wjpmr, 2018,4(12), 78-84

SJIF Impact Factor: 4.639

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

OOCYTE DYSMORPHISM IN WOMEN WITH PCOS AND ITS' EFFECT ON EMBRYO DEVELOPMENT FOLLOWING ICSI

[1]*Muhjah Falah Hassan and [2]Hind Abdul-Kadim

[1]Department of Urosurgery, Clinical Embryology, College of Medicine, University of Karbala / Kerbala – Iraq.
[2]Department of Urosurgery and Infertility, College of Medicine, University of Kufa / Kufa – Iraq.

***Corresponding Author: Muhjah Falah Hassan**

Department of Urosurgery, Clinical Embryology, College of Medicine, University of Karbala / Kerbala – Iraq.

Article Received on 17/10/2018 **Article Revised on 07/11/2018** **Article Accepted on 28/11/2018**

ABSTRACT

Background: Polycystic ovary syndrome (PCOS), with a prevalence of 5% is one of the major causes of ovulatory dysfunction among reproductive age women. Folliculogenesis in the PCOS is often impaired, leading to suboptimal oocyte development, reduced oocyte competence for fertilization, normal embryonic development and achievement of a successful pregnancy. A combination of excess androgen and luteinizing hormone (LH) in PCOS patients might be responsible for follicular atresia and poor quality embryo. The quality of an embryo to a great extent depends on the quality of oocyte from which it was obtained. Morphological deviation of Oocyte as a result of hormonal imbalance can be a reflection of pathophysiological events in this syndrome. ICSI is considered as a treatment option for patients with PCOS after conventional treatments failure. **Aim:** The aim of this research is to study the possible association between polycystic ovary syndrome and certain morphological abnormalities of oocyte and whether this dysmorphism affect fertilization rate and subsequent embryonic development. **Materials and Methods:** One hundred three infertile couples were included in this study. They divided in to two groups. Group I: female partners with PCOS and group II: females without PCOS. Both were included in ICSI program. Microscopic evaluation of oocytes and embryos was done and the results were compared between them. **Results:** The study was showed that the percentage of oocytes with abnormal morphology was significantly more in PCOS women 17.5 % versus 9 % in non-PCOS, p-value=0.046, there was no significant statistical variation regarding both fertilization rate and cleavage rate (71.49 % in PCOS VS 73.38 % in non-PCOS, p-value=0.40 and 93.86 % in PCOS VS 95.77 % in non-PCOS group, p-value=0.59 respectively) .No significant statistical difference between two groups regarding the number of good quality embryos 6.08±4.39 in PCOS VS 4.65±3.43 in non-PCOS, p-value=0.07. **Conclusion:** Women who suffered from PCOS tend to produce larger number of oocytes with several significant dysmorphic features associated with post maturity after controlled ovarian hyper stimulation. These morphological abnormalities had no adverse effect on fertilization rate (FR), cleavage rate (CR) and embryo quality. PCOS women can produce good quality embryos despite higher percentage of poor quality oocytes.

KEYWORDS: PCOS, Oocyte quality and embryo development.

INTRODUCTION

Polycystic ovary syndrome is a condition of chronic an ovulation affecting the women of reproductive age group which usually occurs as a result of an imbalance of reproductive hormones. Women with PCOS produce abnormally high androgen level which leads to failure of ovulation, skipping of menstrual period and difficulty in getting a pregnancy.[1] The syndrome is diagnosed depending upon Rotterdam criteria : menstrual irregularity, elevated male hormones levels (androgens) and polycystic ovarian morphology by trans-vaginal / abdomenal ultrasound.[2]

The syndrome poses an interesting problem for reproductive potential in young women. An ovulation is common among women with PCOS and represents 80-90% of WHO group II an ovulatory sub-fertility.[3]

Several factors may play a role in decreasing fertility in PCOS female other than an ovulation, these including the effects of increased body weight, genetic variations, inflammatory, metabolic and endocrine abnormalities on the quality of oocyte and fetal development.[4-6]

