Original
ISSN 2413-0516

Relation of Serum and Follicular Level of BMP15 with Oocyte Quality, Embryo Grading and Pregnancy Rate

Zainab Hassan Hashim[1*], Lubna Amer[2], Estabraq A. Al-Wasiti[3]

[1]Department of Clinical Reproductive Physiology, High Institution for Infertility Diagnosis and Assisted Reproductive Technologies, University of Al-Nahrain, Baghdad, Iraq.
[2]Institution for Infertility Diagnosis and Assisted Reproductive Technologies, University of Al-Nahrain, Baghdad, Iraq.
[3]Department of Biochemistry, College of Medicine, University of Al-Nahrain, Baghdad, Iraq.
*Correspondence to: Zainab Hassan Hashim (E-mail: zainab.hassan@ierit.nahrainuniv.edu.iq)
(Submitted: 03 March 2022 – Revised version received: 21 March 2022 – Accepted: 07 April 2022 – Published Online: 26 October 2022)

Abstract
Objectives: Use of serum and follicular fluid concentration of oocyte secreted factors BMP15 as biomarkers of oocyte quality, embryo quality and it's relation to pregnancy rate.
Methods: Eighty eight women were included in this study; they were selected from those undergoing intra-cytoplasmic sperm injection.
Results: Positive pregnancy was achieved by 14 women accounting for 19.0% (total number of women that reach embryo transfer was 72). No significant difference in mean serum BMP15 between pregnant and non-pregnant women, but the level of follicular fluid BMP15 was significantly higher in pregnant women. MI oocyte count was not significantly correlated to serum or follicular fluid BMP15 ($P > 0.05$). MII oocyte count showed highly significant positive correlation to serum and follicular fluid BMP15 ($P < 0.01$). Grade 1 embryo count showed highly significant positive correlation to serum and follicular fluid BMP15 ($P < 0.01$), Grade 2 embryo count showed significant positive correlation to serum BMP15 ($P = 0.032$), but the grade 2 embryo count showed non-significant correlation to follicular BMP15 ($P > 0.05$). Also grade 3 embryo count showed non-significant correlation to serum and follicular BMP15 ($P > 0.05$).
Conclusion: The current study revealed that serum and follicular BMP15 could be used as indicator for oocyte maturity, and serum BMP15 could be used as indicator of good quality embryos.
Keywords: Follicular level, BMP15, oocyte, embryo grading, pregnancy rate

Introduction

Infertility is defined as the inability, of a couple to have pregnancy after a period of one year, in those women under 35 years of age or after 6 months in those women above 35 years of age, in spite of regular (3 to 4 times/week), adequate and unprotected sexual intercourse**.** The key limiting factor in female fertility is the oocyte quality, and till now there is poor understanding of what factors that determine the oocyte quality or the mechanisms that governing it.[1,2]

About 35% of infertility cases are caused by female factors, 35% related to male factors, 20% caused by both male and female factors, and 10% by unknown causes.[3] The key limiting factor in female fertility is the oocyte quality; the quality of oocyte greatly affects early embryonic survival, also establishment with maintenance of pregnancy, development of the fetus, and even causes some adult diseases.[1] Bone morphogenetic protein 15 (BMP15) and growth differentiation factor 9 (GDF9), have a unique feature, within the transforming growth factors-b super-family is that the expression of the protein is essentially restricted to the gametes (oocyte). BMP15 and GDF9 are expressed in the oocyte during folliculogenesis, from the earliest stages.[4] They are expressed in high levels by the oocyte throughout folliculogenesis, so they are could be regarded a good indicator for oocyte quality, and measuring them in the serum which is rapid, non-invasive and easy test could give a great clue to female fertility.[5]

This study aimed to use serum and follicular fluid concentration of oocyte secreted factors BMP15 as biomarkers of oocyte quality, embryo quality and then to study it's relation with the pregnancy rate.

Materials and Methods

A prospective study was conducted in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, from Nov., 2020 to July 2021. One hundred and seventy six women were included in this study.

The study subjects involved eighty eight women who were selected from those attended the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, they were enrolled in IVF program.

Inclusion Criteria

- All couples undergoing IVF/ICSI protocols.
- Women at any age from 18 to 47 years old.
- Infertility due to female factors: tubal blockage, unovulatory cycles, and mild-moderate cases of endometriosis that diagnosed laproscopically.
- Couples with male factor infertility.
- Unexplained infertility.

