IVF. The follicular fluid is an important environment because it contains apoptosis and other factors that greatly affect oocyte growth.

This study included 90 women undergoing IVF program ages range from 19 to 45 years, blood sample and Follicular Fluid was obtained from each, Blood samples were taken in CD2 immediately before oocyte separation and FF and the concentration of immune biomarkers was measured using Enzyme-linked immunosorbent assay (ELISA) technology. Patients were classified according to Polycystic ovary syndrome (PCOS) (N = 25), unexplained infertility (N = 20), tubal factors (N = 15) and compared with male factor infertility (N = 30) which was considered as a control group.

The current finding shows there were non-significant ($p > 0.05$) differences in the levels of LH, FSH, PRL and TSH in serum CD2. While a significant ($p < 0.05$) increase was found in level of E2 at day of HCG in unexplained group.

Highly significant differences were found in level of GDF15 in serum CD2 of PCOS group compared with the two groups (unexplained and tubal block) ($p < 0.05$). Moreover, a significant increase was found in the level of CD95/sFas in the serum of the PCOS group compared with all other groups ($p < 0.05$). There was a decrease in the level of APL IgM in PCOS and unexplained groups when compared to all studied groups ($p < 0.05$).

Levels of immune markers in serum and FF at the day of ova pick up (OPU) revealed a significant increase in the level of GDF15 and CD95 in serum and FF of PCOS group compared with the other studied groups ($p \leq 0.001$), in addition, level of APL IgG showed a significant increase only in FF of unexplained and tubal groups when compared between the all studied groups ($p < 0.05$).

In conclusion, GDF15 is bio marker of oxidative stress and increase in PCOS group and CD95/ FAS biomarker of apoptosis and sFas Anti apoptosis increase in PCOS group.

Keywords: Health, Infertile women, *In-vitro*

INTRODUCTION

According to available estimates, the problem of infertility is increasing in the world. Accurately estimating the number of infertile patients is difficult for three reasons: definition of infertility (1, 2 or 5 years of trying to conceive), large variation in selected populations (large populations versus epidemiological studies) and identification of who are infertile. Diagnosis includes (women, couples, or individuals) (Inhorn

and Patrizio,2015). Assisted reproductive techniques (ART), according to the International Society of Monitoring Assisted Reproduction (ICMART) and WHO defined as all treatments or procedures that include the invitro handling outside the body of both oocytes and sperm or of embryos for the needs of a pregnancy.

The releasing and inhibiting Gonadotropin-Releasing Hormone of the hypothalamus controls the biosynthesis and secretion of all anterior lobe hormones)GnRH(from the pituitary gland. The pulsatile hypothalamic secretion of GnRH is responsible for regulation. Since GnRH is secreted every 90 minutes (Monga, 2006).

Gonadotroph hormones affect the ovum which develops, while maturation of oocytes controlled by the pituitary hormones FSH and LH that needed for the development of the egg follicle. The follicle that selected for ovulation has level of FSH in its antral liquid while the selected follicle for atresia not receiving enough FSH to accomplish its maturation. (Johnson *et al.*, 2004).

The Follicular fluid (FF) is a complex extracellular fluid which is semi-viscous and yellow in color occupying the Graafian follicle antrum (Davoodi *et al.*, 2005).

The biochemical composition of FF surrounding the oocyte may play a critical role in determining oocyte quality, the subsequent potential to achieve fertilization and embryo development and an important role in the prediction of success rate of ART (Choi *et al.*, 2003).

CD95 is mainly expressed in immune cells and in many organs such as the thymus, liver, and placenta (Peter *et al.*, 2015). CD95 is activated by the ligand CD95L and by the sCD95L (Fouque *et al.*, 2014).

Growth differentiation factor 15 (GDF-15) and macrophage inhibitory cytokine-1 (MIC-1) is a divergent member of the transforming growth factor (TGF) family of secreted proteins (Verhamme *et al.*, 2019).

GDF-15 can be induced mainly by cellular stress tissue damage, inflammation or else, and particularly by oxidative stress and ROS (Wallentin *et al.*, 2014). Physiologic amounts of ROS in FF explains the reason for healthy oocyte development (Attaran *et al.*, 2000), oxidative stress reported as a possible cause of female infertility (Gupta *et al.*, 2014). GDF-15 present in FF as a soluble biomarker for stress.