Oocytes derived from polycystic ovaries may show reduced developmental competence, reduced ability to complete meiotic division, achieve successful fertilization and develop into a viable good quality embryo. It is believed that the follicular microenvironment is related to oocyte quality. Follicular testosterone levels were significantly high in PCOS,

especially in those with incompetent meiosis.[7] This will badly affect the follicular microenvironment, alter the interactions between granulose cell and oocyte, cause premature luteinization of granulose cell, impair nuclear and/or cytoplasmic maturation of oocytes and lower fertilization rate.[8]

Increased LH concentrations also have been seen in PCOS.[9] This might contribute to decrease the quality of oocyte and increase miscarriage rate.[10]

Treatment focuses on weight reduction and lifestyle modification followed by ovulation induction.[11] Women who fail to ovulate can be stimulated with low dose gonadotropins. For those who remain refractory to these treatments or with coexisting pathologies, assisted reproductive techniques (ARTs) can be an option to be considered with a close supervision to produce the desired outcome of pregnancy with the challengingly sensitive polycystic ovary.[12] Intra cytoplasmic injection ICSI offers an opportunity to assess and evaluate the morphology of oocytes and analyze the correlation between oocyte morphology and subsequent embryonic development.[13] Moreover, detailed evaluation of oocyte morphology gives a new opportunity to analyze the effect of PCOS on oocyte and embryo quality, the effectiveness of different stimulation protocols.[4] Oocyte well being considered as a key factor affecting embryo quality. Currently, evaluation of oocyte morphology is the most objective measure to assess its' quality with multiple morphological features are used to predict the embryo development.[13]

However, variability has been considered and the quality of oocyte, rate of fertilization and embryo quality in PCOS women may not be affected,[4] So, the effect of polycystic ovary syndrome on various aspects of the female reproductive potential is a matter of debate and the ability of assisted reproductive techniques to bypass their fertility problem is a subject of a wide research.

Study design

This is a prospective cohort study that was conducted in the Fertility Center, Al- Sadr Medical City, Al- Najaf AL-Ashraf / Iraq.

MATERIALS AND METHODS

One hundred three sub-fertile couples were included in this study and all of them were involved in intra cytoplasmic sperm injection (ICSI) program throughout the period from October 2017 to June 2018.

The age of females partners was equal to or less than 35 years old. The mean duration of infertility period was 7.72 ± 4.06 years.

Those infertile couples were divided into two groups

- **Group I:** Female partners with PCOS selected depending on Rotterdam criteria which include history of oligo / amenorrhea, clinical and/or biochemical signs of hyper androgenism and investigations : cycle day two hormonal analysis which include (estrogen (E2), luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone and prolactin) and ultrasonic feature of polycystic ovaries (according to Rotterdam/ ESHRE consensus workshop 2004) .
- **Group II:** Female partners with no PCOS.

The male partners of both groups were either have normal semen parameters or with mild to moderate male factor infertility according to WHO criteria 1999. Male partners with sever male factor infertility and frozen sperms obtained from epididymis by percutaneous epididymal sperm aspiration or testicular biopsy had been excluded. Female partners with endometriosis and couples with unexplained infertility also had been excluded.

Male and female partners of both groups had been evaluated by urologist and gynecologist respectively depending on history, physical examination, anthropometric measures (weight, height and body mass index (BMI)) and fertility investigations. All the information about those couples was remained secret. Females of both groups had been subjected to pituitary down regulation using either gonadotropin releasing hormone (GnRH) antagonist or agonist then controlled ovarian hyper stimulation by recombinant FSH (r-FSH) which was done under a close supervision by serial trans-vaginal ultrasound (TVUS) and hormonal assay. Ovulation trigger was done by human chorionic gonadotropin (HCG) injection when the total number of the follicles and their size are adequate. Oocytes pickup was done by the gynecologist under general anesthesia. Assessment of oocytes morphology was done by the embryologist after denudation of the oocytes for better visualization and manipulation.

Oocytes were assessed for their morphological abnormalities microscopically by using an inverted microscope at 400x magnification. It was usually equipped with differential interference contrast optics (Nomarski contrast optics). It was accepted that only mature oocytes that resume their first meiosis (MI) and reaching second meiosis (MII) are appropriate for ICSI.