Exclusion Criteria

- All types of congenital anomalies of the reproductive system.
- Uncontrolled systemic and endocrine disorders.
- Women with BMI more than 30 kg/m^2

Methods and Study Design

A total of eighty eight patients undergoing IVF/ICSI cycle were evaluated:

- Taking full obstetrical, medical, surgical history with assessment of weight and height to obtain (BMI).

- Examinations of the woman clinically and gynecologically to check for any abnormality.
- For male partners, the seminal fluid analysis was assessed according to WHO 2010.
- Doing analysis of female hormones (LH, FSH, E2, Prolactin, Testosterone and TSH) at the second day of the menstrual cycle.
- All women were enrolled to only one type of controlled ovarian hyperstimulation (COH) protocols which is Gonadotropin releasing hormone antagonist protocol.
- Follow up of the patients by doing serial vaginal ultrasound and doing serum level of estradiol (E2) and then accordingly to the result, ovum pick up done.
- Oocyte retrieval done with guidance of trans-vaginal ultrasound after ovulation trigger with HCG about (35–36) hrs.
- At the day of ova pick up, serum and follicular fluid samples were obtained from each woman for measurement of BMP15.

The antagonist protocol involved ovarian stimulation with gonadotropins since the second day of the menstrual cycle followed by the administration of a GnRH antagonist (Cetrorelix acetate for injection 0.25 mg: Cetrotide®, Merk, Switzerland), using flexible method and given when the size of the largest follicles reach (13–14) mm. The initial dose of FSH was 75–300 IU daily according to patient condition. With serial vaginal U/S for checking the number and size of ovarian follicles and for the endometrial thickness (ET), in addition serum level of Estradiol (E2) was done. The serum level of (E2) Estradiol was measured at day of ovulation triggering by (HCG) administration.

The oocyte grading was at retrieval could be either immature oocyte and this is called Germinal vesicle (GV), in which the corona and cumulus cells, are tightly packed around the oocyte, with presence of circular structure inside it, that is called the (germinal vesicle), the other immature oocyte is called Metaphase I (MI). The mature oocyte is called Metaphase II (MII) which has polar body. MI oocyte characterized by the absence of a polar body or a germinal vesicle, and it is intermediate stage between the GV and MII (mature) stages.

ICSI Processes

The aspirated follicles were examined at the IVF laboratory, in petri dish immediately. Flushing was done then kept 1–2 hrs in the (37°C/ CO_2) incubator, all oocytes after that were subjected to denudation and grading in a Laminar Flow Cabinet. The mature eggs were selected by a specialized pipette, and by a very delicate, sharp and hollow needle which is used to held, immobilize and then pick up a single sperm. After that, the sperm was inserted by the needle carefully through egg shell into its cytoplasm. Then the eggs were kept in the CO_2 incubator and carefully monitor the result of cell division, by using Nikon ICSI Microscope.

Embryo Quality and Grading

Zygotes after insemination, were observed after (18–20) hours to check for the presence of (2) pronuclei and after (25–29) hours to observe the presence of early cleavage, which is considered a sign of better implantation rates. The presence of 2 pronuclei at day 1, was regarded as a good prognostic sign. Then at day two (43–45 hours after insemination) and day three (67–69 hours after insemination) the embryos were evaluated. Good quality embryos were considered when they were homogeneous, with normal kinetics (4) cells at day 2 and (7–9) cells at day 3, and containing <10% of cytoplasmic fragments.

The embryos at the third day, were classified as being with or without compaction, which referred to all embryos that underwent the compaction process, the embryos could be at the beginning of compaction when the fusion of the membrane was visible, in this stage the counting of the number of cells is still possible, and those embryos with full compaction, in those embryos the distinguishing of cell boundaries was not possible.

Embryo Transfer: The dividing embryos were then replaced into the uterine cavity under pelvic ultrasound guidance and by an embryo transfer catheter.

Results

The pregnancy rate in infertile women enrolled in the current study is shown in Figure 1. Positive pregnancy was achieved by 14 women accounting for 19.0%. Total number of patient was 88, a number of cases were not included in counting pregnancy rate this included five cases of empty follicles, four cases of embryonic developmental arrest, six cases of failed fertilization and one patient refuse embryo transfer. So the number of cases that were included in counting pregnancy rate was 72 patients.