The antiphospholipid syndrome (APS) can be identified by the occurrence of arterial and venous thromboses, and multiple fetal losses, usually comes with thrombocytopenia, with presence of antiphospholipid antibodies (aPL) (Cervera *et al.*, 2009)

Cardiolipin Antibodies (ACA) are presented by IgG, IgA, and IgM. ACA can exist with cardiac phosphatide, phosphatidyl serine, or phosphatidyl inositol, The target antigen of ACA is different from place to place. It is phospholipids in the heart; phospholipid-binding proteins in the plasma, such as β2GPI; or compounds such as protein and phospholipid, and the phospholipids during the formation of β2GPI (Giannakopoulos *et al.*, 2007).

MATERIALS AND METHODS

This study was designed on a sample of infertile women through their review of the Kamal al-Samaria Hospital, the Infertility and IVF Center. (Baghdad/Iraq) during the period from November 2019 to the end of April 2020 Ninety infertile women) case and control) have been enrolled in this study and enter their ICSI cycle, The subjects' ages range from 19 to 45 years. Blood sample and Follicular Fluid taken from serum and FF and concentration of (LH, FSH, PRL, TSH) measured by Minividas instrument and immune markers (GDF15,CD95, ACL,APL) measured using ELISA technique. Patients were classified as PCOS (N= 25), unexplained infertility (N = 20), tubal factor (N= 15) and compared with male factor infertility (N= 30) that was the control group. Blood samples taken in CD2 directly before the pickup of the oocyte. FF samples were inhaled from mature follicles ($\geq$ 14 mm diameter) exactly at the time of transvaginal oocyte pickup. Blood and FF were handled directly after the collection. Blood samples then centrifuged at 4000 rpm for 10 min and supernatant was used for measuring the concentration of (LH, FSH, PRL, TSH, E2 hormones) and (GDF-15, CD95, ACL, APL) and stored at -70°C until assayed. All FF belonging to mature follicles were collected and pooled every patient. FF was centrifuged at 3000 rpm for 7 min to separate cellular cantents and debris and supernatant was a liquoted and stored at −70°C until assayed.

RESULTS AND DISCUSSION

Levels of Hormones in Serum CD2 and Day of HCG

The levels of hormones (LH, FSH, PRL and TSH) in the serum CD2 as well as the level of E2 in day of HCG are shown in Table 1. The results revealed that there were non-significant ($p > 0.05$) differences in the hormones levels in all studied groups (PCOS, unexplained, and tubal block) as following LH (4.99 ± 0.74, 4.16 ± 0.48 and 4.05 ± 0.53 mIU/mL) respectively: FSH (6.56 ± 0.53, 6.39 ± 0.77 and 6.54 ± 0.53 mIU/mL) respectively, PRL (19.51 ± 2.78 , 17.48 ± 2.14 and 14.13 ± 1.41 mIU/mL) respectively, and TSH (1.971 ± 0.202, 1.909 ± 0.342 and 4.491 ± 2.284 mIU/ml) respectively in comparison to male factor group which revealed these results, LH (4.36 ± 0.76 mIU/mL), FSH (6.56 ± 0.89 mIU/mL) PRL (16.65 ± 1.55 mIU/mL) and TSH (2.845 ± 0.384 mIU/mL).

The results showed that there were a significant ($p < 0.05$) increase in level of E2 in unexplained group (2498.93 ± 672.67 mIU/mL) when compared between the three studied groups with each other (PCOS, unexplained and tubal block) (1428.03 ± 191.79, 2498.93 ± 672.67 and 1158.33 ± 229.50 mIU/mL) respectively and significant ($p < 0.05$) increase in unexplained group (2498.93 ± 672.67 mIU/mL) when compared with male factor (1410.40 ± 189.32 mIU/mL), and there were non-significant ($p > 0.05$) differences when compared between PCOS and tubal block group with male factor.

The current study shows that there is no change in the concentration of hormones (LH, FSH PRL, TSH) in all studied groups. These results are disagree with Wdowiak *et al.* (2020) who indicated a significant differences between groups

according to the FSH, LH, PRL and TSH on the third day of the cycle. While the current result agree with Hashemi *et al.* (2016) who indicated that the results revealed that there is a non-significant ($p > 0.05$) difference in the levels of FSH, TSH, E2, progesterone, and PRL in infertile women diagnosed with PCOS and healthy women. The difference in the current result and a previous study may be due, the current study is designed for women with different types of infertility.