Four morphological abnormalities, two extracellular abnormalities: wide PVS and morphologically abnormal IPB and two intracellular abnormalities: cytoplasmic granularity and large multiple cytoplasmic vacuoles were studied because they were the most frequently seen.

Most defective oocytes were exhibit more than one of the above mentioned abnormalities. All these observations had been assessed and recorded. They help later on for analysis of the fertilization rate (FR), cleavage rate (CR), embryo development and quality after ICSI.

Figure 1: Normal size denuded MII oocyte with homogenous, slightly clear cytoplasim and normal IPB extruded within normal size PVS 400 x magnification.[14]

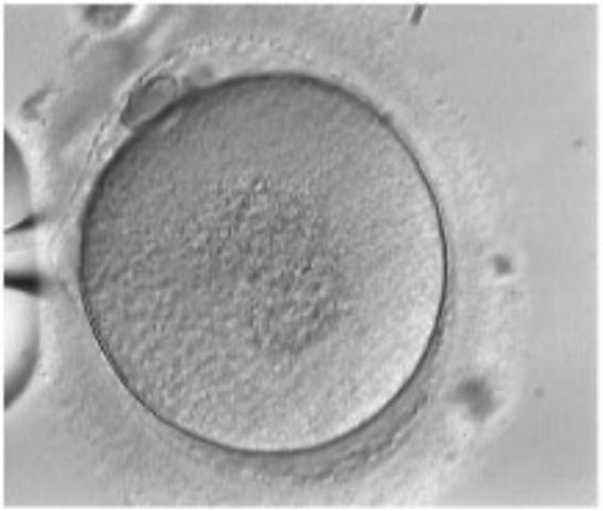

Figure 2: Denuded MII oocyte with central cytoplasmic granularity (400 x magnification).[15]

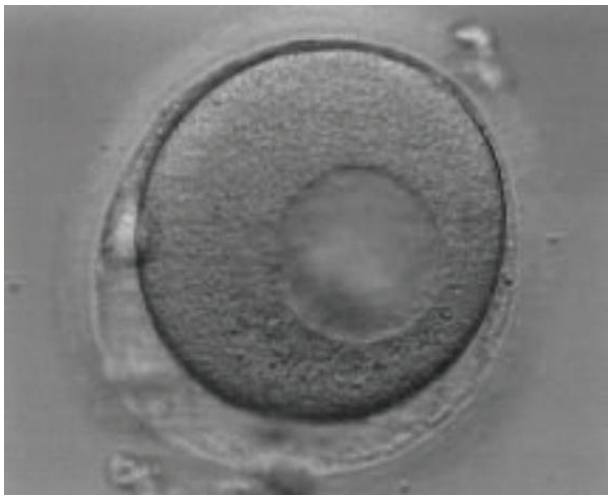

Figure 3: Denuded MII oocyte with large central cytoplasmic vacuole (400 x magnification).[14]

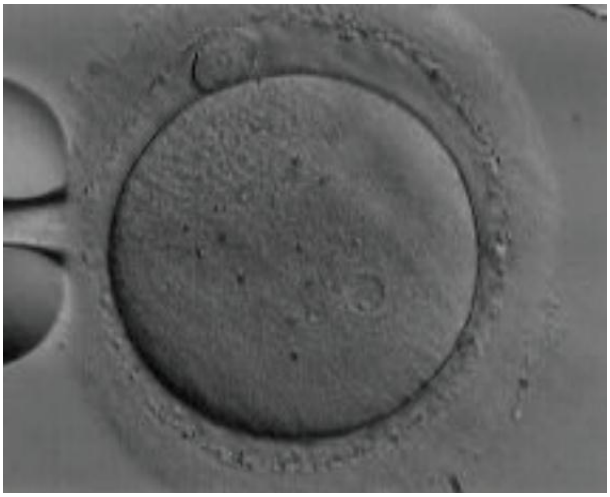

Figure 4: Denuded MII oocyte with wide PVS and debris within it (400 x magnification).[15]