The characteristics of infertile women enrolled in this study are shown in Table 1. The mean age of all enrolled women 32.25 ± 6.41 years and the mean age of women with positive pregnancy was significantly lower than that of non-pregnant women (29.14 ± 4.54) years versus (32.76 ± 6.55) years, respectively (P = 0.050). The mean duration of infertility of all enrolled women was (7.89 ± 3.87) years and the mean duration of infertility of pregnant women was lower than that of non-pregnant women (6.93 ± 3.08) years versus (8.05 ± 3.98) years; however, the difference did not reach statistical significance (P = 0.319). Out of all enrolled women, primary infertility was seen in 65 (74.0%) women, whereas, secondary infertility was seen in 23 (26.0%) women and there was no significant difference in the frequency distribution of women according to type of infertility with respect to pregnancy outcome (P = 1.000). The mean BMI for pregnant women was (26.71 ± 2.60), and for non-pregnant women (26.72 ± 3.01), there was no significant difference in the frequency distribution of women according to BMI with respect to pregnancy (P = 0.958).

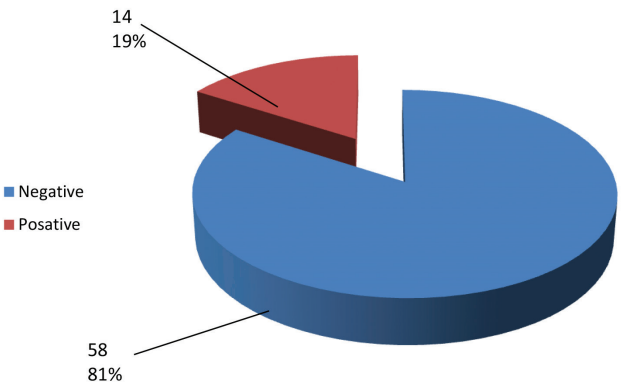

Fig. 1 **Pie chart showing pregnancy rate of women undergoing ICSI.**

Relation of Pregnancy Rate to Serum and Follicular Level of BMP15

At day of ova pick up the bone morphogenetic protein 15 (BMP15) serum and follicular levels are shown in Table 2. There was no significant difference in mean serum BMP15 (169.79 ± 23.22) versus (155.95 ± 54.13) between pregnant and non-pregnant women. But the level of follicular fluid BMP15 was higher in pregnant women in compression with non-pregnant women in a highly significant manner (179.71 ± 29.44) versus (144.74 ± 38.12) ($P = 0.001$).

Receiver Operating Characteristic (ROC) Curve Analysis to find the Cutoff Value of BMP15 that can Predict a Positive Pregnancy Outcome

Receiver operating characteristic (ROC) curve analysis was carried out to find the cutoff value of BMP15 that can predict a positive pregnancy outcome and the results are shown in Figure 2 and Table 3. The cutoff value of BMP15 was > 129 but with poor accuracy (56.6%) since the area under curve (AUC) was less than 0.7.

The Correlations of Serum and Follicular Fluid BMP15 to Oocytes Maturity

The correlations of serum and follicular fluid bone morphogenetic protein 15 (BMP15) that measured at day of ova pick up to oocytes maturity are shown in Table 4. MI oocyte count was not significantly correlated to serum (0.195) or follicular fluid BMP15 (–0.005). MII oocyte count showed highly significant positive correlation to serum (0.270) and follicular fluid BMP15 (0.413) ($P < 0.01$).

The Correlations of Serum and Follicular Fluid BMP15 to Embryo Grading

The correlations of serum and follicular fluid bone morphogenetic protein 15 (BMP15) to embryo grading are shown in Table 5. Grade 1 embryo count showed highly significant positive correlation to serum (0.273) and follicular fluid (0.301) BMP15 ($P < 0.01$), Grade 2 embryo count showed significant positive correlation to serum BMP15 (0.215) ($P = 0.032$). While grade 2 embryo count showed non-significant correlation to follicular BMP15 (0.133) ($P > 0.05$). Also grade 3 embryo count showed non-significant correlation to serum (–0.099) and follicular (–0.043) BMP15 ($P > 0.05$).