A significant increase in level of E2 in unexplained group compared with other groups in this study disagree with Wdowiak *et al.* (2020), who indicated a significantly lower level of E2 in the study group, both during ovulation and after ovulation. Follicular maturation usually evaluated by measuring serum E2 concentration. Serum E2 is used to find out the approximate time of ovulation of infertility patients, and when the date of ovulation approaches, the level of E2 changed in natural cycle in IVF particularly. In women with pre-menopausal, E2 is secreted mainly by follicle granulosa cells. As these cells divide and increasing in number when the follicle grows, the E2 levels already increases. E2 concentration is an important indicator for follicules maturation (Segawa *et al.*, 2015). This result disagrees with Khmil *et al.* (2020) who reported a significant increase in estradiol levels in serum of PCOS patients. Reports on estradiol levels in PCOS differ in opinion. The ovulation is paired with low estradiol secretion, the reason for that is peripheral extra glandular conversion and lower progesterone secretion (Homer *et al.*, 2017). Some studies found decreasing of estradiol levels in PCOS, while other studies reported high estradiol levels (Hashemi *et al.*, 2016). The increased estradiol level in PCOS is because of the reduced sex hormone-related globulin that associated with the levels of obesity and testosterone (Bergh *et al.*, 1994).

Levels of Immune Markers in Serum CD2

The levels of immune markers (GDF-15, CD95, ACL, APLIgM and APL IgG) in serum CD2 are shown in Table 2 which indicates that there were a significantly ($p \le 0.001$) increase in level of GDF 15 in PCOS group (6.88 ± 0.71 ng/L) when compared with the unexplained group and tubal block group (2.70 ± 0.66, 3.09 ± 0.66 ng/L) respectively. When compared between three studied groups with male factor the results revealed that there were no significant difference ($p > 0.05$) between PCOS group and male factor (5.09 ± 0.82 ng/L). Still, there were a significant ($p < 0.001$) decrease in level of GDF-15 in unexplained and table block in comparison to male factor group.

As shown in the Table 2 and 3 there was a statistically significant ($p \le 0.001$) increased in level of CD95 in PCOS group (8.91 ± 1.23 ng/L) when compared with each other (unexplained group and tubal block group), (3.99 ± 1.24, and 2.04 ± 1.03 ng/L) respectively, while when compared between the three studied groups with male factor, the result revealed that there was highly significant ($p \le 0.001$) increased in level of CD95 in PCOS in comparison to male factor group (2.63 ± 0.72 ng/L), and there were non-significant ($p > 0.05$) differences in unexplained and tubal block group in comparison to male factor group.

On the other hand, the results showed that there was non-significant ($p > 0.05$) differences in level of ACL in all studied groups (PCOS, unexplained and tubal block), (1.41 ± 0.18, 1.13

Table 1: Levels of hormones in serum CD2 and day of HCG of the studied groups

Groups	Serum CD2 (Mean ± SE)				Day of HCG
	LH (mIU/mL)	FSH (mIU/mL)	PRL (mIU/mL)	TSH (mIU/mL)	E2(mIU/mL)
PCOS	4.99 ± 0.74^a	6.56 ± 0.53^a	19.51 ± 2.78^a	1.971 ± 0.202^a	1428.03 ± 191.79^b
Unexplained	4.16 ± 0.48^a	6.39 ± 0.77^a	17.48 ± 2.14^a	1.909 ± 0.342^a	2498.93 ± 672.67^a
Tubal block	4.05 ± 0.53^a	6.54 ± 0.53^a	14.13 ± 1.41^a	4.491 ± 2.284^a	1158.33 ± 229.50^b
Male factor	4.36 ± 0.76^a	6.56 ± 0.89^a	16.65 ± 1.55^a	2.845 ± 0.384^a	1410.40 ± 189.32^b
LSD value	2.289	2.366	6.598	2.356	988.482
P-value	0.794	0.998	0.434	0.19	0.042^*

*Mean that carrying similar letters is indicate the non-significant difference (p > 0.05).
*Mean that carrying different letters is indicate the significant difference (p < 0.05).

