

**OOCYTE AND EMBRYO QUALITY OF INFERTILE
PATIENTS WITH CLASSIC PHENOTYPE POLYCYSTIC
OVARIAN SYNDROME AFTER CONTROLLED OVARIAN
HYPERSTIMULATION**

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Received on January 3, 2021

Presented by I. Ivanov, Member of BAS, on January 29, 2021

Abstract

The polycystic ovary syndrome (PCOS) is a combination of conditions, closely associated with female infertility and metabolic syndrome. It is recognized mainly with a polycystic ovary morphology (PCOM) of increased ovarian volume and pearl chain-like follicles on ultrasound, producing a large cohort of growing follicles during controlled ovarian hyperstimulation (COH) that are frequently heterogeneous in size and with poor quantity or quality of mature oocytes. However, recent data has proposed controversial opinions. The objective of the present study was to evaluate the relationship between the classical phenotype PCOS and the oocyte and embryo quality after COH for intracytoplasmic sperm injection (ICSI).

An observational case control study was conducted for a four years period at the IVF department of a private gynecological hospital. Infertile females under 39, who performed COH for ICSI were included. A total of 496 ICSI cycles were divided into two groups – a PCOS group with 109 ICSI cycles and a control group with tube infertility that encompasses 387 ICSI cycles. The main outcome measures were the quantity and quality of the retrieved oocytes and embryos as well as the ICSI outcomes (fertilization rate, implantation rate and clinical pregnancy rate). The statistical analyses were performed, using SPSS

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DOI:10.7546/CRABS.2023.02.07

version 12.0 and MEDCALC[®] statistical software and statistical significance was considered at $p < 0.05$.

Significantly higher mean number of total oocytes (14 ± 6.02 SD vs. 9 ± 3.25 SD; $p < 0.0001$) and meta-phase II oocytes (10 ± 1.94 SD as opposed to 6 ± 2.89 SD; $p = 0.0001$), as well as immature and atretic ones were retrieved in the PCOS group compared to the controls ($p < 0.0001$). However, the fertilization rate did not differ among the two groups (66.8% and 62.4%, respectively). Despite obtaining significantly more embryos in the PCOS patients (9 ± 5.17 SD vs. 6 ± 2.96 SD; $p < 0.0001$), there were no differences between the two groups regarding the figures of the top quality embryos (25.6% and 29%, respectively), implantation rate (19.7% and 19.95%, respectively) and clinical pregnancy rate (28.4% and 33.6%, respectively, NS).

Patients with classic phenotype PCOS are not associated with poor oocyte and embryo quality or with unfavourable ICSI outcome after controlled ovarian hyperstimulation.

Key words: PCOS, ICSI, oocyte quality, embryo quality, pregnancy rate

Introduction. Polycystic ovary syndrome (PCOS), which affects 5–7% of women of reproductive age, is a well-recognized reproductive endocrine disorder mainly because of the polycystic ovary phenotype of increased ovarian volume and pearl chain-like follicles [1]. It is an extensively studied pathology and at the same time it is widely misunderstood, mostly because it does not represent one single condition. Many attempts for classification of this disorder have been made, but consensus was never really achieved. The last one from 2012, which represents an extension of “Rotterdam criteria”, was accompanied by four phenotypes with a detailed description and better distribution of the morbidity of PCOS. The first two phenotypes A and B, the so-called “Classic types” PCOS, are with a more pronounced ovarian dysfunction, clinical or biochemical hyperandrogenism, higher rates of insulin resistance and a risk for metabolic syndrome. On the contrary, the other two types of PCOS (C and D types) generally demonstrate intermediate levels of the above described signs, with ovulating ovaries and even are hypoandrogenic [2–4].

Precisely hyperandrogenism, as a specific feature of PCOS, is considered to be the main reason for disturbances in folliculogenesis with significantly enlarged cohort of early-growing and recruitable follicles [5,6]. PCOS patients are typically characterized by producing a large cohort of growing follicles during the controlled ovarian hyperstimulation (COH) which are frequently heterogeneous in size and produce numerous oocytes that are regarded poor [6,7]. It is considered that impaired oocyte maturation and embryonic development in PCOS women are possibly linked with abnormal endocrine/paracrine factors, metabolic dysfunction and alterations in the intrafollicular microenvironment during folliculogenesis and follicle maturation [8–10].