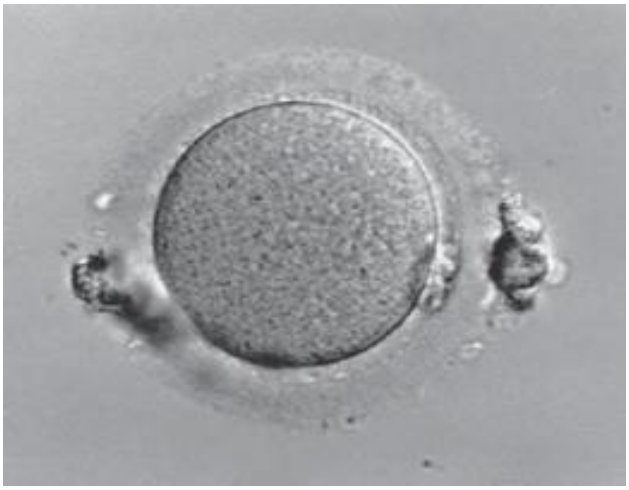

Figure 5: Denuded MII oocyte with fragmented IPB (400 x magnification).[14]

Females of both groups were subjected to ICSI. Assessment of embryo development represented by fertilization rate, cleavage rate and embryo quality was done (embryos with higher cell numbers, regular appearing cells, and little or no fragmentation were considered as good quality (grade I and II), while those with abnormal cell numbers, irregular shape and with high percentage of fragmentations were considered as bad quality (grade II and III).[16]

RESULTS

One hundred three infertile couples were included in this study and divided in to two groups:
Group I In which female partner has PCOS.
Group II In which female partner has no PCOS.

Table (1) shows the means of age, BMI, duration and the type of infertility in both groups. There were no significant statistical differences regarding these parameters between both groups. p-value > 0.05.

Table 1: A comparison between both groups regarding age, BMI, duration and type of infertility.

Parameter	Non-PCOS (N=50) Mean ±SD	PCOS (N=53) Mean ±SD	P-value
Age (years)	27.96 ± 4.06	27.98 ± 3.85	0.55
BMI (Kg/m^2)	27.83 ± 4.87	28.92 ± 4.51	0.69
Duration(years)	7.74 ± 4.06	7.28 ± 3.43	0.37
Primary infertility	34	40	0.399
Secondary infertility	16	13	

Table (2) shows the types of induction protocols which were used during COH and the total dose of gonadotropins. Three types were used : antagonist protocol, short agonist protocol and long agonist protocol. For the non-PCOS group, the short agonist protocol was the preferred one and used for 30 out of 50 females, the antagonist protocol was the second choice and used in 17 females, while only 3 women were stimulated by long agonist protocol. In the PCOS group, the antagonist protocol was the preferred one and used in 41 females out of 53. The short agonist protocol was used in 12 females with no female was stimulated by long agonist protocol in this group. Thus, there was a significant statistical difference between the two groups, p-value = 0.001. There were no significant statistical difference regarding the total dose of gonadotropins, p-value = 0.945.

Table 2: A comparison between PCOS and non-PCOS regarding stimulation protocols and total dose of gonadotropins.

Protocol	Non-PCOS (Total no.)	PCOS (Total no.)	Total	P-value
Antagonist	17	41	58	0.001*
Short agonist	30	12	42	
Long agonist	3	0	3	
Total	50	53	103	
Total dose of gonadotropin (IU)	**Mean ± SD** 1925.2 ± 1968.7	**Mean ± SD** 1663.20 ± 615.2		0.945

Table (3) shows the fertility potential of the male partner in both groups according to their semen parameters. There was no significant statistical difference between them, p-value = 0.45.

Table 3: Shows the fertility potential of male partner in both groups.

SFA	Non-PCOS	PCOS	Total	P-value
Abnormal	31	29	60	0.454
Normal	19	24	43	
Total	50	53	103	

Table (4) compares CD2 hormonal profile and endometrial thickness (ET) in both groups. There was no significant statistical variation regarding cycle day 2 E2, LH, FSH and ET between females in both groups (p-value = 0.08 , 0.60 , 0.89 and 0.09) respectively. The only exception was CD2 serum prolactin which showed a significantly higher level in women with poly cystic ovary syndrome with a mean of 19.05±13.36 in non-PCOS and 25.55 ± 11.69 in PCOS women (p-value = 0.01).

