

# Evaluation of Oocyte Quality in Polycystic ovarian syndrome (PCOS) Women Undergoing Controlled Ovarian Stimulation with Antagonist Protocol Followed by Frozen Embryo Transfer

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## ABSTRACT

**Background:** Polycystic ovarian syndrome (PCOS) is a highly prevalent hormonal disorder noted among 5-8 percent of women in the age group of 15-40 years. Genetics, diet and lifestyle play a crucial role in modulating PCOS by managing a healthy weight and abdominal circumference. In treatment procedure GnRH antagonist protocol was used as a preferred mode of stimulation to reduce the side effects of GnRH agonist procedure. The objective of this study is to evaluate the oocyte quality under controlled ovarian stimulation along with antagonist regimen, ensued by the transfer of frozen embryos. **Materials and Methods:** This research is retrospective and analytical. The data was analysed utilizing 100 PCOS and 100 non PCOS subjects. From the retrieved data, base line characteristics, quality of oocyte, M2 conversion rate, blastocyst formation rate, clinical viable pregnancy rate was noted. Antagonist protocol followed by frozen embryo transfer was assessed between PCOS and Non-PCOS women. **Results:** The M2 conversion rate was significantly higher in the Non PCOS group while the need for double antag was significantly more in the PCOS

group. No variation was observed in the rate of fertilization, cleavage rate, blastocyst conversion rate and frozen embryo rate between PCOS and non PCOS group. **Conclusion:** Antagonist protocol would be a better protocol option for PCOS women helping in reducing the risk of OHSS without compromising on quality of embryo, fertilization rate and clinical pregnancy rate.

**Keywords:** PCOS, Infertility, OHSS, Antagonist protocol, Oocyte Quality, Frozen embryo Transfer.

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## INTRODUCTION

Polycystic Ovarian Syndrome (PCOS) is a commonly prevalent hormonal disorder observed in 5-8 % women in the age group of 15-45 years. PCOS has been linked to chronic anovulatory cycles.<sup>1</sup> At every level of the reproductive system, several factors have been identified as contributing to the pathophysiology of anovulation in PCOS. Diet, lifestyle, and genetics all have a significant impact on PCOS. There is a higher likelihood of having PCOS if a close family member already has the condition.<sup>2</sup> When exposed to blood sugar levels, the fats and protein from food can become advanced glycation end products. When exposed to blood sugar, the diet can produce advanced glycation end products. Foods that are low on the glycemic index such as vegetables, fruits, whole grains and milk contain relatively few advanced glycation end products, even after cooking all of which modulate PCOS.<sup>3</sup> Obesity is known to accentuate the PCOS problem. Consequently, maintaining a healthy weight, in particular regulating the circumference of abdomen, is vital for PCOS therapy.<sup>4</sup>

PCOS patients as well as patients with infertility problems choose assisted reproductive techniques like controlled ovarian stimulation and frozen embryo transfer. Following controlled ovarian stimulation by follicle stimulating hormone, the exposure of ovaries to human chorionic gonadotropin or luteinizing hormone in many instances lead to Ovarian Hyper Stimulation Syndrome (OHSS).<sup>5</sup> There are no specific tests and no specific symptoms for OHSS. Meta-analysis data indicated a lower risk of OHSS in *in vitro* fertilisation (IVF) cycles using Gonadotropin hormone-releasing hormone (GnRH) antagonist compared to cycles using GnRH agonist as part of the treatment protocol.<sup>6</sup> PCOS is one of the major risk factors for development of OHSS and thus this sub-group of women

can be targeted for mono follicular ovulation. GnRH agonist protocol is linked with enhanced risk of OHSS in PCOS patients, therefore GnRH Antagonist protocol is used as a preferred mode of stimulation.<sup>7</sup>

Studies from multiple centres have revealed that women who have frozen embryo transfers experience much more live births than those who receive fresh embryo transfers.<sup>8</sup> Therefore, the objective of the current study was to evaluate the quality of oocyte, M2 conversion rates, blastocyst formation rates, clinical viable pregnancy rates in an intracytoplasmic sperm injection (ICSI) cycle and Antagonist protocol followed by frozen embryo transfer in PCOS and non-PCOS women.

## MATERIALS AND METHODS

### Study population

This retrospective analytical study reviewed the results of 209 patients who had undergone ICSI treatment from January 2020 – December 2021 in ARC branches (Avadi, Perambur, Egmore, Perungudi and Saveetha). As this was a retrospective study, patient's participation in the analysis was not required. After getting the IEC approval of ARC International research and fertility center, Chennai, patient data collection was initiated.