Despite the information above there are insufficient and controversial studies focused on the topic and almost none of them have specified the phenotype PCOS

in which the observation has been made. Taking this into account we aimed to investigate patients with classic type PCOS in terms of oocytes and embryo quality after COH for ICSI procedure.

Materials and methods. An observational case control study was conducted for a period of four years at the IVF department of a private gynecological hospital. The inclusion criteria were infertile female, who had performed a controlled ovarian hyperstimulation for intracytoplasmic sperm injection (ICSI) with age under 39, BMI under 32; with more than five oocytes retrieved and with classic phenotype polycystic ovarian syndrome (PCOS) or obstructive (tube) type infertility. A total of 496 ICSI cycles fit the described above criteria which were divided into two groups – a PCOS group with 109 ICSI cycles and a control group with tube infertility, encompasses 387 ICSI cycles.

The diagnosis of PCOS was made according to NIH 2012 classification by the presence of two of the following three criteria: polycystic ovarian morphology (PCOM), ovarian dysfunction and clinical or biochemical sign of hyperandrogenism. At the observation only PCOS patients with phenotype A or B were included [2]. PCOM was characterized as the presence of 12 or more pearl chain-like follicles, each measuring 2–9 mm in diameter, and an increased ovarian volume at transvaginal ultrasonography [13].

All patients were examined for baseline hormonal levels, including androgens and Anti-Müllerian hormone (AMH) (pmol/L) on day 3 to 5 of the menstrual cycle before starting ICSI procedure.

ICSI/embryo transfer procedure was performed in all patients. The GnRH antagonist protocol was used for the ovarian stimulation. After confirming baseline blood levels and excluding any functional ovarian cysts, COH with Follitropin alpha was started on day 2 of the menstrual cycle in both groups. Regular transvaginal ultrasound scan and serum concentrations of luteinizing hormone (LH), estradiol (E2) and progesterone level monitoring were accomplished during the ovarian stimulation. The criteria for human chorion gonadotropin (hCG) administration was the presence of three or more follicles exceeding 17 mm in diameter. Ovum pick-up (OPU) was performed 34–36 h after the administration of 5000–6500 IU hCG subcutaneously for the ovulation trigger and the matured oocytes were fertilized by ICSI. Standard embryo transfer procedures were carried out and only top quality embryos, at the cleavage or blastocyst stage, were transferred using soft catheter. Luteal support was performed via transvaginal administration of progesterone, starting on the day of OPU.

The main outcome measures were quantity and quality of the retrieved oocytes and embryos as well as ICSI outcomes (fertilization rate, implantation rate and clinical pregnancy rate).

Oocyte morphology evaluation: Approximately 2–3 h after the oocyte retrieval, the oocytes were assessed and grouped according to their maturity status (MII, MI) or data for abnormality. Metaphase II (MII) oocyte was defined

as having a spherical shape, enclosed with a uniform zona pellucida, a uniform translucent cytoplasm and an extruded size appropriate 1st polar body (PB). A Metaphase I (MI) oocyte was defined as an oocyte with no germinal vesicle (GV) and no PB. The other abnormal or degenerated oocytes were classified as “atretic oocytes” (A).

Embryo morphology evaluation: Normal diploid fertilization was estimated 16–18 h after the injection. Only oocytes with two visible pronuclei were considered normal and were consequently estimated. Embryo quality was assessed according to morphological criteria based on the assessment of the blastomeres and the degree of blastomeres’ fragmentation. Good quality cleavage stage embryos were considered to have seven or eight adequate-sized blastomeres with < 10% of cytoplasmic fragmentation and no multinucleation. Top-quality embryos on day 5 were defined as an expanded and hatching blastocyst with the highest quality inner-cell-mass (ICM) and the highest quality trophectoderm (TE) cells.