The Correlations of Serum BMP15 to Peri-follicular and Endometrial Blood Flow Doppler

The Resistive Index (RI) and pulsatility index (PI) of perifollicular blood flow measured by pulsed Doppler ultrasound at the day of ova pickup showed no significant correlation to serum BMP15, RI (0.312), PI (0.309) ($P > 0.05$).

Table 1. **Characteristics of infertile women enrolled in this study**

Characteristic	Total $N = 72$	Positive pregnancy $N = 14$	Negative pregnancy $N = 58$	P
Age (years)				
Mean ± SD	32.25 ± 6.41	29.14 ± 4.54	32.76 ± 6.55	0.050 I S
Range	20–47	23–40	20–47	
Duration of Infertility (years)				
Mean ± SD	7.89 ± 3.87	6.93 ± 3.08	8.05 ± 3.98	0.319 I NS
Range	1–17	2–12	1–17	
Type of infertility				
Primary, *N* (%)	65 (74.0%)	10 (71.4%)	55 (74%)	1.00 Y NS
Secondary, *N* (%)	23 (26.0%)	4 (28.6%)	19 (26%)	
BMI (kg/m²)				
Mean ± SD	26.72 ± 2.81	26.71 ± 2.60	26.72 ± 3.01	0.958 I NS
Range	20.44–30.75	21.46–30.75	20.44–30.70	

N, number of cases; SD, standard deviation; I, independent samples *t*-test; Y, Yates correction for continuity; NS, not significant at $P > 0.05$; S, significant at $P \leq 0.05$.

Table 2. **BMP15 serum and follicular levels and it's relation to pregnancy rate**

Characteristic	Total $N = 72$	Positive pregnancy $N = 14$	Negative pregnancy $N = 58$	P
Serum BMP15				
Mean ± SD	157.89 ± 51.08	169.79 ± 23.22	155.95 ± 54.13	0.350 I NS
Range	14–296	130–213	14–296	
Follicular fluid BMP15				
Mean ± SD	149.64 ± 38.86	179.71 ± 29.44	144.74 ± 38.12	0.001 I HS
Range	24–253	121–253	24–197	

N, number of cases; SD, standard deviation; I, independent samples *t*-test; NS, not significant at $P > 0.05$; HS, highly significant at $P \leq 0.01$.

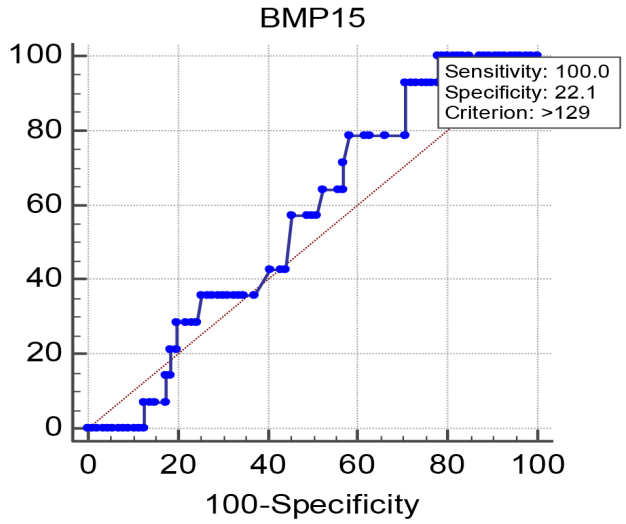

Fig. 2 **Receiver operating characteristic (ROC) curve analysis to find the cutoff value of BMP15 that can predict a positive pregnancy outcome.**

Table 3. **The results of receiver operating characteristic (ROC) curve analysis to find the cutoff value of BMP15 that can predict a positive pregnancy outcome**

Characteristic	BMP15
Cutoff	>129
AUC	0.566
95% CI	0.463 to 0.664
P-value	0.350 NS
Sensitivity %	100.0
Specificity %	22.1
Accuracy %	56.6

AUC, area under curve; CI, confidence interval; NS, not significant.

Table 4. **Correlations of serum and follicular fluid BMP15 oocytes maturity**

Characteristic	Correlation Index	Serum BMP15	Follicular fluid BMP15
Immature metaphase I (MI) oocytes	*R*	0.195	–0.005
	P	0.052	0.962
Mature metaphase II (MII) oocytes	*R*	0.270	0.413
	P	0.007*	<0.001*

*, highly significant at $P \leq 0.01$.

