Evaluation of Hormones Serum Level among Women with Different Infertility Causes

Dhuhaa Qasim Neamah Fhamah[1], Abeer Thaher Naji Al-Hasnawi[1], Ali Ibrahim Rahim[2],

[1]College of Medicine, University of Karbala, Karbala, Iraq;[2]College of Medicine, University of Al-Ameed, Karbala, Iraq,
Corresponding author: DhuhaaQasimNeamah Fehamah, g-mail: dhuhaa1988@gmail.com.

ABSTRACT

Background: Infertility is the failure to establish a clinical pregnancy after 12 months of regular and unprotected sexual intercourse. Infertility may occur due to male factor or female factor or both. The most common causes of infertility are ovulatory dysfunction, including polycystic ovaries (PCO) and poor ovarian reserve (POR).

Methods: A cross sectional study includes 100 participants, male factor (n=40) ,PCOS (n=30) and POR (n=30) .The samples of blood were collected at cycle day two and detected the hormonal levels by MINI VIDAS system.

Results: The result of present study showed that follicle-stimulating hormone (FSH), estradiol (E2) , anti-Mullerian hormone (AMH) and Estradiol hormone (E2) at day of human chorionic gonadotropin (HCG) injection in POR were significantly different from male factor and PCOS cases with (*P=0.005, P=0.008, P=0.005, P=0.005*) respectively. About the luteinizing hormone (LH) in Male factor cause was significantly different from PCOS and POR patients with (*P=0.006*). While, prolactin in PCOS cause was significantly different from male and POR causes with(*P=.0.005*).In addition, Total follicle number, Embryo Grade l(GI), Embryo Grade ll (GII) of POR women were significantly different from male factor and PCOS cases with (*P=0.005,P=0.005 and P=0.003*) respectively. Whereas, Embryo Grade III (Glll) of PCOS women was significantly different from male factor and POR patients with (*P=0.030*).

Conclusion: The serum hormonal levels of FSH, E2,AMH and E2 HCG were significant associated with POR infertile women, whereas, prolactin hormone was significant related with PCOS women. Also, the total follicle number, Embryo Grade l(GI), Embryo Grade ll (GII) significantly related with POR women, while, Embryo Grade III (Glll) more related with PCOS infertile women.

Keyword: FSH, E2, Prolactin, AMH and E2 HCG serum level hormones, ELISA test, Infertility causes

تقييم مستوى مصل الهرمونات بين النساء المصابات لاسباب العقم المختلفة

خلاصة

الخلفية: العقم هو الفشل في إثبات الحمل السريري بعد 12 شهرا من الجماع المنتظم وغير المحمي. قد يحدث العقم بسبب العامل الذكوري أو العامل الأنثوي أو كليهما. الأسباب الأكثر شيوعًا للعقم هي ضعف التبويض، بما في ذلك تكيس المبايض (PCO) وضعف احتياطي المبيض (POR).

الطرق: دراسة مقطعية تشمل 100 مشارك، عامل الذكور (العدد = 40)، متلازمة تكيس المبايض (العدد = 30) و POR (العدد = 30). تم جمع عينات الدم في اليوم الثاني من الدورة وتم الكشف عن المستويات الهرمونية بواسطة نظام MINI VIDAS.

النتائج: أظهرت نتائج الدراسة الحالية أن الهرمون المنبه للجريب (FSH)، الاستراديول (E2)، الهرمون المضاد للمولر (AMH) وهرمون الاستراديول (E2) في يوم حقن موجهة الغدد التناسلية المشيمية البشرية (HCG) في POR كانت مختلفة بشكل كبير عن عامل الذكور وحالات متلازمة تكيس المبايض عند (*P = 0.005، P = 0.008، P = 0.005، P = 0.005*) على التوالي. فيما يتعلق بالهرمون اللوتيني

(LH) في العامل الذكوري، كان السبب مختلفًا بشكل كبير عن مرضى متلازمة تكيس المبايض وPOR عند (*P = 0.006*). في حين أن البرولاكتين في سبب متلازمة تكيس المبايض كان مختلفًا بشكل كبير عن الذكور وأسباب POR عند (*P=.0.005*). بالإضافة إلى ذلك، كان إجمالي عدد الجريبات، درجة الأجنةI (GI)، درجة الأجنةII (GII) لدى النساء POR مختلفًا بشكل كبير عن عامل الذكور وحالات متلازمة تكيس المبايض عند (*P = 0.005*، *P = 0.005* و *P = 0.003*) على التوالي. حيث أن الأجنة من الدرجة الثالثةIII (GIII) لدى نساء متلازمة تكيس المبايض كانت مختلفة بشكل كبير عن عامل الذكور ومرضى POR عند (*P = 0.030*).