Fertilization rate (FR) was calculated by dividing the number of fertilized oocytes by the number of M2 oocytes. Implantation rate (IR) was defined by the ratio between the number of gestational sacs with fetal heart activity and the number of embryos transferred. Clinical pregnancy rate (PR) was defined as the presence of at least one gestational sac exhibiting fetal heart activity.

The statistical analyses were performed, using SPSS version 12.0 and MED-CALC[®] statistical software. The chi-squared and Fisher exact tests were used to analyze nominal variables as well as the Mann–Whitney U test or Student test to compare two groups according to the distribution of the variables. Statistical significance was considered at $p < 0.05$.

Results. The baseline characteristic of all patients are shown in Table 1. AMH levels (39.3 ± 9.2 vs. 9.8 ± 5.9 ; $p < 0.0001$) and BMI (27.36 ± 4.66 vs. 22.39 ± 2.14 ; $p = 0.0001$) were significantly higher in PCOS women which is expected due to the affiliation of these patients to the classical phenotype of the syndrome.

T a b l e 1

Baseline characteristics of the PCOS classical phenotype patients and control group

Characteristic	PCOS group	Control group	<i>p</i> value
Number of cycles ICSI	109	387	
Age (y)	30	32	NS
BMI (kg/m ²)	27.36 (±4.66)	22.39 (±2.14)	0.0001
AMH (pmol/L)	39.3 (±9.2)	9.8 (±5.9)	< 0.0001
PCOS type I or II	yes	no	

PCOS = US characteristics of PCO morphology, clinical and/or biochemical hyperandrogenemia and ovarian dysfunction

T a b l e 2

The control ovarian hyperstimulation response

Characteristic	PCOS group	Control group	<i>p</i> value
E ₂ level at the day of hCG (pmol/L)	18711.1 (±4137.2)	10976.3 (±4397.9)	< 0.001
LH level at the day of hCG (mIU/mL)	2.89 (±2.46)	2.06 (±1.99)	0.001
Number of follicles > 17 mm in diameter at hCG day	9 (±4.4)	7 (±3.2)	0.001
Number of follicles < 17 mm in diameter at hCG day	8 (±4.9)	5 (±3.6)	< 0.001

At the day of hCG a significantly higher serum E₂ level (18711.1 ± 4137.2 as opposed to 10976 ± 4397.9 ; $p < 0.001$) and higher number of follicles with different size was attained in the PCOS patients ($p = 0.01$ and $p < 0.001$, respectively) (Table 2).

Significantly more total oocytes (14 ± 6.02 SD vs. 9 ± 3.25 SD; $p < 0.0001$) and meta-phase II oocytes (10 ± 1.94 SD vs. 6 ± 2.89 SD; $p = 0.0001$), as well as immature and atretic ones was retrieved in the PCOS females ($p < 0.0001$) (Table 3). However, the fertilization rate did not differ between the two groups (66.8% and 62.4%, respectively).

T a b l e 3

Oocytes and embryos characteristic and pregnancy outcome after Controlled ovarian hyperstimulation in PCOS and control group

Characteristics	PCOS group	Control group	<i>p</i> value
Total oocytes (<i>n</i>)	14 (±6.02 SD)	9 (±3.25 SD)	< 0.0001
M II oocytes (<i>n</i>)	10 (±1.94 SD)	6 (±2.89 SD)	0.0001
M I oocytes (<i>n</i>)	2 (±4.98 SD)	1 (±6.02 SD)	< 0.0001
Atretic oocytes (<i>n</i>)	2 (±1.79 SD)	2 (±1.79 SD)	< 0.0001
M I + A oocytes (<i>n</i>)	2 (±2.50 SD)	1 (±1.81 SD)	< 0.0001
Fertilization rate (%)	66.8	62.4	NS
Embryos (<i>n</i>)	9 (±5.17 SD)	6 (±2.96 SD)	< 0.0001
Top quality embryos (%)	25.6	29	NS
Embryos transferred (<i>n</i>)	2 (±1.14 SD)	2 (±0.99 SD)	NS
Implantation rate (%)	19.7	19.95	NS
Clinical pregnancy rate per cycle (%)	28.4	33.6	NS