Also the Resistive Index (RI) pulsatility index (PI) of perifollicular blood flow measured by pulsed Doppler ultrasound at the day of ova pickup showed non-significant correlation to follicular BMP15, RI (–0.068), PI (–0.110) ($P > 0.05$).

Furthermore the Resistive Index (RI), pulsatility index (PI) of endometrial blood flow measured by pulsed Doppler ultrasound at the day of embryo transfer showed no significant correlation to serum BMP15, RI (–0.002), PI (0.202) ($P > 0.05$).

Table 5. **The correlations of serum and follicular fluid BMP15 to embryo grading**

Characteristic	Correlation Index	Serum BMP15	Follicular fluid BMP15
Grade 1 embryo	*R*	0.273	0.301
	P	0.006**	0.002**
Grade 2 embryo	*R*	0.215	0.133
	P	0.032*	0.188
Grade 3 embryo	*R*	–0.099	–0.043
	P	0.089	0.114

*, Significant at $P \leq 0.05$; **, highly significant at $P \leq 0.01$.

Also showed non-significant correlation to follicular BMP15, RI (–0.143), PI (0.034) ($P > 0.05$), as shown in Table 6.

Discussion

The main aim of this study was to find an easy, fast, not expensive and available outpatient test to be an indicator for female fertility.

Various studies regarding AMH, FSH, LH, prolactin, E2 and other hormones beside genetic variants investigations have been performed and obtained as a good and specific biomarkers determined in last decade. All of these efforts were used to predict female reproductive potential and they used for different female sexual activities and are investigated with their receptors in different female sexual system disorders affected.[6-10] These hormones are used to estimate growing follicles number in the ovary and to estimate and predict the response of ovaries to stimulation by gonadotropin. These biomarkers provide only an indirect evaluation of oocyte function and yield no information about quality of the oocyte, because they are not derived from the oocyte itself.[11]

BMP15 is known to be secreted only by the oocyte, essential for process of folliculogenesis, quality of the oocyte and female fertility, so these factors could be regarded as oocyte function biomarkers.[12]

Positive pregnancy was achieved by 14 women accounting for 19.0%. The rate was low when compared with other studies like a study done by De Geyter et al. that found that pregnancy rate was 28%,[13] also other study done by Jassim WH, et al. found pregnancy rate to be 25.4%.[14] The pregnancy rate was low because 4 cases of testicular biopsy, 2 cases of moderate endometriosis and also there were 14 case with age above 40 years were included in the this study, furthermore the SARS-CoV-2 (Covid-19) pandemic also might be one of the causes of decrease in pregnancy rate, this is supported by study result done by Maya, W. D. C, et al., that found that germ cell destruction and testicular damage was clearly observed in patient with Covid-19,[15] and the testes that infected with SARS-CoV-2-showed extensive peritubular fibrosis, vascular congestion with extensive destruction of germ cell.[16] Furthermore, SARS-CoV-2 could cause ovarian tissue damage and decline in the function of the ovary and oocyte quality, causing female infertility and may cause miscarriage.[16] A number of cases were not included in counting pregnancy rate, this included five cases of empty follicles, four cases of embryonic developmental arrest, six cases of failed fertilization and one patient refuse embryo transfer. So the number of cases that were included in counting pregnancy rate was 72.

Table 6. **The correlations of serum and follicular BMP15 to perifollicular and endometrial blood flow doppler**

Characteristic	Correlation index	Perifollicular RI	Perifollicular PI	Endometrial RI	Endometrial PI
Serum BMP15	*R*	0.312	0.309	–0.002	0.202
	P	0.207	0.211	0.989	0.217
Follicular fluid BMP15	*R*	–0.068	–0.110	–0.143	0.034
	P	0.788	0.782	0.397	0.839

Relation of Pregnancy Rate to Serum and Follicular Fluid Levels of BMP15

There was no significant difference in mean serum BMP15 between pregnant and non-pregnant women, but the level of follicular fluid BMP15 was higher in pregnant women in compression with non-pregnant women in a highly significant manner.

