

Embryo Quality and Intracytoplasmic Sperm Injection (ICSI) Outcome in Iraqi Women with Polycystic Ovary Syndrome (PCOS): A Cohort Prospective Study

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Polycystic ovary syndrome (PCOS) is a major cause of ovulatory dysfunctions among reproductive-aged women. PCOS impairs folliculogenesis leading to suboptimal oocyte maturation, impaired embryonic development and pregnancy failure. Intracytoplasmic sperm injection (ICSI) is a popular option for PCOS patients to attain pregnancy. However, there is no specific determinant to ascertain successful pregnancy outcome in PCOS women undergoing ICSI. The purpose of this study was to determine the influence of PCOS on embryo quality and subsequent pregnancy rate in Iraqi women who had undergone ICSI. Over the course of three months, one hundred and three infertile couples who were referred to Al-Sadr Medical City, Kufa, Iraq between October 2017 and June 2018 were enrolled in this study. The couples were divided into two groups: those with PCOS, and those who did not have PCOS. The amounts of hormones were determined. The evaluation of embryo attributes with grading, as well as the determination of the fertilization rate, cleavage rate, and pregnancy rate, were carried out. The difference in fertility and cleavage rates between the PCOS ($P=0.40$) and non-PCOS ($P=0.59$) groups was not statistically significant. When comparing the two groups, the mean number of good quality embryos in the PCOS group was higher ($P=0.07$), whereas the pregnancy rate in the former was considerably lower ($P=0.02$) than in the latter. According to our findings, PCOS had no negative impact on the quality of the embryos produced by Iraqi women who underwent ICSI treatment. Because PCOS is a complicated disorder characterized by a variety of endogenous physiological variables that may either directly or indirectly interfere with conception, the low likelihood of pregnancy in these patients suggests that good embryo quality is not the only predictor of successful pregnancy.

Keywords: Embryo Quality; Fertilization Rate; Hyperandrogenemia; ICSI Outcome; PCOS; Pregnancy Outcome.

Polycystic ovarian syndrome (PCOS) is a disorder of chronic anovulation in women of reproductive age and usually occurs due to imbalance of reproductive hormones¹. It reflects heterogeneous collection of signs and symptoms, with wide spectrum of disorders. For some, there are mild symptoms while others experience severe disturbances in reproduction, endocrine and metabolic functions². Its prevalence varies between 2% and 26% among women across different populations^{3,4}, and constitutes of 80% to 90% of group II anovulatory sub-fertility, as per the World Health Organization (WHO) [1]. PCOS is diagnosed as per the Rotterdam 2003 criteria: menstrual problems (oligo- and/or anovulation), elevated levels of male hormones (clinical and/or biochemical hyperandrogenism) and by transvaginal ultrasound of ovaries⁵.

PCOS accompanies various associated factors that account for compromised fertility, which is not restricted only to anovulation², and these factors include increased body weight, inflammatory conditions, metabolic and endocrine defects with subsequent impairments of oocyte quality, embryo development and future fetal wellbeing^{6,7}. Increased luteinizing hormone (LH) in PCOS⁸ also adversely affects the quality of embryo (early developmental delay and arrest) and increases the rate of miscarriages^{9,10}. The incidence of miscarriage in PCOS is three times higher than normal women and is believed to be a result of hypersecretion of LH, and insulin, as well as excess body weight¹¹. However, this tends to be diverse as PCOS women with normal androgen levels still have the ability to produce developmentally normal embryos⁹. Impairment of endometrial blood flow, growth factors, cytokines, and adhesive molecules also may contribute to fertility disruptions in PCOS patients¹². Moreover, high serum androgen level may also serve as causative factors owing to its adverse effect on the normal endometrial development by reducing expression of endometrial protein¹³.