Table 2: Level of immune markers in serum CD2 of the studied groups

Groups	Serum CD2 (Mean ± SE)				
	GDF-15 (ng/L)	CD 95 (ng/L)	ACL (ng/L)	APL IgM (ng/L)	APL IgG (ng/L)
PCOS	6.88 ± 0.71^a	8.91 ± 1.23^a	1.41 ± 0.18^a	26.50 ± 1.03^b	26.79 ± 0.94^a
Unexplained	2.70 ± 0.66^b	3.99 ± 0.93^b	1.13 ± 0.09^a	26.17 ± 0.72^b	27.54 ± 1.04^a
Tubal block	3.09 ± 0.66^b	2.04 ± 1.03^b	1.26 ± 0.25^a	29.64 ± 1.72^a	27.79 ± 1.36^a
Male factor	5.09 ± 0.82^{ab}	2.63 ± 0.72^b	1.30 ± 0.17^a	30.29 ± 0.98^a	29.09 ± 1.14^a
LSD value	2.378	3.062	0.561	3.076	3.466
P-value	0.001^*	0.001	0.727	0.05*	0.448

*Mean carrying similar letters indicate a non-significant difference ($p > 0.05$).
*Mean carrying different letters indicate a significant difference ($p < 0.05$).

Table 3: Levels of immune markers in serum at day of ova pickup of the studied groups

Groups	Serum day OPU (Mean ± SE)				
	GDF-15 (ng/L)	CD 95 (ng/L)	ACL (ng/L)	APL IgM (ng/L)	APL IgG (ng/L)
PCOS	7.79 ± 0.71[a]	9.97 ± 1.18[a]	1.15 ± 0.08[a]	28.48 ± 0.87[a]	28.14 ± 0.92[a]
Unexplained	2.48 ± 0.79[c]	3.29 ± 1.07 [b]	1.11 ± 0.06[a]	27.82 ± 0.66[a]	26.46 ± 1.25[a]
Tubal block	2.19 ± 0.70[c]	1.31 ± 0.53 [b]	1.29 ± 0.11[a]	28.17 ± 1.07[a]	25.68 ± 1.36[a]
Male factor	4.94 ± 0.76 [b]	3.16 ± 0.84 [b]	1.17 ± 0.06[a]	27.09 ± 0.64[a]	27.40 ± 0.99[a]
LSD value	2.174	2.734	0.203	2.022	3.114
P-value	0.001[*]	0.001[*]	0.484	0.568	0.48

*Mean carrying similar letters indicate a non-significant difference ($p > 0.05$).
*Mean carrying different letters indicate a significant difference ($p < 0.05$)
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Table 4: Levels of immune markers in follicular fluid at day of ova pickup of the studied groups

Groups	FF day OPU(Mean ± SE)				
	GDF-15 (ng/L)	CD 95 (ng/L)	ACL (ng/L)	APL IgM (ng/L)	APL IgG (ng/L)
PCOS	6.73 ± 0.61[a]	12.43 ± 1.21[a]	1.00 ± 0.08[a]	26.75 ± 0.89[a]	24.10 ± 1.06[b]
Unexplained	2.27 ± 0.59[c]	5.56 ± 1.48[b]	1.05 ± 0.04[a]	26.18 ± 0.98[a]	26.37 ± 1.28[a]
Tubal block	2.88 ± 0.64[c]	3.41 ± 1.03[b]	1.03 ± 0.05[a]	28.75 ± 0.77[a]	27.38 ± 1.04[a]
Male factor	4.68 ± 0.67[b]	3.19 ± 0.96[b]	1.07 ± 0.07[a]	28.01 ± 0.56[a]	25.72 ± 0.71[b]
LSD value	1.959	3.238	0.195	2.181	2.581
P-value	0.001[*]	0.001[*]	0.88	0.149	0.041

*Mean carrying similar letters indicate a non-significant difference ($p > 0.05$).
*Mean carrying different letters indicate a significant difference ($p < 0.05$).