But a study done by Li et al., on gene expression found that BMP15 mRNA expression levels were closely associated with pregnancy outcomes.[17] Many factors might affect pregnancy rate other than oocyte quality like male factors for example abnormality in DNA as in sperm retrieved by testicular biopsy, also due to bad endometrial receptivity and psychological problems.

Correlations of Serum and Follicular Fluid BMP15 to Oocyte Maturity

The current study showed that MI oocyte count were not significantly correlated to serum or follicular fluid BMP15 ($P > 0.05$) Table 3. While MII oocyte count showed highly significant positive correlation to serum and follicular fluid BMP15 ($P < 0.01$).

The result correspond to study result done by Li et al. on gene expression found that the mRNA expression levels of BMP15 were closely related to maturation of the oocyte, fertilization and outcomes of the pregnancy.[17] Furthermore a study done by others stated that a beneficial synergistic effects are exerted by OSFs on the maturation of nucleus and cytoplasm, rapid energy utilization and oxidative stress management.[18,19] BMP15 is secreted by the oocyte in a primary follicle, which, together organize the granulosa and theca cells that surround the oocyte into oocyte–cumulus–follicle complex. The granulosa at this time secretes AMH, that affects the oocyte. Throughout the development of the follicle, this autocrine–paracrine dialogue between the somatic cells and the oocyte continues and is regarded essential for establishing the fertilization potential and oocytes developmental competency.[20]

The correlations of serum and follicular fluid and bone morphogenetic protein 15 (BMP15) to embryo grading are shown in Table 4. Grade 1 embryo count showed highly significant positive correlation to serum and follicular fluid BMP15 ($P < 0.01$). Grade 2 embryo count showed significant positive correlation to serum BMP15 ($P = 0.032$).

This results were supported by study results of Canosa, S., et al., that found that the blastocyst group (BL) of embryos showed faster kinetic in a significant manner, and the expression of BMP15 mRNA was higher in CCs of this group with significant value, as compared to arrested embryos.[21] Also supported by study done by Daneshjou, D. et al., that found that there is positive correlation between the expression level of BMP15 mRNA with the fertilization rate and grade I embryos.[22]

Conclusion

Accordingly the observed data conclude that:

1. Serum and follicular BMP15 could be used as indicator for oocyte maturity.
2. Serum BMP15 could be used as indicator of grade I embryos.

Conflict of Interest

None.

References

1. Adhikari, D., Lee, I. W., Yuen, W. S., and Carroll, J. (2022). Oocyte mitochondria—key regulators of oocyte function and potential therapeutic targets for improving fertility. Biology of Reproduction, 106(2): 366–377.
2. Mustafa, M., Sharifa, A. M., Hadi, J., Illzam, E., and Aliya, S. (2019). Male and female infertility: causes, and management. IOSR Journal of Dental and Medical Sciences, 18: 27–32.
3. Aghajanova, L., Hoffman, J., Mok-Lin, E., and Herndon, C. N. (2017). Obstetrics and gynecology residency and fertility needs: national survey results. Reproductive sciences, 24(3): 428–434.
4. Sanfins, A., Rodrigues, P. and Albertini, D. F. (2018). GDF-9 and BMP-15 direct the follicle symphony. Journal of assisted reproduction and genetics, 35(10): 1741–1750.
5. Da Broi, M. G., Giorgi, V. S. I., Wang, F., Keefe, D. L., Albertini, D. and Navarro, P. A. (2018). Influence of follicular fluid and cumulus cells on oocyte quality: clinical implications. Journal of Assisted Reproduction and Genetics, 35(5): 735–751.
6. Al-Tu'ma, F.J.; Farhan, N. H. and Al-Safi, W.G. (2015) Association between fat mass and obesity gene (re9939609) polymorphism with PCOS women in Iraqi population. Human Int. J. Pharm. Pharm. Res., 5(1): 62–72.
7. Hassan, MF. (2020) Original research the frequency of elevated prolactin level in polycystic ovary syndrome women and it's effect on pregnancy rate. Global J. Public Health Med., 2(1): 109–117.
8. Al-Lami, H.B.; Al-Tu'ma, F.J. and Al-Safi, W.G. (2020). Association between anti-Mullerian hormone and other biomarkers with ovarian function in polycystic ovarian syndrome of Iraqi women, J. Contem. Med. Sci, 6(4): 168–175.
9. Wand, F.; Dai, W.; Yang, XH.; Guo, YH. And Sun, Yp. (2016). Analyses of optimal body mass index for infertile patients with either polycystic or non-polycystic ovary syndrome during assisted reproductive treatment in China. Sci. Rep., 6(1): 1–9.
10. Al-Faris, N.N; Al-Tu'ma, F. J. and Al-Safi, W.G. (2017). Assessment of CRP and its correlation with total antioxidant capacity in women with polycystic ovarian syndrome. Iraqi Nat. J. of Chem., 17(1): 44–57.
11. Victoria, M., Labrosse, J., Krief, F., Cédrin-Durnerin, I., Comtet, M. and Grynberg, M. (2019). Anti Müllerian hormone: more than a biomarker of female reproductive functions. Journal of Gynecology Obstetrics and Human Reproduction, 48(1): 19–24.