Infertile women with PCOS are usually successfully treated with first line ovulation inducing agents such as clomiphene citrate and insulin-sensitizing medications. Women who fail to conceive even first line treatment, are candidates for gonadotrophin treatment or laparoscopic ovarian drilling. Assisted reproductive

technologies (ART) are offered to women with PCOS failing to ovulate with these protocols¹⁴. In addition, ART may be considered when there is a severe accompanying infertility factor, such as severe male factor necessitating intracytoplasmic sperm injection (ICSI)¹⁵. Excessive response to gonadotropins manifested by possibly life-threatening ovarian hyperstimulation syndrome (OHSS) is a potential complication of controlled ovarian hyperstimulation (COH) in these patients¹⁶. The ART performance of patients with PCOS, employing either *In vitro* fertilization (IVF) or ICSI, has been reported to be comparable to control groups mainly consisting of tubal factor or male factor infertility^{17,18}. Further in-depth research is required to unveil the overall impact of PCOS on various aspects of the female reproductive potential, and the use of ART to bypass their fertility problems is a subject of scientific and ethical debate. It is essential to understand the exact effects of PCOS on various fertility parameters across different population. There are inadequate reports on Iraqi population pertaining to embryo quality in PCOS women. Thus, the present study, conducted on Iraqi women, aimed at evaluating the influence of PCOS on the embryonic quality, embryonic development, and major pregnancy-associated parameters, following ICSI.

MATERIALS AND METHODS

Ethical considerations and study population

This is a prospective cohort study that was conducted at IVF Center, Al-Sadr Medical City, Kufa, Iraq. The Institutional Medical Ethics Committee of University of Kufa has approved (FOM/8/10.10.17) the study proposal. One hundred three infertile couples were included in this study and all of them were involved in ICSI program throughout the period from October 15, 2017 to June 30, 2018. The age of female partners was >35 years old. The infertile couples were divided in two groups: Group 1 females with PCOS selected according to Rotterdam 2003 criteria¹⁹ with male partners having mild-to-moderate semen quality impairment, and Group 2 females were without PCOS (normal ovulatory women who attended the fertility clinic for mild-to-moderate male factor infertility or women with tubal obstruction. Females with (a) endometriosis, (b)

abnormal renal or hepatic functions, (b) individuals with hyperprolactinemia/hypothyroidism, (c) individuals with secondary causes of androgen excess, (d) individuals with gynecological age less than three years, (e) women who suffered from genetic disorders, such as Turner's syndrome, primary hypopituitarism, primary premature ovarian failure, (f) primary insulin resistance (IR), (g) male partners with severe impairment of semen quality, or frozen sperms (from testis or epididymis), (i) couples with unexplained infertility (normal female and females with no identified cause of sub-fertility) had been excluded from this study.

Preparation of subjects, anthropometric and hormonal measurements

Male and female partners of both groups had been evaluated by urologists and gynecologists. Females of both groups had been subjected to pituitary downregulation using either gonadotropin releasing hormone (GnRH) antagonist; Cetrotide 0.25 mg (Serona) from Day-6 (fixed protocol) or agonist; Decapeptyle 0.1 mg (Serona) (depending upon the treatment protocol which was followed by the specialists in the center which is individualized according to each couples' characteristics), *i.e.* antral follicle counts (AFC), body mass index (BMI), earlier response to the treatment in previous cycle and the male semen parameters, from the Day-2 of cycle (CD2) then controlled ovarian hyperstimulation by recombinant follicle stimulating hormone (r-FSH); Follitrope 75×2 IU(Merck) which was done under a close supervision by serial trans-vaginal ultrasound (TVUS) and hormonal assay for 10-14 days. Ovulation trigger was done either by human chorionic gonadotropin (HCG); Pregnyl 5000 IU×2 (Merck) injection or Decapeptyle 0.2 mg (depending upon the risk of OHSS; clinical symptoms of nausea, vomiting and abdominal discomfort, ultrasound; more than 20 follicles and some free fluid in the abdomen, hormonal; high serum estrogen >2500 pg/ml at the day of trigger and the preference of fresh embryo transfer) when the total number of the follicles and their size are adequate (7-12 follicles of more than 16 mm size). Oocyte pickup was done by the gynecologist under general anesthesia using trans-vaginal approach. The oocytes were denuded by hyaluronidase enzyme; 80 IU/l and mechanical way by repeated