الاستنتاج: كانت مستويات هرمون FSH وE2 وAMH وE2 HCG في الدم مرتبطة بشكل كبير بالنساء المصابات بالعقم POR، في حين كان هرمون البرولاكتين مرتبطًا بشكل كبير بالنساء المصابات بمتلازمة تكيس المبايض. أيضًا، يرتبط العدد الإجمالي للجريب، درجة الجنينI (GI) ، درجة الجنينII (GII) بشكل كبير بالنساء POR، في حين أن درجة الجنينIII (GIII) أكثر ارتباطًا بالنساء المصابات بالعقم PCOS.

INTRODUCTION:

Infertility can be described as a couple based disease that prevents women from becoming pregnant after one year during regular, sexual relations without protection (**1,2**). The most common causes of infertility are Female factor 40% and male factor 30% (**3**). Male infertility is defined as the inability of a male partner to accomplish a pregnancy in a fertile female partner(**4**). It is accounting for 40–50% of all infertility cases (**5**). Female infertility has many causes, ovulation disorders is one of this causes(**6**).Disorders of ovulation are often present with irregular periods (oligomenorrhoea) or an absence of periods (amenorrhoea). Studies done worldwide prove that the most common causes of female infertility are polycystic ovarian syndrome (PCOS) and primary Ovarian insufficiency (**7,8)**. PCOS is a heterogeneous disorder recognized by a triad of hyperandrogenism, irregular of menstrual cycle and polycystic ovaries morphology. Therefore, patients can present with various manifestations of this triad depending on the patient's age, lifestyle and disease phenotype (**9**). Also, this syndrome is characterized by variation in the levels of different types of hormones such as follicle-stimulating hormone (FSH), luteinizing hormone (LH) and others (**10**).Poor ovarian reserve (POR) is another ovulatorydisorderwhich defined as a decrease in the number and/or quality of oocytes in the ovary, accompanied by decline in the level of anti-Müllerian hormone (AMH), a decrease in the number of antral follicles, and an increase in the level of follicle-stimulating hormone (FSH) (11). Regarding theIntra-Cytoplasmic Sperm Injection (ICSI), which is a single healthy sperm is injected directly into a mature egg.It is a type of assisted reproductive technology (ART). (12)

MATERIALS AND METHODS:

The current study included 100 participants divided into three groups depended on the most common causes of infertility.Male factor representthe first group, which composed of 40 patients. The second group is PCOS, which includes of 30 patients. The third group is POR, which represented 30 patients whose performed Intra-Cytoplasmic Sperm Injection (ICSI) attended to the Fertility Center in AL-Sader Teaching Medical City, Al-Ameer International Center for Fertilization and IVF and Al-Kafeel hospital. All patients have undergone an antagonist protocol and were diagnosed by specialized Gynecologists and Embryologists . The patients age ranged from (18-40) years old.At cycle day two, 5 ml of blood samples were collected from the patients in gel tube and allowed to clot after that serum was separated by centrifugation at 3000 rpm for 5 minutes. Serum was used to determine the FSH, LH, prolactin, E2, A.M.H,E2 day of HCG injection and B.HCG concentrations by MINI VIDAS system.

RESULTS

Demographic characteristics of the infertility causes among study groups:

The patients age ranged from **(**18-40) with mean values (30.00), (29.97) and (31.50) among male factor, PCOS and POR patients respectively with no significant difference between them (*P* =0.439). The mean value of body mass index (BMI) in male factor patients were (28.82) and (26.80) of PCOS while the BMI of POR with mean value (28.69). There were no significant differences in BMI between patients groups (*P*=0.474). About Duration of infertility, the mean values of male factor, PCOS and POR were (9.19), (8.00) and (9.20) respectively with a non significant differences (*P=0.532*).

Concerning the types of infertility, the male factor count of primary infertility was (33), while the counts of PCOS and POR were (23), (22) respectively. Whereas, the male factor count of secondary infertility was (7), while the counts of PCOS and POR were (7), (8) respectively. There was no significant differences about infertility types between patients groups (*P* =0.684). Demographic characteristics of the subjects according to the cause of infertility are presented in table (1).

Table (1): Demographic characteristics according to the cause of infertility.

	Cause of infertility						*P value*
	Male (40)		PCOS (30)		POR (30)		
	Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	
Age (year)	30.00	5.88	29.97	4.72	31.50	5.36	0.439
BMI (Kg/m^2)	28.82	10.36	26.80	3.47	28.69	5.53	0.474
Duration (y)	9.19	4.93	8.00	4.73	9.20	5.06	0.532
	Count	**%**	**Count**	**%**	**Count**	**%**	
Infertility pri.	33	41.0	23	30.8	22	28.2	0.684
Infertility sec.	7	31.8	7	31.8	8	36.4	

Infertility pri.: Primary infertility, Infertility sec.: Secondary infertility, ANOVA test (LSD test), Chi-square test, SD: Standard deviation.