Table 4: A comparison of cycle day 2 hormones and endometrial thickness between the studied groups.

Parameter	Non-PCOS (N = 50) Mean±SD	PCOS (N=53) Mean ± SD	P-value
E2 (pg/ml)	34.75 ± 15.70	41.33 ± 22.15	0.089
LH (IU/L)	4.12 ± 7.93	4.73 ± 3.44	0.608
FSH (IU/L)	4.94 ± 2.23	4.89 ± 1.46	0.895
Prolactin (ng/l)	19.05 ± 13.36	25.55 ± 11.69	0.010*
ET (mm)	3.48 ± 0.86	3.79 ± 0.98	0.091

Table (5) shows that there was a significant statistical difference between both groups regarding the morphological abnormality of oocytes , p-value = 0.046.

Table 5: A comparison between both groups regarding the morphological abnormality of oocytes.

Parameter	Non-PCOS (N=50)	PCOS (N=53)	P-value
Granularity	17(3.9%)	29(4.5%)	0.046*
Vacuoles	9(2%)	17(2.6%)	
Wide PVS	8(1.86%)	38(5.9%)	
Abnormal PB	5(1.16%)	29(4.5%)	
To. No. of morphologically abnormal oocytes	39(9%)	113(17.5%)	

The effect on fertilization rate (FR), cleavage rate (CR) and embryo quality (good or bad) can be demonstrated in table (6). There was no significant statistical difference in both groups regarding FR and CR despite of being less in PCOS group, p-value = 0.40 and 0.59 respectively. While the mean total number of good quality embryos was higher in PCOS group than non-PCOS group with no significant statistical difference, p-value = 0.074.

Fertilization rate was calculated by dividing the total number of zygotes / total number of injected MII oocytes. The total number of zygotes was represented by the total number of oocytes who develop two pronuclei (2PN) and two polar bodies (2PBs) after sperm injection. CR was calculated by dividing the total number of embryos / the total number of zygotes.

FR = (To. number of 2PN oocytes / To. no. of injected MII oocytes)* 100%.
CR = (To. number of embryos / To. no. of 2PN oocytes)* 100%.

Table 6: A comparison of FR, CR, mean total no. of embryos and their quality between our studied groups.

Parameter	Non-PCOS Mean ± SD	PCOS Mean ± SD	P-value
FR	73.38 ± 24.32	71.49 ± 21.84	0.402
CR	95.77 ± 14.81	93.86 ± 20.55	0.597
To. No. of embryos	5.12 ± 3.89	6.46 ± 4.49	0.113
To. No. of good quality embryos	4.65 ± 3.43 (90.8%)	6.08 ± 4.39 (94.04%)	0.074
To. No. of bad quality embryos	0.46 ± 1.01 (9.16%)	0.38 ± 1.00 (5.95%)	0.679

DISCUSSION

The main purpose of this observational, prospective cohort study is answering the question: Dose altered intra follicular microenvironment within the polycystic ovary have an adverse effect on the oocytes quality in form of dysmorphism then subsequently on embryos development, (fertilization rate, cleavage rate and embryos quality) in those women who had been subjected to ICSI as a fertility treatment measure. To answer this question, a comparison with women without PCOS was done.

Excess androgen in PCOS stimulates multiple ovarian follicles in an equivalent time thus prevents a single dominant follicle to be developed and leads to ovulation failure. The hyper androgenic state also leads to disturbed meiotic division as well as mitotic division of the oocytes and in turn results in improper maturation of the oocytes and developmental compromise.[17]

As the most important factor which has been used for determining the outcome of embryo development in patients who are subjected to ARTs is oocyte quality. Thus, multiple prognostic factors depends on morphological features of the oocyte have been introduced and might be used as predictors of oocyte quality, its' fertilizing ability, subsequent embryo development.[18]

The study was showed that 90% of the retrieved oocytes from the non-PCOS women were morphologically normal versus 82.4 % from PCOS group. So the percentage of morphologically abnormal oocyte is significantly higher in PCOS women.