aspiration through a sequence of denuding pipettes. Then the oocytes were washed with the culture medium and the maturity of oocytes was assessed. ICSI was commenced in all cases as the center uses ICSI in nearly 99% of cases, fertilization was assessed 16-18 hrs. after injection, Subsequent evaluation of the embryo quality was done depending on blastomere number, their shape, equality, mono-nucleation, and the percentage of fragmentations. Embryos were classified as good quality (grade I and II) when they have 4 cells at 48 hrs. after injection or have 6-8 cells 72 hrs. with even sized blastomeres, little or no fragmentation (for good 10-20% and for bad more than 20%) and single, clearly visible nuclei per each blastomere. Anything else were classified as bad quality embryos (grade III and IV)²⁰.

All participants had their body weight and height was measured in bare feet on a plane surface and with minimal garments. The body mass index (or Quetelet Index) was measured by using the following formula: $BMI = \text{weight (kg)} / (\text{Height in m})^2$.²¹ Hormonal parameters of the subjects were measured following the standard protocol.

Statistical analysis

Statistical analysis was done assuming a confidence level of 95%, and obtained data were arranged in Microsoft Office Excel spreadsheet 2007, analyzed by SPSS (v. 22.0, IBM, Chicago, IL, USA) and MedCalc (v. 19.05, Ostend, Belgium). Data of the continuous variables are expressed in means±standard deviation (SD). Independent sample Students' t-test and Chi-square test were applied to analyze the obtained data. P-value <0.05 was set as statistically significant.

RESULTS

Age, anthropometry and induction protocol

The descriptive information about age, BMI, durations of primary and secondary infertility of the study group and control subjects have been expressed in Table 1. It reflects that the age of female respondents of both groups (P=0.55) and the durations of infertility (P=0.40) are not statistically significant. The BMI, which is one of the key anthropometric predictors of PCOS was also reported to be non-significant between the two groups (P=0.37). The types of induction protocols that were used during COH was statistically

significant between the two groups ($P=0.001$), whereas no significant difference was recorded between the total doses of gonadotropins ($P=0.945$) (Table 2).

Hormonal profiles, endometrial thickness and ICSI outcome

Comparison of hormonal profiles on cycle day-2 (CD2) did not show any significant difference for LH ($P=0.608$), FSH ($P=0.895$) and estradiol (E2) levels ($P=0.089$) between PCOS and non-PCOS groups. Contrarily, serum prolactin levels ($P=0.01$) and total testosterone ($P=0.008$) were reported to be significantly higher in PCOS women than control subjects. Endometrial thickness (ET) of PCOS women on CD2 was also not significantly different from non-PCOS subjects (Fig. 1).

The ICSI outcomes in form of fertilization rate (FR), cleavage rate (CR) and embryo quality and pregnancy rate have been expressed in Table 3. There was no statistically significant difference between the two groups regarding FR and CR despite being less in the PCOS group ($P=0.40$ and 0.59 , respectively). While the mean total number

of good quality embryos was higher in the PCOS group than the non-PCOS group with no significant statistical difference ($P=0.074$). Regarding pregnancy rate (PR) in both groups, it was higher in the non-PCOS group 52.17% compared to 27.5% in the PCOS counterparts ($P=0.02$). Four non-PCOS women and thirteen PCOS women were not included in PR calculation due to the development of OHSS and the cancellation of fresh embryo transfer.

So, the total number of females included in the calculation of PR in PCOS and non-PCOS woman was 40 out of 53 and 46 out of 50 respectively, and the rate of developing OHSS was 24.5% and 8% respectively.

DISCUSSION

PCOS represents a major cause of infertility, and its prevalence varies across various population^{4,7}. It is imperative to understand the influence of this disease on the reproductive system, particularly at oocyte and subsequent embryo development on specific population¹⁴.