12. Dayanir, D., Ruso, H., Kalem, Z., Gurgan, T. and Ozogul, C. (2019). Rewarding Conversation Between Oocyte and Cumulus Cells Directs the Process of Folliculogenesis. Gazi Medical Journal, 30(4).
13. De Geyter, C., Calhaz-Jorge, C., Kupka, M. S., Wyns, C., Mocanu, E., Motrenko, T. and Goossens, V. (2018). ART in Europe, 2014: results generated from European registries by ESHRE: The European IVF-monitoring Consortium (EIM) for the European Society of Human Reproduction and Embryology (ESHRE). Human reproduction, 33(9): 1586–1601.
14. Jassim, W. H., Al-Obaidi, M. T. and Ghazi, H. F. (2021). The Effect of Intrauterine Infusion of Peripheral Blood Mononuclear Cells Culture on Subendometrial Blood Flow in Patients Undergoing ICSI Cycles. Iraqi Journal of Embryos and Infertility Researches, 10(2): 53–72.
15. Maya, W. D. C., Du Plessis, S. S. and Velilla, P. A. (2020). SARS-CoV-2 and the testis: similarity with other viruses and routes of infection. Reproductive biomedicine online, 40(6): 763–76.
16. Duarte-Neto, A. N., Teixeira, T. A., Caldini, E. G., Kanamura, C. T., Gomes-Gouvêa, M. S., Dos Santos, A. B., and Hallak, J. (2022). Testicular pathology in fatal COVID-19: A descriptive autopsy study. Andrology, 10(1): 13–23.
17. Li, J., Li, C., Liu, X., Yang, J., Zhang, Q., Han, W., and Huang, G. (2022). GDF9 concentration in embryo culture medium is linked to human embryo quality and viability. Journal of Assisted Reproduction and Genetics, 39(1): 117–125.
18. Chandra, V. and Sharma, G. T. (2020). In vitro strategies to enhance oocyte developmental competence. Frontiers in Bioscience-Scholar, 12(1): 116–136.
19. Romaguera, R.; Morató, R.; Jiménez-Macedo, A.R.; Catalá, M Roura, M; Paramio, M.T.; Palomo, M.J.; Mogas, T. and Izquierdo, D. (2010). Oocyte secreted factors improve embryo developmental competence of COCs from small follicles in prepubertal goats Theriogenology, Oct 1; 74(6): 1050–9.
20. Michael, J. D., Campanile, G., and Baruselli, P. S. (2020). Transforming growth factor-β superfamily and interferon-τ in ovarian function and embryo development in female cattle: review of biology and application. Reproduction, Fertility and Development, 32(6): 539–552.
21. Revelli, A., Gennarelli, G., Sestero, M., Canosa, S., Carosso, A., Salvagno, F., and Benedetto, C. (2020). A prospective randomized trial comparing corifollitropin-α late-start (day 4) versus standard administration (day 2) in expected poor, normal, and high responders undergoing controlled ovarian stimulation for IVF. Journal of Assisted Reproduction and Genetics, 37(5): 1163–1170.
22. Daneshjou, D., Mehranjani, M. S., Zadehmodarres, S., Shariatzadeh, S. M. A. and Mofarahe, Z. S. (2022). Sitagliptin/metformin improves the fertilization rate and embryo quality in polycystic ovary syndrome patients through increasing the expression of GDF9 and BMP15: A new alternative to metformin (a randomized trial). Journal of Reproductive Immunology, 150: 103499.