Note: Values are expressed as means (range). M II oocyte – metaphase II oocyte cumulus complex; M I oocyte – metaphase I oocyte cumulus complex; A oocyte – atretic oocyte

The mean number of the embryos transferred was comparable among the groups (2 ± 1.14 SD and 2 ± 0.99 SD, respectively). Despite obtaining significantly more embryos in the PCOS group (9 ± 5.17 SD vs. 6 ± 2.96 SD; $p < 0.0001$), there were no differences between the two groups regarding the figures of the top quality embryos (25.6% and 29%, respectively), implantation rate (19.7% and 19.95%, respectively) and clinical pregnancy rate (28.4% and 33.6%, respectively) (Table 3).

Discussion. In the present study, we have found out that patients with PCOS have had even more favourable results in terms of numbers of oocytes retrieved and quality compared to controls with tube factor infertility. Overall it seems that PCOS is not associated with poor embryo quality or with unfavourable ICSI outcome.

Intrinsic abnormalities of the oocytes and abnormal endocrine profile have been proposed as a reason for the disordered folliculogenesis and oogenesis with detrimental impact over oocytes and embryo quality. Recent data have suggested that mature oocytes in PCOS patients have an altered gene expression profile, especially for genes implicated in meiosis and mitosis, cell-cycle check-points, genes containing androgen receptor binding sites, and genes of primary follicle recruitment [10–12]. Additionally, a microarray analysis demonstrated differential expression of dysregulated genes in the granulosa cells of PCOS women involved in oxidative stress, lipid metabolism and insulin signalling, which hypothesized that these genes may be involved in the follicular growth arrest and metabolic disorders associated with the different phenotypes of PCOS [13]. Despite these findings, several studies and meta-analyses found that patients with PCOS produce at least enough or even better competent oocytes than controls [14,15].

Considering abnormal endocrine milieu with increased LH and insulin levels, the patients with classic PCOS have been associated with an abnormal granulosa cells (GC) function. It is known that Insulin-like growth factor I (IGF-I) influences E2 synthesis at GC and act with synergism with FSH and LH. IGF operate through two membrane receptors (IGFR-1 and IGFR-2) and six IGF-binding proteins (IGFBPs 1-6) which can potentiate or inhibit IGF effect. IGFBP-3 in follicular fluid correlate with a better oocyte maturation. Higher levels of expression of IGFBP-3 mRNA in GC of growth follicles have been found during COH in patients with PCOS [11,16]. Not surprisingly, we found significantly more oocytes retrieved and more MII oocytes available in PCOS, which is in accordance with other published studies [17,18]. Simultaneously, there were more immature and atretic oocytes in the observed group. Nevertheless, metaphase II oocyte quality, in terms of fertilization and embryo development, was not impaired following ICSI in both of the groups. In contrast, lower FR has been noted in other studies [19].

Regarding embryo quality, we did not find any difference in the rate of top-quality embryos between the groups, but the mean number of achieved embryos was higher in the PCOS. Despite described abnormalities of folliculogenesis and

granulosa cell function, oocyte and embryo quality appear to be not impaired. As a result, implantation and clinical pregnancy rate have been noted to be similar to the controls, which is in agreement with earlier literature [18]. These results could be explained by the fact that we have excluded from the study patients with poor ovarian response and those with BMI over 32 who are at high risk of low implantation and high miscarriage rates.

It is known that women with PCOS are with raised serum AMH levels due to the increased number of small antral follicles in the polycystic ovaries. This excessive follicle number is linked to disturbances in folliculogenesis, which are thought to be the consequence of intraovarian hyperandrogenism [5,20]. Abnormally high anti-Müllerian hormone seems to be the most typical single finding in all PCOS phenotypes but is still not included in any classification [3]. The higher number follicles with heterogeneous size and huge serum levels of AMH and serum E2 on the day of hCG in PCOS group was not a surprising observation in this study.

In conclusion, patients with classical PCOS represent the ability to produce more MII oocytes but also more MI and atretic ones. Nevertheless, it seems that controlled ovarian hyperstimulation with these patients is not related to adverse effect on oocyte and embryo quality and ICSI outcome in terms of fertilization, implantation and clinical pregnancy rates.

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