Table 1. Comparison of patient information for PCOS and non-PCOS groups

Data	Non-PCOS (n=50)	PCOS (n=53)	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	0.40
Secondary infertility	16	13	
Spermiogram	0.45		
Normal	19	24	
Abnormal	31	29	

values are expressed in mean±SD; $P<0.05$

Table 2. Comparison of stimulation protocols and total dose of gonadotropins in PCOS and non-PCOS females

Protocol	Non-PCOS (n=50)	PCOS (n=53)	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)	1925.2±968.7	1663.20±615.2		0.945

values are expressed in mean±SD; $P<0.05$

Table 3. Comparison of fertilization rate, cleavage rate, mean total number of embryos, their quality and pregnancy rate between the studied groups

Parameters	Non-PCOS (n=46)	PCOS (n=40)	P-value
Fertilization rate	73.38±24.32	71.49±21.84	0.402
Cleavage rate	95.77±14.81	93.86±20.55	0.597
Total number of embryos	5.12±3.89	6.46±4.49	0.113
Good quality embryos, Total no. (%)	4.65±3.43(90.8)	6.08±4.39(94.04)	0.074
Bad quality embryos, Total no. (%)	0.46±1.01(9.16)	0.38±1.00(5.95)	0.679
Pregnancy rate, Total no. (%)			
Pregnant	24/46(52.17)	11/40(27.5)	0.02*
Not pregnant	22/46(47.80)	29/40(72.5)	

values are expressed in mean±SD; P<0.05

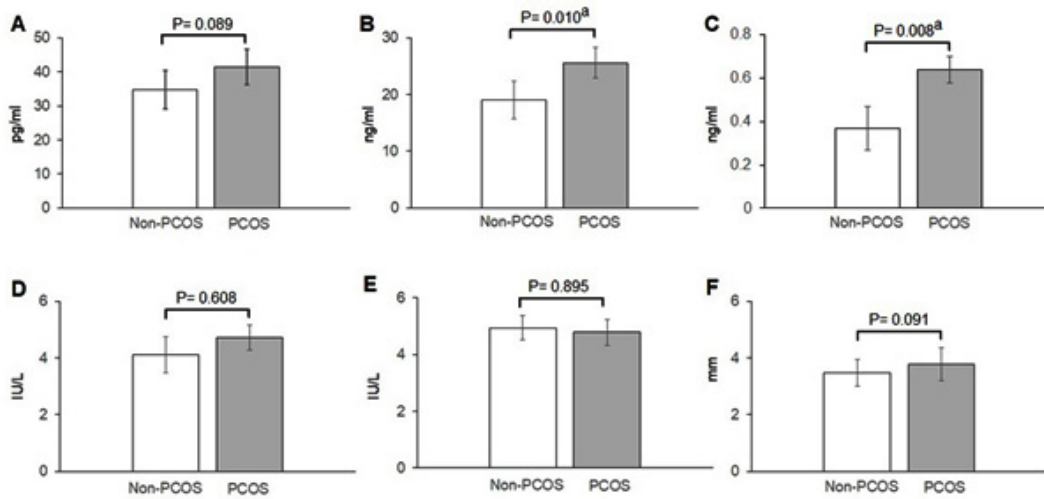


Fig. 1. Comparison of hormonal profiles, (A) estradiol (pg/ml), (B) prolactin (ng/ml), (C) total testosterone (ng/ml), (D) LH (IU/L), (E) FSH (IU/L), and (F) endometrial thickness (mm) between the PCOS and non-PCOS groups

Thus, the present study, conducted in Iraq, aimed to investigate impact of altered intrafollicular microenvironment in women with PCOS, on pregnancy rate, embryo quality, and embryonic development following ICSI as a fertility treatment measure.