In this study we focused on four morphological abnormalities: cytoplasmic granularity, cytoplasmic vacuoles, wide perivitelline space (PVS) and morphologically abnormal first polar body (IPB). The percentage of oocytes with each abnormality in PCOS women was more than those in non-PCOS group. Wide PVS was the most prevalent one in PCOS group 5.9% followed by morphologically abnormal IPB 4.5%, cytoplasmic granularity 4.5% and cytoplasmic vacuoles 2.6%. While in non-PCOS women cytoplasmic granularity is the most 3.9% followed by cytoplasmic vacuoles 2%, wide PVS 1.86% and morphologically abnormal IPB 1.16%.

Both wide PVS and large IPB are considered as a sign of post maturity which results from shrinkage of the oocyte in relation to zona pellucida (ZP) creating a gap between them. It may also result from extrusion of excess cytoplasm with the chromosomal set during the formation of IPB which leads to large both PVS and IPB.[19] Increased E2 to androgen ratio may also leads to large PVS.[20] Large IPB can also indicates abnormal positioning of meiotic spindle which usually associates with aging process[14] Some studies had been showed that abnormally wide PVS were observed in up to 35% of

normal women and an isolated wide PVS with normal other oocyte morphology leads to lowering FR and negatively affect embryo quality.[21] One study was showed that oocytes with PVS abnormalities had a negative effect on the embryo development in infertile couples with male factor cause.[19] However, different studies had been failed to find an exact correlation between PVS abnormalities and the prognosis of ARTs.[22] and PVS abnormalities were not resulted in a lowered FR and unfavorable ICSI outcome.

These findings may indicate that the probability of oocyte post maturity may be higher in PCOS women. This could be explained by excessive LH secretion in those women as high LH usually associated with higher rate of pre-mature resumption of meiosis.[23] Other adverse effect of it on oocyte quality by causing changes in the steroidogenic environment in the antral follicles resulting in pre-mature progesterone release. In addition, the aged oocytes might not be related to polycystic ovaries per se as the type of induction protocol and the type and time of trigger might play a role. Early triggering of ovulation may lead to retrieval of immature oocyte while late triggering results in retrieval of post mature oocyte. Both are considered under the term as dysmature oocyte.

While both cytoplasmic granularity and vacuoles are considered as a part of normal physiological processes during oocyte development and maturation and only extensive, dark, central granularity and large vacuoles more than 14 mm in size are considered abnormal and could affect embryo development and decrease FR and PR and might necessitate extensive studying.[24] Also, some studies was suggested that cytoplasmic granularities is a usual result of any stimulated cycle regardless the cause of infertility.[25] Researchers had been showed that oocytes with cytoplasmic granularity had the same chance of fertilization as normal oocyte and it had no effect on the embryo quality but might affect further embryo development.[26] Regarding IPB morphology, although it could be considered as an indicator of oocyte viability and could be used as a predictor of oocyte performance during ICSI but data from most of these studies were showed that there was no correlation between FR, embryo quality and it.

This study was showed that both FR and CR were lower in PCOS women in comparison to non-PCOS ones (71.5% VS 75.4% and 93.8% VS 95.7%) but with no significant statistical difference. Similar results were obtained from different studies which had been showed insignificantly low FR.[27,28] While others were showed significantly low FR and high CR in PCOS women.[29,30]

Although the study was showed that the percentage of morphologically abnormal oocytes was more in PCOS women, the percentage of good quality embryos was also more thus abnormal oocyte morphology seems to have no effect on the quality of developing embryos or this may be related to ICSI procedure that enable the embryologist to choose morphologically normal oocyte to be injected and exclude severly dysmorphic oocytes or those with multiple abnormality especially when the patient had been produce a sufficient number of oocytes. Also it indicates that not all morphological deviations of oocyte associate with clinical consequences (*The ugliest Oocyte can give the most beautiful embryo)*.

Oocyte aging can significantly increase premature separation of chromosomes ends in aneuploidy.[31] Failure of normal alignment, scattering with degenerating meiotic spindle and decondensation of chromatids is also noticed in aged oocytes. Good quality embryos (grade I and II) which had been produced from post mature (aged) oocytes or oocytes with multiple dysmorphic features are classified depending upon microscopical observation but their genetic constituents were unkown and could not be predicted by visual observation. So, It has been suggested that not all good quality embryos are chromosomally competent and a significant number of embryos generated by ARTs are chromosomally abnormal,[32] and further investigations in this direction is recommended.