Serum levelof Hormones among patient groups and their associationwith the causes of infertility:

The follicle stimulating hormone mean in male factor was (5.63) and in PCOS group was (5.72), while in POR group was (7.42). However, POR was significantly different from male and PCOS causes (*P=0.005*), as found in table (2).

Also, the luteinizing hormone mean in male factor group was (3.43) and PCOS group was (6.27), while in POR group was (7.01). However, Male factor cause was significantly different from PCOS and POR causes with (*P=0.006*). On the other hand, the mean of prolactin in male factor was (18.79), in PCOS cases were (31.29) and in POR was (16.74). However, PCOS cause was significantly different from male and POR causes with (*P=0.005*).

Regarding Estradiol hormone (E2)at cycle day two, the mean in male factor was (48.07), in PCOS cases was (51.97) and in POR was (33.59). However, POR was significantly different from male and PCOS causes with (*P=0.008*). The anti mullerian hormone (AMH) mean in male factor was (3.28), in PCOS group was (4.13) and in POR was (0.87). However, Male causes was significantly different from PCOS and POR. Also, PCOS was significantly different from POR (*P=0.005).* About the mean of Estradiol hormone (E2) at day of HCG injection in male factor was (2262.58), in PCOS cases was (1951.83) and in POR was (1282.20) However, POR was significantly different from male and PCOS causes with (*P=0.005*).

Table (2): Hormonal study associated to the causes of infertility.

Variables	Cause of infertility						P value
	Male (40)		PCOS (30)		POR (30)		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	
FSH (mIU/ml)	5.63	1.16	5.72	1.84	7.42	3.75	**0.005***
LH (mIU/ml)	3.43	0.99	6.27	4.09	7.01	7.70	**0.006***
Prolactin (ng/ml)	18.79	6.62	31.29	16.32	16.74	7.04	**0.005***
E2 day 2 (pg/ml)	48.07	18.93	51.97	35.56	33.59	11.48	**0.008***
AMH (ng/ml)	3.28	1.17	4.13	2.09	0.87	0.29	**0.005***
E2 HCG (pg/ml)	2262.58	832.43	1951.83	770.88	1282.20	705.68	**0.005***

FSH: follicle stimulating hormone, LH: Luteinising Hormone, E2at day 2:Estradiol hormone at cycle day two, AMH: Anti Mullerian Hormone, E2 HCG: Estradiol Hormone at Human Chorionic Gonadotropin injection . ANOVA test (LSD test).

Clinical characteristics that related with the causes of infertility:

The mean of Intracytoplasmic sperm injection (ICSI) attempt in male factor cases was (0.41) and in PCOS group was (0.35), while in POR group was (0.77) . However, no significant difference between groups (*P=0.060*). About the mean of Total follicle number in male factor cases was (12.33) and in PCOS group was (13.65), while in POR group was (7.43). However, POR was significantly different from male and PCOS causes with (*P=0.005*).

Regarding the mean of Maturity rate in male factor cases was (84.37) and in PCOS group was (76.74), while in POR group was (77.35). However, no significant difference was detected (*P=0.109*). The mean of Fertilization rate in male factor cases was (80.74) and in PCOS group was (79.52), while in POR group was (83.70). However, no significant difference was detected (*P=0.623*).

Also, the mean of embryo Grade l(GI) in male factor cases was (3.28) and in PCOS group was (2.48), while in POR group was (1.20). However, POR was significantly different from male and PCOS causes (*P=0.005*). In addition, the mean of embryo Grade ll (GII) in male factor cases was (3.31) and in PCOS group was (3.39), while in POR group was (2.00). However, POR was significantly different from male and PCOS causes (*P =0.003*). About the mean of embryo Grade III (Glll) in male factor cases was (1.62) and in PCOS group was (2.29), while in POR group was (1.23). However, PCOS was significantly different from POR and male factor causes (*P=0.030*). Concerning the mean of Transferred embryo in male factor cases was (3.69) and in PCOS group was (3.39), while in POR group was (3.07). However, POR was significantly different from male causes (*P=0.015*).

On the other hand, The count of Beta-Human Chorionic Gonadotropins (B.HCG) test, the male factor count of Negative resulte was (28), while the counts of PCOS and POR were (18), (21) respectively. Whereas, the male factor count of positive test was (12), while the counts of PCOS and POR were (12), (9) respectively .There were no significant difference between groups (*P=0.439*), as present in table (3).