± 0.09 and 1.26 ± 0.25 ng/L) in comparison to male factor group (1.30 ± 0.17 ng/L), but the result revealed that there were a significant ($p < 0.05$) decrease in level of APL IgM in PCOS and unexplained groups when compared between three studied groups with each other (PCOS, unexplained and tubal block), (26.36 ± 1.03, 26.17 ± 0.72 and 29.64 ± 1.72 ng/L) respectively and when compared between three studied groups with male factor group the results reveled that there were a significant ($p < 0.05$) decrease in level of APL IgM in PCOS and unexplained groups (PCOS and unexplained) (26.36 ± 1.03, 26.17 ± 0.72 ng/L), (30.29 ± 0.98 ng/L) respectively and there were non- significant ($p > 0.05$) difference in the level APL IgM in tubal block (29.64 ± 1.72 ng/L) when compared with mal factor group (30.29±0.98 ng/L). Also, there were non-significant ($p > 0.05$) differences in the level of APL IgG in all studied groups PCOS, unexplained, tubal block (26.79 ± 0.94, 27.54 ± 1.04 and 27.79 ± 1.36 ng/L) respectively in comparison to male factor group (29.09 ± 1.14 ng/L)

A noval previous study showed increase in level of GDF-15 in PCOS group when compared with the studied groups and with male factors and these results is approaching to (Souček *et al.*, 2018). In which the first determination for the concentrations of GDF-15 is done by ELISA in FF and serum of patients and healthy persons. The concentrations were varied from 35-571 ng/mL, GDF-15 found significantly higher in FF then in serum. To our knowledge the current study, is the noval first study in Iraq to detect GDF-15 in serum and FF of infertile women who will undergo an IVF program. Souček *et al* . (2018), conducted to clarify the presence or absence of GDF in the serum and FF of women who will undergo the IVF program regardless of the cause of infertility, but in this study,

the patients were divided on the basis of the cause of infertility. Also, this study disagree with (Clark *et al.*, 2020) which done on mice which reported that metformin elevates circulating levels of GDF15, which are necessary for its beneficial effects on energy balance and body weight, major contributors to its action as a chemopreventive agent. The differences between the current study and previous study may be due to women in current study didn't receive Metformin before the beginning of the program.

The result of current study showed increase in level of CD95 in PCOS group, and when compared of the three studied groups with male factor, and this finding disagree with Pekel *et al.* (2015) who found in his study that when detect the serum and FF levels of sFas and sFas ligand in unexplained infertility patients, the PCOS and tubal factors had lower sFas levels significantly as compared with their controls .Serum and FF sFas levels and antioxidant activity were decreased in infertility, meaning there was increased apoptosis, and in unexplained infertility studied group, the changes in these parameters were more clear. Both sFas and sFas ligand are an important apoptotic markers, because Fas is a mediator for extrinsic pathway of apoptosis (Brown and Attardi, 2005). Fas need FasL to begin the apoptosis, Fas/FasL receptor interaction is the starting key of the extrinsic pathways of apoptosis (Pfeffer,2003).

This finding is agree with Al Hadithi *et al.,* (2018) who reported there was a highly significant increase in serum concentration of sFas of patient with uterine leiomyoma (mean 0.326 pg/mL ± SE 0.028) compared to control healthy subjects (mean 0.126 pg/mL ± 0.025).

Apoptosis regulating homeostasis by cell death in living tissues, Means that apoptosis is related to reproductive physiology processes, such as implantation, endometrium proliferation and follicular atresia. Its work is coordinated by Fas, Fas ligand, sFas, and bcl-2 molecules. (Mor and Straszewski, 2002). Fas mediated apoptosis need the receptor-ligand interaction that modulated by sFas to be balanced in order to acts as an antagonist of FasL mediated apoptosis (Onalan *et al.*, 2005).

There are few data about soluble apoptosis markers in IVF patients. Low levels of serum sFas were found in women undergoing IVF compared with those of normal females (Mor and Straszewsk, 2002).These low levels reveal increasing in apoptosis rate and a down regulation of the patient's immune system (Onalan *et al.*, 2005), who found no significant difference in case of FF of sFas and sFasL ,serum sFasL in these groups. Also, the current study disagree with (Onalan *et al.*, 2006),who found increased serum levels observed of sFas and decreased FF levels of sFasL observed in IVF patients who take Metformin therapy compared to those who didn't. Studies reported that Metformin drug has an anti-apoptotic effect in PCOS women. The same results found in infertility patients because of endometriosis. The differences between the current study and previous studies may due to women with PCOS don't received Metformin therapy.