REFERENCES

1. Mahesh G., Daneshwar S., Manju T., Angelin P., Soumitra S. and Preeti G. A cross sectional study of polycystic ovarian syndrome among young women in Bhopal, Central India. *Int. J. Community Med. Public Health. Jan*, 2018; 5(1): 95-100.
2. Fauser B., Tarlatzis B. and Chang J. The Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum. Reprod.*, 2004; 19: 41–7.
3. Adam H. Polycystic Ovary Syndrome, Management–Diagnosis and Treatment. In: Infertility in practice.4th ed. CRC Press is an imprint of Taylor & Francis Group, an Informa business, 2014; 201-236.
4. Susie J., Thomas H. and Adam H. Polycystic ovary syndrome and assisted reproduction. In: Textbook of assisted reproductive techniques. David K. and Colin M. (eds.).5th ed., 2018; 1: 60.
5. Al-Faris N. N., Al-Tu'ma F. J. and Al-Safi W. G. Assessments of C-Reactive Protein and its Correlation with Total Antioxidant Capacity in Woman with Poly Cystic Ovary Syndrome Iraqi National Journal of Chemistry, 2017; 17(1): 44-57.
6. Al-Faris N. N. , Al-Tu'ma F. J. and Al-Safi, W. G. Total Antioxidant Capacity and Homocysteine Levels in Obese Women with Polycystic Ovary Syndrome. International Journal of Pharmacy and Pharmaceutical Research, 2017; 8(3): 78-86.
7. Teissier MP., Chable H., Paulhac S. and Aubard Y. Comparison of follicle steroidogenesis from normal and polycystic ovaries in women undergoing IVF: relationship between steroid concentrations, follicle