Table (3): Mean of clinical characteristics according to the infertility causes

Variables	Cause of infertility						P value
	Male (40)		PCOS (30)		POR (30)		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	
ICSI attempt	0.41	0.64	0.35	0.66	0.77	0.90	0.060
Total follicles	12.33	4.14	13.65	7.18	7.43	4.52	**0.005***
Maturity rate	84.37	14.53	76.74	18.52	77.35	17.70	0.109

Fertilization rate	80.74	14.59	79.52	18.06	83.70	19.40	0.623
Embryo GI	3.28	2.05	2.48	1.61	1.20	1.24	**0.005***
Embryo GII	3.31	1.64	3.39	2.22	2.00	1.31	**0.003***
Embryo GIII	1.62	1.55	2.29	1.68	1.23	1.43	**0.030***
Transferred embryo	3.69	0.61	3.39	0.92	3.07	1.08	**0.015***
	Count	**%**	**Count**	**%**	**Count**	**%**	
B HCG result -ve	28	71.8	18	58.1	21	70.0	0.439
+ve	12	28.2	12	41.9	9	30.0	

ICSI: Intracytoplasmic sperm injection, GI: Grade l, B HCG: Beta-Human Chorionic Gonadotropins, ANOVA test (LSD test), Chi-square test

DISCUSSION:

The age of patients ranged from (18-40) with no significant result (*P=0.439*) between the studied groups.This results were compatible with other studies such as the study accomplished by (**13**),who found the mean age of male factor and PCOS were (32.6), (34.8) respectively and a study conducted by (**14**), in which the mean age (27.7) which reported a non significant result between the patients and control groups about age of PCOS group. Concerning the POR group the mean age of women in the our study wasidentical to the study of (**15**), in which mean age was(32.00) with a non significant differences between the two groups (P > 0.05). While the study by (**16)**,in which the mean age was (36.68) and there was significantly lower in the control group compared with the POR patients group with (*p< 0.001*).

In addition, the study showed there was no significant differences in BMI between patients groups with (*P=0.474*). The results of current study were related with other studies such as the study by (**17**) and **(18**),whose reported the BMI means were (23.4), (23.6) of male factor and PCOS respectively with no significant association was observed (P > 0.05) . Also, a study by (**19**), in which BMI mean of male factor was (23.97) withno significant result and the study by **(20**), in which BMI mean was (27.19) in PCOS groupwithno significant difference (*P>0.05*). Several studies have proven the obesity prevalence among PCOS, ranging from 42% to 62.5%. (**21**),reported that BMI mean as (34.3). At the same time, (**22**) and a study of (**23**), reported that BMI were (27.4) and (25.5), respectively. There is broad variability in the prevalence of overweight and obese women in PCOS populations in different countries. The BMI mean of women with the POR group in the present study was (28.69) .This finding is related with the study by(**15**), in which the BMI mean was(32.00) with no significant differences between the two groups (P > 0.05).

About duration of infertility, there were a non significant differences (*P=0.532*) between cases.It is related with other studies such as the study by (**24**), in which the mean of infertility duration was (7.51) with no significant result. Also, the study by (**25**),in which mean of infertility duration was (5) with no significant difference. The mean of infertility duration of women with the PCO group in our study was incompatible with the (**26**), who found the mean of infertility duration was (2.9). Whereas, the mean of infertility duration of women with the POR group in this study was (9.20) which is similar to the (**15**) in which mean of infertility duration was (9.3) with no significant differences (P > 0.05)

Regarding the types of infertility, there was no significant differences about infertility types between patient groups with (*P=0.684*) in current study . Several studies such as the study by(**25**),whose reported that primary infertility count was 37 (74%) and secondary infertility was 13 (38%) of male factor infertility. The study by (**24**),whose found the primary infertility was (34) and secondary infertility was (11) with no significant difference. In addition, the study by (**13**),who demonstrated that primary count was (42) of male factor and count of secondary was(25), while, in PCOS the primary count was (6) and secondary count was(2). These results were incompatible with the results of present study might be due to ethnic differences or small samples size.

About the hormonal levels according to the cause of infertility in patients groups. The POR patients were significantly different from the male and PCOS causes with (*P=0.005*). The mean of FSH in male factor and PCO were no significant, it is associated with other studies such as the study of (**27**), in which fifty PCOS women were recruited for estimation in which serum FSH mean was (6.10) and a study by(**13**) who reported the mean of FSH in male factor was (5.6), in PCOS group was (5.7) , with no significant result, the levels of FSH was normal in these groups. Also, a study by(**28**), who found the FSH mean in PCOS cases was (5.03) with *(P*=0.045) and the study by (**29**), whose found the mean of FSH was (5.7) in PCOS cases. The FSH determinations are characterized by some difficulties such as the inconvenience of the required blood draw on day 2 or 3 of menses(**30**).