While, other researchers found that there was decreased sFas levels in serum of endometriosis group when compared with the group of male factor infertility. They showed that low levels of serum sFas is in relationship with increased apoptosis in endometriosism, other study mentioned that the serum FF sFas levels in IVF women ranged with different patients diagnosis (Abdelmeged *et al.,* 2011).

The current study shows no change in the concentration of ACL in all the studied groups, and these results agreed with Caccavo *et al.* (2007) who indicated ACL levels detected in patients were not significantly ($p > 0.05$) different with the control group.

The current result reveled significant decrease in level of API IgM in PCOS and unexplained group when compared with male factor but non-significant defferances in level of APL IgG in all studied groups ,this study is closed to Buckingham *et al.* (2006) who reported that 19.2% of 99 women undergoing IVF, one APL at least was found in their serum and/or FF, and the antibody levels in FF as the same as that in serum.

Ulcova-Gallova *et al.,* (2012) reported that APL antibody is associated with fertility autoimmune diseases not only in SLE women. Some studies reported that there was no significant association between APL and pregnancy failure in women with SLE. Other studies showed significant relationship between them (Kalunian *et al.*, 1988). APL may presented in 5% of healthy controls and up to 37% in SLE women. (Cervera *et al.*, 2002).

Level of Immune Markers in Serum at Day of Ova Pickup

Level of immune markars in serum at day of Ova pick up are shown in the Table 3. This table indicates a significant increase ($p \leq 0.001$) in level of GDF 15 in serum of PCOS group when compared between the three studied groups (PCOS, unexplained and tubal block) as fallowing (7.79 ± 0.71, 2.48 ± 0.79 and 2.19 ± 0.70 ng/L) respectively ,while when compered between three studied groups with male factor group, the result shows there were a significant increase ($p < 0.001$) in PCOS group (7.79 ± 0.71 ng/L) in comparison to male factor (4.94 ± 0.76 ng/L) , and there were a significant ($p < 0.001$) decrease in level of GDF15 in unexplained and tubal block groups in comparison to male factor group.

As shown in Table 3, there was a statistically significant ($p \leq 0.001$) increased in level of CD95 in PCOS group (9.97 ± 1.18 ng/L) when compared between the three studied groups with each other (PCOS, unexplained and tubal block) as fallowing (9.97 ± 1.18 , 3.29 ± 1.07, and 1.31 ± 0.53 ng/L) respectively. When compared between the three studied groups with male factor the result revealed that there were highly significant ($p \leq 0.001$) increase in level of CD95 in PCOS (9.97 ± 1.18 ng/L) in comparison to male factor group (3.16±0.84 ng/L). At the same time, there were non-significant ($p > 0.05$) differences in unexplained and tubal block in comparison to male factor groups .

The statistical analysis shown there were non- significant ($p > 0.05$) difference in the level of ACL in PCOS, unexplained and tubal block as fallowing (1.15 ± 0.08, 1.11 ± 0.06 and 1.29 ± 0.11 ng/L) in compassion to male factor (1.17 ± 0.06) Regarding levels of APL IgM the result revealed non- significant ($p > 0.05$) difference between all studied groups PCOS group (28.48 ± 0.87 ng/L) , unexplained group (27.82 ± 0.66) and tubal block group (28.17 ± 1.07 ng/L) and male factor group (27.09 ± 0.64 ng/L). Also, non- significant ($p > 0.05$) difference where found when the comparison was made regarding the level of APLIgG between the studied groups PCOS group (28.14 ± 0.92 ng/L), unexplained group (26.46 ± 1.25) and tubal block group (25.68 ± 1.36 ng/L) and male factor group (27.40 ± 0.99 ng/L).

The results of thcurrent study showed increase in level of GDF15 in PCOS group and this finding approached to (Souček *et al.*, 2018) who reported that clarify the presence or absence of GDF in the serum and FF of women who will undergo the IVF program regardless of the cause of infertility, but in this study the patients were divided on the basis of the cause of infertility.GDF15 can measured by ELISA in FF and in serum of patients and healthy women, the results of a research showed that the concentrations ranged from 35 to 571 ng/mL, and GDF-15 levels showed significant higher in FF compared with corresponding serum (Souček *et al* .,2018).