size, oocyte quality and fecundability, 2000; 15: 2471–7.
8. Marie L., Angela E., Jenny A., Visser, Jackson C., Kirkman B., Teresa K. and Wiebke A. The steroid metabolome in the isolated ovarian follicle and its response to androgen exposure and antagonism. Endocrinology, 2017; 158(5): 1474-1485.
9. Al-Tu'ma F. J., Ferhan N. H. and Al-Safi W. G. (Spring) Polymorphism of tumor necrosis factor-alpha 308 G/A gene in Iraqi patients with polycystic ovarian syndrome *Iraq Med J*, 2017; 1(2): 45–49.
10. Amir H., Hossein M. and Anoosh N. Comparison of the levels of LH, FSH, TSH, Prolactin, Progesterone and Estradiol hormones between Iranian infertile women with PCOS and healthy women. *Int. J. of Medical Research and Health Sciences,* 2016; 5(12): 370-375.
11. Anju KN., Bindu N., Streekumary R. and Jayasree L. Effectiveness of lifestyle package among overweight and obese adolescent girl between 15-19 years with PCOS. *Int. J. Med. Public health.,* 2017; 4(1): 84-90.
12. Chaitra S., Abinaya K., Mridula D., Jayanthi, Satish D. and Renuka R. Polycystic ovary syndrome and pregnancy outcome. *Int. J. Reprod. Contracept. Obestet. Gynecol,* 2017; 6(9): 3804-3807.
13. Azita F., Mohammad Ali K. and Sareh A. Oocyte morphology and embryo morphokientics in an intra-cytoplasmic sperm injection programmer. Is there a relationship? Online, 2017; 25(*2):* 190-196.
14. Laura R., Basak B., Thomas E. and Jacqueline M. Human Reproduction, 2012; 27(S1): i2–i21.
15. Mina A. Morphological expression of human egg and embryo quality. In: Textbook of clinical embryology. Coward K and Wells D. (eds.), 2013; 30.
16. Denny, S. and David, K. Evaluation of embryo quality, analysis of morphology and physiology. In Textbook of assisted reproductive techniques. David K. and Colin M. (eds.).5th ed. 2018(2): 17.
17. Dumesic DA., Meldrum DR., Katzjaffe MG., Krisher RL. and Schoolcraft WB. Oocyte environment: follicular fluid and cumulus cells are critical for oocyte health. *Fertil. Steril*., 2015; 103: 303–316.
18. Faramarzi A., Khallili MA. and Ashourzadeh S. Oocyte morphology and embryo morphokinetics in an intra-cytoplasmic sperm injection program. Is there a relationship? *Zygote; Cambridge*, 2017; 25(2): 190-196.
19. Hassa H., Aydin Y. and Taplamacioglu F. The role of perivitelline space abnormalities in the developmental potential of embryos. *J. of The Turkish German Gynecological Association,* 2014; 15(3): 161-163.
20. W., Gong F. and Lu G. Correlation of serum Anti-müllerian hormone concentrations on day 3 of the in vitro fertilization stimulation cycle with assisted reproduction outcome in polycystic ovary syndrome patients. *J. Assist. Reprod. Genet*, 2012; 29: 397-402.
21. Rienzi L., Ubaldi FM., Iacobelli M., Minasi MG., Romano S., Ferroro S., Sapienza F., Baroni E., Litwicka K. and Greco E. Significance of metaphase II human oocyte morphology on ICSI outcome. *Fertil. Stertil*., 2008; 90: 1692-1700.
22. Rienzi, L.; Ubaldi, F.M.; Lacobelli, M.; Minasi, M.G.; Romano, S. and Greco, E.: Meiotic spindle visualization in living human oocytes. *Reprod. Biomed. Online*, 2005; 10: 192-198.
23. BenRafael Z., Neri L., Shlomo M. and Joseph GS. Advances in assisted reproductive technologies. *Springer Science and Business Media. Dec.,* 2012; 6: 320-323.
24. Philipe M., Rosalie C., Karen C., Elodie H., Florence S., Naima-belhadri M., Aviva D., Hikmat C., Veronique B., Henri C. and Moncef B. Impact of oocytes with CLCG on ICSI outcomes and their potential relation to pesticide exposure. *J. of Ovarian Research,* 2017; 10: 42.
25. Mohammad K., Mahdieh M. and Abdul-Munaf S. Role of oocyte morphology on fertilization and embryo formation in assisted reproductive techniques. *Middle East Fertility Society Journal,* 2005; 10(1).
26. Fancsovits P., Zsuzsa G., Tóthné Á., Murber J., Rigó JR. and Urbancsek J. Importance of cytoplasmic granularity of human oocytes in in vitro fertilization treatment. *Acta. Biologica. Hungarica,* 2012; 63(2): 189–201.
27. Yahia W., Mahmoud S. and Tamer F. Prediction of intracytoplasmic sperm injection outcome in patients with polycystic ovary syndrome using follicular anti-mullerian. AAMJ, 2013; *10(4).*
28. Pellatt L., Rice S., Dilaver N. and Heshri A. Anti-müllerian hormone reduces follicle sensitivity to follicle-stimulating hormone in human granulosa cells. *Fertil Steril*, 2011; 96: 1246-1251.
29. Krishnapillai J., Divya N. and Jesin FL. Comparison of ovarian response between PCOS and Non-PCOS patients undergoing ICSI with antagonist protocol. *J. of Infertility and Reproductive Biology,* 2015; 3*(*3): 199-207.
30. Okohue JE., Onuh SO., Ikimalo JI. and Onuh SO. Challenges in running/establishing an assisted conception centre in the Niger-Delta region of Nigeria. *West African J. of Assist. Reprod.*, 2011; 1: 1-17.
31. Steuerwald NM., and Maihes JB. Post-ovulatory aging of mouse oocytes leads to decreased MAD2 transcripts and increased frequencies of premature centromer separation and anaphase. *Mol. Hum. Reprod. Sep.*, 2005; 11(9): 623-30.
32. Farzaneh F., Fatemeh M., Mohammed Hassan S., Hojjatollah S., Razieh DF. and Seyed Mehdi K. Frequency of chromosomal aneuploidy in high quality embryos from young couples using preimplantation genetic screening. *Int. J. Reprod. Biomed. May*, 2017; 15(5): 297-304.