About the mean of luteinizing hormone (LH), the male factor cause was significantly different from PCOS and POR causes with (*P=0.006*).Our result agree with the study of (**31**),who noted mean of LH was (3.8) with no significant result . Also, a study accomplished by(**32**), who demonstrated LH mean was (3.2) with no significant result (*P=0.20*). Regarding the PCOS group mean was higher than male factor this consistent with many studies such as the study by (**33**), who found the endocrinological disorder which is linked to hypersecretion of LH and ovulatory dysfunction is attributed to increased levels of LH . And the study by **(29**),revealed that mean of LH in PCO was (6.95). While, current study disagree with the study conducted by (**34**), whose reported the association were non significant (*P*=0.429) between PCOS patients and the control.

Regarding the prolactin hormone, the PCOS women were significantly different from male and POR cases with (*P=0.005*).It is related with other studies such as the result of (**35**),who found higher level of prolactin in PCOS of a group of Bangladeshi women and a study by(**28**), whose demonstrated a highly significant result (*P=0.001*) in PCOS causeconcerning serum prolactin. Also, a study conducted by (**36),** in Bangladeshi, observed hyperprolactinemia in 18.6% of PCOS patients. But this study incompatible with the study by (**34**), whose foundthat mean of prolactin in PCOS cases was (25.24) and(20.93) in healthy control with non significant result (*P=0.111*).

About the Estradiol hormone (E2)at cycle day two, the POR women was significantly different from the male and PCOS causes with *P=0.008*). It is similar with other studies like the study of (**37**), who found the Estradiol hormone mean in non PCO was (53.8) and in the PCOS cases was (51.97) with no significant result.Concerning Estradiol hormone (E2) at cycle day two in POR patients, several studies conducted by (**15**) and (**31**), whose revealed that mean of Estradiol hormone were (30.10) and (4.9) respectively with non significant result.

Concerning the anti mullerian hormone (AMH), the male factor was significantly different from PCOS and POR. Also, PCOS was significantly different from POR with (*P=0.005).* A study by (**29**) found the mean of AMH was (7.04) in PCOS patients. Another study accomplished by (**15**) revealed the mean of AMH of POR cases was (0.58) and control was (2.56) with significant association between groups (*P=0.001*).

About the mean of Estradiol hormone (E2) at day of HCG injection, the POR caseswere significantly different from male and PCOS causes with (*P=0.005*). The mean of Estradiol hormone (E2) at day of HCG injection in male factor was non significant .It is similar to the study by **(37**), who found the mean of E2 level on HCG of non PCOS patients was (3911.0) with (*P= 0.001*), while the mean of this hormone in PCOS was (1951.83). Another study by(**29**), whose reported the mean of Estradiol hormone at day of HCG injection was (3565.92). The difference was not found to be significant. In present study the mean of Estradiol hormone at day of HCG injection in POR was significantly different from male and PCOS causes with (*P=0.005*). A study conducted by (**38**) revealed that mean of E2 on HCG day was (684.66) in POR cases. However, The differences between these studies and our study might be due to the a small sample size or differences in study design and geographical areas.

About clinical characteristics according to the cause of infertility, there were no significant result between patient groups (*P=0.060*) regarding Intracytoplasmic sperm injection (ICSI) attempt. A study conducted by (**39**), reported that the patients with previous ICSI attempts have a higher number of large follicles proportion compared with patients undergoing their first ICSI trial.

Likewise, fertilization rate was higher (74.4% versus 59.7%) in patients with previous ICSI attempts and first attempt respectively. This study incompatible with present study and this may be due to small samples size or different methods procedure used in present study.

Concerning the mean of Total follicle number , the POR was significantly different from male and PCOS patients with (*P=0.005*). In male factor and PCO was good number of follicles because stimulation by antagonist protocol and good timing of the administration of HCG. A study conducted by (**33**), found the mean of total follicles number in PCOS patients was (6.945). And a study by (**25**),whose reported the median of total oocytes was (12) associated with good stimulation of E2,AMH and FSH .

Regarding the mean of maturity rate, no significant difference was detected (*P=0.109*). Our result was inconsistent with (**40**), who found a significant result with (*P=0.006*) between maturity rate and PCOS cause.