While, this study disagree with (Clark *et al.,* 2020) which done on mice which reported that metformin elevates circulating levels of GDF-15.The differences between the current study and previous study may be due to women in current study don't receive metformin drugs before beginning the program.

The result of current study showed increase in level of CD95 in serum of PCOS at the day of OPU, this finding disagree with (Pekel *et al.*, 2015), found that serum and

FF levels of sFas and sFas L in Patients with unexplained infertility, PCOS and tubal factor, were significantly lower sFas levels when compared with the control group,. These findings were disagreed with Onalan *et al*. (2005) ,who reported that FF sFas and sFasL concentrations were similar in different groups of IVF patients except for women with PCOS, while sFas concentrations were lower in serum. Metformin therapy in the PCOS group increased serum concentrations of sFas and decreased FF concentrations of sFasL means it gives an anti-apoptotic effect. The differences in current study and previous study may be due to PCOS group didn't received Metformin therapy.

Also, disagree with (Malamitsi-Puchner *et al*., 2004), who reported the Concentrations of sFas were found to be similar between poor responders receiving different therapy regimens, the same results found between patients with and without clinical pregnancies .

This study demonstrated non- significant difference in the level of ACL in serum at day of OPU and this result is agree with (Radojcic *et al*., 2004) who indicated the absence of elevated aCL in the sera of fertile subjects ,that study reported that in 4% of studied women the control group have an elevation of IgG aCL , the dispersal of aCL in the general population from USA, Canada, various European and some Arab countries showed geographic variation, ranging between of 1–4% for IgG, 0.5–5% IgM, and 2–5% for IgG/IgM .On the other hand ,the current result disagree with (Saeed *et al*., 2012) who shows an incidence of aCL antibodies in the group of patients with unexplained infertility. And disagree with the other published data that reported by Radojcic *et al.* (2004), the incidence of aCL IgG was 17% in unexplained infertile patients is comparable to 21.15 % reported by Radojcic *et al*. (2004) ,and 24 % that reported by (Balasch *et al.,*1996).

There is no significant differences in the level of APL IgG,IgM in serum at day of Opu this result closed to (Buckingham *et al*., 2006) who found that in 19.2% of 99 women undergoing IVF, at least one APL was showed in their serum and/or FF, while the levels of the antibody in their follicular fluid found not higher than that in serum.

Buckingham *et al.* (2006) found one woman of 99 patients sample whose contained significantly more IgG anti-β2GPI antibodies in her FF than in her serum.

Levels of Immune Markers in Follicular Fluid at Day of Ova Pickup

Table 4 shows the levels of immune markers in FF at day of OPU. This table indicates a highly significant ($p \leq 0.001$) increase in level of GDF-15 in PCOS group when compared between the three studied groups with each other (PCOS, unexplained and tubal block) as fallowing (6.73 ± 0.61, 2.27 ± 0.59 and 2.88 ± 0.64 ng/L), respectively.

Also when compared between the three studied groups with the male factor, the statistical analysis showed a significant ($p \leq 0.001$) increase in the level of GDF-15 in PCOS (6.73 ± 0.61 ng/L) in comparison to male factor (4.68 ± 0.67 ng/L), while a significant ($p \leq 0.001$) decreed in unexplained (2.27 ± 0.59

ng/L) and tubal block (2.88 ± 0.64 ng/L) in comparison to male factor group.

The results shown that there were a highly significant ($p \leq 0.001$) increase in level of CD95 in PCOS group when compered between three studied groups with each other as fallowing (PCOS, unexplained and tubal block) (12.43 ± 1.21, 5.56 ± 1.48) and (3.41 ± 1.03 ng/L), respectively . Also, when compered between three studied groups with male factor, the result showed a significantly ($p \leq 0.001$) increase in level of CD95 in PCOS (12.43 ± 1.21 ng/L) in comparison to male factor (3.19 ± 0.96 ng/L) , and a significant ($p \leq 0.001$) increase was found in unexplained group (5.56 ± 1.48) in comparison to male factor (3.19 ± 0.96 ng/L) ,and non- significant ($p > 0.05$) decrease in tubal block group (3.41 ± 1.03 ng/L) in comparison to male factor.