The study revealed that 94.04% of embryos derived from PCOS women were of good quality in comparison with 90.8% in the non-PCOS group (P=0.074) (Table 3). This might be related to the ICSI procedure itself which enables the embryologist to select the best quality gametes to be injected so the probability of producing good quality embryos is augmented. A study by Hassan

et al., is consistent with the current results²² but, contrasting report is also available demonstrating significantly higher total number of embryos in PCOS women compared to non-PCOS control, but with significantly lower percentage of good quality embryos²³. Some researchers believed that only lean PCOS women produced good quality embryos while obese women did not^{24,25}. A recent study had done morphokinetic analysis and showed that embryos from PCOS patients developed earlier to the nine-cell staged form as compared to the controls²⁶. It is known that human embryo compaction is mediated via high degree integrated cell-to-cell signaling. Cells

bind tightly to each other, having no distinct cell borders, with the synchronized actions of the desmosomes, gap junctions, tight junctions, and various other adhesion molecules, to form the embryo. Followed by these processes, cellular polarity develops and cell continues differentiating resulting in the formation of inner cell mass and trophectoderm²⁷. Not much is revealed regarding these mechanisms, but there are few key proteins, such as the connexins and cadherins, have been shown to play critical roles^{27,28}. In PCOS patients, nonetheless, connexins (Cx43) and E-cadherins expressions reportedly increase which may aid the robust embryo formation and differentiation^{28,29}.

Our results also depict 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%) (**Table 3**). Similar results were obtained from different studies that showed lower FR in PCOS patients³⁰⁻³². There are also reports showing significantly lower FR and higher CR in PCOS women compared to the non-PCOS control^{33,34}. Moreover, among the studies pertaining to PR in PCOS women, some demonstrated significant lower PR while few could find no significant difference in PR between PCOS and the control³⁰⁻³². In the present study, PR was observed to be significantly lower in PCOS women (27.5% vs. 52.17%) despite a high yield of good quality embryos. There must be other factors related directly or indirectly to PCOS, responsible for the failed conception and pregnancy loss. PCOS is associated with overproduction of ovarian androgens, aberrant hypothalamic-hypophyseal signals, environmental and genetic variables and others that are integral to the ultimate pathways similar to various metabolic disorders such as insulin resistance, glucose intolerance, and obesity²⁷⁻²⁹. High LH and prolactin levels, low glycoprotein level, infertility treatments and protocols of induction together with other intra-ovarian factors might also play role in the implantation failure and miscarriage in females with PCOS¹¹. Our result shows significantly higher total testosterone levels in PCOS patients (**Fig. 1**), which is reportedly linked with disrupted endometrial growth during the luteal phase leading to failed implantation and pregnancy loss¹³. Although the mechanism underlying these observations is yet unknown, it might be linked to changes in aromatase activity and cumulus

cell-oocyte interactions³⁵. Because of a recent link discovered between embryo morphokinetics and cumulus cell gene expression in women with PCOS, this might be an intriguing area to pursue³⁵.

In women with PCOS, prolactin levels during pregnancy may be relevant as a long-term risk marker for metabolic health. Currently, androgen status and obesity are proposed as predictors of individual risk of metabolic disorders in PCOS; however, metabolic disturbances are also enhanced in non-hyperandrogenic PCOS, and there are presently no ideal predictors for detecting those at higher risk of metabolic and cardiovascular complications³⁶. Prolactin promotes β -cell proliferation in pancreatic islets as a physiological response to the development of insulin resistance during pregnancy³⁷. In a large population-based cohort, high prolactin within the normal range was linked to a reduced prevalence of diabetes and poor glucose tolerance³⁸. However, elevated prolactin level beyond physiological levels leads to pregnancy loss and lowering its level by medication before ICSI improves implantation³⁹. In the present study, PCOS women had a significantly higher prolactin level (**Fig. 1**) and the induction protocol of choice was the antagonist protocol which has negative impact on implantation⁴⁰.

CONCLUSIONS

Overall, this study reveals that PCOS women undergoing IVF had good embryo quality and fast embryonic growth. However, embryo quality is not a sole predictor of successful pregnancy, as PCOS is a multifaceted disorder with variable phenotypes. Various factors, such as insulin intolerance, disrupted endocrine axes, hyperandrogenemia, intrinsic embryonic factors, etc. may directly or indirectly affect pregnancy rate in PCOS women. Further research into the mechanisms is needed to better understand and act in order to improve the oocyte health of PCOS women undergoing IVF and to enhance viability of the future offspring.

Competing Interest

The authors declare that they do not have any conflict of interest.

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