About the mean of Fertilization rate, no significant difference was detected (*P=0.623*). These results confirm the importance of ICSI treatment in ensuring a high fertilization rate regardless of the causes of fertility. The present study result compatible with a study accomplished by (**41**),who found the fertilization rate of infertile patients was (88.02). ICSI is effective and improve fertilization rate of oocytes .

Also, the mean of Embryo Grade l(GI) and Grade ll (GII), POR was significantly different from male and PCOS cases with (*P=0.005*) and (*P=0.003*). Current study result agreed with study of (**13**) who reported good quality embryos (embryo Grade I and ll) of male factor was (3.8) , PCO was (2.9) and POR was (1.0). POR patients was significantly different from other patients groups. In addition, the mean of Embryo Grade III (Glll), PCOS was significantly different from POR and male factor causes with (*P=0.030*). A study conducted by (**42**), revealed to that decrease of AMH was correlated with bad quality of embryos (Embryo Grade III) .

On the other hand, the mean of transferred embryo in POR women was significantly different from male cases with (*P=0.015*). Current study result was agree with a study of (**43**), who found a non significant result with (*P >0.05*) between the PCOS (4.0± 1.5) and non PCOS (4.0± 1.2) women regarding quality of embryos. Regarding POR in present study, the low number of Oocytes and their bad quality result in a decrease number of embryos that suitable for transfer. This result compatible with a study result of (**44**), who demonstrated the associated with number and quality of oocytes and embryos.

Furthermore, the count of Beta-Human Chorionic Gonadotropins (B.HCG) test with no significant difference between groups (*P=0.439*). The pregnancy rate not depended on quality and number of embryos but also associated with others factor effect on implantation rate. In this study no significant between B.HCG test and causes of infertility groups, this is proof that treatment for ICSI is the best option for pregnancy. This result disagree with study by (**45**), who appreciate the relation between outcome of ICSI and various infertility causes and reported the different success ICSI rate in various infertility causes.

Conclusion: Current study found that serum levels of FSH, E2,Prolactin, AMH and E2 at HCG injection hormones were significantly different between PCOS and POR cases. Further studies with large samples size are needed for recognizing the role of these hormones among infertile women.

REFERENCES

1. Thoma, M., Fledderjohann, J., Cox, C. and Adageba ,R. K. . Biological and Social Aspects of Human Infertility: a global Perspective. 2021;*In Oxford research encyclopedia of global public health.*
2. World Health Organization. Infertility.2022; WHO. Available (Accessed: 7 July 2022).
3. El Adlani , S., Benksim , A., KaddourM, Y. A. B., Soummani A.,&Cherkaoui, M. Infertility: knowledge and attitudes of Moroccan young people—gender approach . 2021;*Middle East Fertility Society Journal* 26(1), 1-5.
4. Shah, K.M., Gamit, K. G., Raval, M. A., & Vyas, N. Y. Male infertility: A scoping review of prevalence, causes and treatments. 2021;Asian Pacific Journal of Reproduction ,10(5), 195-202.