On the other hand, the statistical analysis showed non-significant difference ($p > 0.05$) in the level of ACL when compared between three studied groups (PCOS, unexplained and tubal block) as fallowing (1.00 ± 0.08, ,1.05 ± 0.04 and 1.03 ± 0.05 ng/L) respectively in comparison to male factor (1.07 ± 0.07 ng/L) ,and non- significant ($p > 0.05$) difference were found in the level of APL IgM, in (PCOS, unexplained and tubal block) as fallowing (26.75 ± 0.89, 26.18 ± 0.98 and 28.75 ± 0.77 ng/L) respectively in comparison to male factor (28.01 ± 0.56 ng/L) . Also non- significant ($p > 0.05$) difference were found in the level of APL IgG in (PCOS, unexplained and tubal block) as fallowing (24.10 ± 1.06, 26.37 ± 1.28 and 27.38 ± 1.04 ng/L) respectively in comparison to male factor (25.72 ± 0.71 ng/L) .

The current study shows a significant increase in the concentration of GDF-15, It is the first study in Iraq to detect GDF-15 in serum and ff in infertile women who will undergo an IVF program , this is agree with Santoso *et al*., (2021) novel study demonstrated an elevated concentration of GDF-15 in the peritoneal fluids of infertile women with endometriosis. Furthermore, these molecules positively correlate with pelvic adhesion in patients. The examination of serum GDF-15 concentration also revealed its capacity to discriminate between early- and late-stage endometriosis, which indicates a potential use as a noninvasive biomarker in endometriosis-related infertility. Serum GDF-15 is a potential biomarker to differentiate the severity of endometriosis. Further studies are warranted to evaluate the role of these molecules in cellular and molecular levels (Santoso *et al*,2021).

The differences between the current study and previous study may be due to the previous study sample is peritoneal fluids of infertile women with endometriosis. Also, current study concluding with (Souček *et al*., 2018), this considered the first determination for the concentrations of GDF-15 by ELISA in FF and serum of patients and healthy persons.

The current study shows a significant increase in the concentration of CD95 in the PCOS group, and this result disagreed with Onalan *et al*. (2005) who reported that FF sFas and sFasL concentrations were found similar in different study groups of IVF patients except for those with PCOS

where sFas concentrations were lower in them. Concentrations were similar between responders receiving different therapy regimens poorly, as well as between patients with and without clinical pregnancy, show lack of predictive value of FF sFas and sFasL on successful IVF outcome

While the current finding agreed with. Santos *et al.* (2021) postulated that low concentrations of sFas and high concentrations of sFasL in FF are related to apoptosis and poor oocyte and embryo quality. Concentrations of sFas were significantly higher in FF containing immature oocytes compared with those containing atreic oocytes.

Malamitsi-Puchner (2006), who reported that Fas–FasL system in cells and fluids from gonadotropin-stimulated human ovaries

The current study disagree with (Onalan *et al.*, 2005),who reported Metformin therapy in the PCOS group increased serum concentrations of sFas and decreased FF concentrations of sFasL, showed an anti-apoptotic effect .The differences between current study and (Onalan *et al.*, 2005) the PCOS group in current study received Metformin therapy before beginning IVF program.

A non-significant difference in the present study and this is level of ACL and this result is agree with (Radojcic *et al.*, 2004),who mentioned to the absence of elevated aCL in the sera of fertile persons studied and repoted that in one woman (4%) of the control group there is elevation of IgG aCL. These results considered as accepted results as the prevalence of aCL in the general population from the USA, Canada, various European and some Arab countries that showed considerable geographic variation, ranging between 1–4% for IgG, 0.5–5% IgM, and 2–5% for IgG/IgM (Radojcic *et al.*, 2004).

The current study shows that non- significant different in APLIgM, IgG when compered between three studied groups and male factor this study disagree with Buckingham *et al.* (2006) who reported that in 19.2% of 99 women analyzed, had about one aPL in their serum and/or FF. The most available antibody found was β2GPI IgG, in 16 women where Beta-2-Glycoprotein I (β2GPI) IgM antibodies were found in 4 women, and 4 women had IgG and 2 had IgM to CL. Phosphatidyl serine PS IgG antibodies were found in 5 women and no women showed significant levels of IgM in their serum or FF, also found that there is no women had IgM in their follicular fluid. Only 2 of the 19 women with antibodies had more than one type of antibody in their follicular fluid or serum (Buckingham *et al.*, 2006).

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