5. Bold, J., & Swinburne, D. Pre-Conceptual Guidelines for Men: A Review of Male Infertility Experience, including Nutrition and Lifestyle Factors .2022;*Dietetics , 1*(3),164-181.
6. Sala U., G.M., Wahed, M.I., Haque, A. and Nejum, M.R. .Current ConSequence and Research of Human Infertility in Bangladesh. 2018;Journal of Reproductive Endocrinology & Infertility
7. Deshpande, P. S., & Gupta, A. S. Causes and Prevalence of Factors Causing Infertility in a Public Health Facility. 2019;Journal of Human Reproductive Sciences, 12(4), 287.
8. Man , L.; LustgartenGuahmich, N.; Vyas, N.; Tsai, S.; Arazi, L.; Lilienthal, D.; Schattman,G.; Rosenwaks, Z.; James, D.. Ovarian Reserve Disorders, Can We Prevent Them? A Review.2022*; Int. J. Mol. Sci.* 2, 23, 15426.
9. Meier, L., Joham ,A.E., Norman, R.J., Stener –Victorin ,E.,…& Teede, H.J.. Polycystic ovary syndrome. 2022;The Lancet Diabetes & Endocrinology, 10(9),668-680.
10. Teede, H.J.; Misso, M.L.; Boyle, J.A.; Garad, R.M.; McAllister, V.; Downes, L.; Gibson-Helm, M.; Hart, R.J.; Rombauts, L.; Moran, L.; *et al.* International Evidence-Based Guideline for the Assessment and Management of Polycystic Ovary Syndrome 2018; Monash University: Melbourne, Australia, 2018
11. Cohen, J., Chabbert-Buffet, N., Darai .E. Diminished ovarian reserve, premature ovarian failure, poor ovarian responder—a plea for universal definitions.2015; .*Journal Assised Reproductions andGenetic*,32,(12), pp.1709-1712.
12. Geng, T., Cheng, L., Ge, C., & Zhang, Y. . The effect of ICSI In infertility couples with non-male factor: a systematic review and Meta-analysis. 2020;*Journal of Assisted Reproduction and Genetics*, 37(12), 2929–2933.
13. Sarapik, A., Velthut, A., Haller-Kikkatalo, K., Faure, G. C., Béné, M. C., de CarvalhoBittencourt, M., … &Salumets, A. . Follicular proinflammatory cytokines and chemokines as markers of IVF success. 2012;*Clinical and Developmental Immunology*, 2012, 606459.
14. Al-Musawy SH, Al-Saimary I, Sherif M. Levels of cytokines profile in polycystic ovary syndrome. 2018; *Med J Babylon* ,15(2)
15. Peihao L.1, Xiruo Zhang, Jingmei Hu, Linlin Cui, ShidouZhao, Xue Jiao, Yingying Qin . Dysregulated cytokine profile associated with biochemical premature ovarian insufficiency . 2020;*AmericanJournal of Reproductive Immunology* ,84(4),e13292.
16. Bouet,P., Thomas Boueilh, Juan Manuel Chao de la Barca, Lisa Boucret, Simon Blanchard, VéroniqueFerré-L'Hotellier, Pascale Jeannin, Philippe Descamps, Vincent Procaccio, PascalReynier, Pascale May-Panloup. The cytokine profile of follicular fluid changes during ovarian ageing . 2020; *Journal of gynecology obstertrics and human reproduction*,49(4),101704.
17. Spanou, S.,Kalogiannis, D., Zapanti, E.,…&Mastorakos, G.. Interleukin 15 concentrations in follicular fluid and their effect on oocyte maturation in subfertile women undergoing intracytoplasmic sperm injection.2018;*Journal of assisted reproduction and genetics*, 35,1019-1025.
18. Martinez, R. M., Baccarelli, A. A., Liang, L., Dioni, L., Mansur, A., Adir, M., … &Machtinger, R. Body mass index in relation to extracellular vesicle–linked microRNAs in human follicular fluid. 2019;*Fertility and sterility*, 112(2), 387-396..
19. Mehta, B. N., Chimote, M. N., Chimote, N. N.,Nath, N. M., &Chimote, N. M. Follicular-fluid anti-Mullerian hormone (FF AMH) is a plausible biochemical indicator of functional viability of oocyte in conventional in vitro fertilization (IVF) cycles. 2013; *Journal of human reproductive sciences*, 6(2), 99.
20. Kudsy, M., Alhalabi, M., & Al-Quobaili, F. Follicular fluid Vascular Endothelial Growth Factor (VEGF) could be a predictor for pregnancy outcome in normo-responders and polycystic ovary

syndrome women undergoing IVF/ICSI treatment cycles.2016;*Middle East Fertility Society Journal*, 21(1), 52-56.

21. Sangabathula, H. & Varaganti, N.. Clinical profile polycystic ovarian syndrome-100 cases. 2017;*Journal of Contemporary Medical Research*, 4, 1249-53.
22. Joshi, A., Yonzon, P. &Tandukar, S. Clinical profile of patients with polycystic ovarian syndrome in Nepal. 2017;*EndocrinolMetabInt J*, 4, 00083.
23. Alakananda, Prasad and Goel, . A Study on Clinical Profile of Patients with Polycystic Ovarian Syndrome. 2015;*Int J Sci Res* [Internet], 6, 2319-7064.
24. Ali R.A. , Al-Murshidi S. Y. and Al-Jarrah.D. M. Effect of Interleukin-1beta in the Serum and Follicular Fluid on Intracytoplasmic Sperm Injection Outcome in Women.2018;*Thesis for the Degree of Master of Science in Clinical Embryology*
25. Swadi,N.N., Edan,S.B.,& Al-Dulaimi, A.R.. Serum and Follicular Fluid Thyroid Hormone Levels and Intra-Cytoplasmic Sperm Injection (ICSI) Outcomes . 2023;Thesis, *for the Degree of Master of Science in Medical Physiology.*
26. Agarwal , A., Mulgund, A., Hamada, A., and Chyatte, M.R. .. A unique view on male infertility around the globe. 2015;*Reproductive. Endocrinology*, 13(1), 11-9.
27. Anwary, Alfazzaman and Begum . A clinical study on PCOS patients in a Tertiary Hospital. 2010;*Medicine today*, 22, 34-37.
28. Mehde, A.A. and Resan, A.K.. Study of Several Biochemical Features in Sera of Patients with Polycystic Ovaries and Compared with the Control Group. 2014;*Australian Journal of Basic and Applied Sciences* ,8(10): 620-627.
29. Jain N, Malik S, Prakash V.. Impact of various PCOS phenotypes on oocyte competence in an ART cycle. 2022;*Clin J Obstet Gynecol*.; 5: 067-071.
30. Hehenkamp, w. j., looman, c. w., themmen, a. p., de jong, f. h., tevelde, e. &broekmans, f. j.. anti-mullerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. 2006;*the Journal of Clinical Endocrinology & metabolism*, 91, 4057-4063.
31. Ye, H., Huang, G.N., Zeng, P.H., Pe,i L. IVF/ICSI outcomes between cycles with luteal estradiol (E2) pre-treatment before GnRH antagonist protocol and standard long GnRH agonist protocol: a prospective and randomized study. *J Assist Reprod Genet*. 2009 Mar;26(2-3):105-11
32. Liu M, Liu S, Li L, Wang P, Li H and Li Y. .LH Levels May Be Used as an Indicator for the Time of Antagonist Administration in GnRH Antagonist Protocols—A Proof-Of-Concept Study.2019; Front. Endocrinol. 10:67.
33. Lisi, F., Rinald,i L., Fishel S, Caserta D, Lisi R and Campbell A . Evaluation of two doses of recombinant luteinizing hormone supplementation in an unselected group of women undergoing follicular stimulation for in vitro fertilization. 2005;Fertil Steril 83, 309–315
34. Rawdhah,,.H.K.K.M. , AL Hasnawi ,A.T.N., Hadi ,Z.J.H .Role of Interleukin 17A Gene Polymorphism and Serum Level in Patients with Polycystic Ovary Syndrome. 2023; .*Human Reproduction*, Volume 15, Issue 6, 1 June 2000, Pages 1221–1224.
35. Begum, F. Clinical and hormonal profile of polycystic ovary syndrome. 2009;*Journal of SAFOG*, 1, 22-25.
36. Islam, S., Pathan, F.,& Ahmed, T..Clinical and Biochemical Characteristics of Polycystic Ovarian Syndrome among Women in Bangladesh. 2015; *medical journal* :MMJ,24(2),310-318.
37. Zhang W, Tian Y, Xie D, Miao Y, Liu J, Wang X. The impact of peak estradiol during controlled ovarian stimulation on the cumulative live birth rate of IVF/ICSI in non-PCOS patients.2019; J Assist Reprod Genet. 2019 Nov;36(11):2333-2344.
38. Kavrut M, Kahraman S, Kumru P. Automated measurement of follicle volume for the determination of trigger day in poor responder IVF patients. 2022;Med J;53(2):53–57.

39. Alhilali, M.J.S., Parham, A., Attaranzadeh, A., Amirian, M., &Azizzadeh, M. . IL-5 in follicular fluid as a negative predictor of the intracytoplasmic sperm injection outcome. 2019;*Cytokine*, 113,265-271.
40. Ms, K, Am M, Km M, K G.Gonadotrophin releasing hormone antagonist in IVF/ICSI. 2008;*J Hum Reprod Sci*. Jan;1(1):29-32.
41. Xu, J., Yu Y, Xue M, Lv X. Intracytoplasmic Sperm Injection Improves Normal Fertilization Rate and Clinical Pregnancy Rate in Male Infertility. 2022*;Contrast Media Mol Imaging*. 31;2022:1522636
42. Lin, W.Q., Yao, L.N., Zhang, D.X., Zhang, W., Yang, X.J., Yu, R.The predictive value of anti-Mullerian hormone on embryo quality, blastocyst development, and pregnancy rate following in vitro fertilization-embryo transfer (IVF-ET).2013;J Assist Reprod Genet.,30(5):649-55
43. Homburg,R.,Berkowitz,D.,Levy,T.,Feldberg,D.,Ashkenazi,J.,&Ben-Rafael,Z..In vitro fertilization and embryo transfer for the treatment of infertility associated with polycystic ovary syndrome. 1993;Fertility and sterility, 60 (5) , 858-863.
44. Opsahl, MS, Blauer KL, Black SH, *et al*.The number of embryos available for transfer predicts successful pregnancy outcome in women over 39 years with normal ovarian hormonal reserve testing. 2001;*J Assist Reprod Genet.* 2001;18:551–556.
45. Ashrafi, M., Sadatmahalleh, S. J., Akhoond, M. R., Ghaffari, F., &Zolfaghari, Z. ICSI outcome in infertile couples with different causes of infertility: a cross-sectional study. 2013;*International journal of fertility & sterility*, 7(2), 88.