The inclusion criteria were patients diagnosed with PCOS as per the Criteria of Rotterdam<sup>9</sup> Non-PCOS women with AFC > 8 having tubal factor or male factor infertility served as controls. The women with history of continuous inflammation of the reproductive system like chronic endometriosis, endometritis, adenomyosis, presence of fluid, other ovarian pathology / surgery, structural deformity, low ovarian

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reserve, recurrent implantation failure, husband age >45 years were excluded from the study. 200 patient's data fulfilled the inclusion criteria and 100 were allocated to the PCOS and non PCOS group individually.

## Study Procedure

The patient's basic information, like maternal age, height, body mass index (BMI), weight, period of infertility, causes of infertility, preconditions, and prior IVF attempts and pregnancies, as well as number of oocytes retrieved, rate of M2 conversion, and fertilisation rate, were collected from the patient's database file after receiving ethical committee approval for the study. Patients' data was categorized based on Rotterdam criteria as PCOS as study participants ( $n=100$ ) and Non-PCOS women with AFC >8 with tubal factor or male factor infertility as control participants ( $n=100$ ). All the participants had undergone controlled ovarian stimulation with antagonist protocol triggered by GnRH, followed by oocyte retrieval and oocyte insemination. Oocyte quality and embryo grading was noted followed by freeze all strategy after which frozen embryo transfer was done. Oocyte retrieval was done 35 hrs after the trigger and the count of oocytes retrieved, count of M2, blastocyst formation rates were analyzed using vaginal ultrasound guidance under light anaesthesia. Frozen embryo transfer was done. The value of beta Human chorionic gonadotrophin (HCG) on day 15 and the evidence of heart rate in first scan was noticed.

Ovarian stimulation with recombinant human follicle stimulating hormone was carried out on the second or third day of the menstrual cycle. According to the ovarian response doses were altered. During the ovarian stimulation the goal was to form a minimum of 3 mature follicles having a diameter of 17 mm or more. Once this was achieved, Follicle-Stimulating Hormone (FSH) and other medications were discontinued, and a single injection of HCG was given to mature the eggs to allow fertilization. HCG is administered 3 hr before the scheduled egg retrieval such to allow adequate maturation of eggs. HCG was used for triggering of ovulation following Controlled ovarian hyperstimulation (COH). In a single dose of 5000 -10000IU, Urinary HCG has proved an excellent substitute for the luteinizing hormone (LH) surge.<sup>10</sup>

The timing of the egg retrieval was based on the initiation of endogenous LH surge. Approximately 4-6 h after follicular aspiration by ICSI, oocytes were inseminated depending on the quality of the sperm. Embryo scoring was done based on the morphologic criteria. After 3 to 6 days of culture, high quality embryos were taken for freezing process and stored. The frozen embryo transfer was done after the endometrial thickness reaching >7mm and the supplement of progesterone was started.<sup>11-13</sup> Follow up was done consequently and HCG level was done on 15<sup>th</sup> day and heart rate was monitored in the first scan.

## Statistical Analysis

Categorical variables were presented with frequency and proportion whereas continuous variables were presented with mean and standard deviation. The statistical significance of the cross tabulation between categorical variables was examined using the chi square test. Independent *t*-test was used to compare continuous variables between two groups. *P* value < 0.05 was considered statistically significant. The statistical analysis was performed using RStudio version 1.2.1093.

## RESULTS

The baseline characteristics of the PCOS( $n=100$ ) and non-PCOS ( $n=100$ ) was analysed. There were nil significant differences found in the age, husband age, history of previous pregnancy, hypothyroidism, and diabetes mellitus. Whereas significantly enhanced weight and BMI was observed in the PCOS compared to the non-PCOS group (Table 1).

**Table 1: Comparison of baseline characteristics between PCOS and non-PCOS group.**

Baseline characteristics		PCOS (n=100)	Non-PCOS (n=100)	P-value
Age (Mean ± SD)		28.73±3.71	28.34±3.68	0.456
Weight (Mean ± SD)		68.41±9.63	65.57±9.91	0.041
BMI (Mean ± SD)		28.89±4.96	27.07±4.31	0.006
Husband age (Mean ± SD)		33.02±4.42	33.06±4.48	0.949
Previous pregnancies	Yes	14(14.0%)	9(9.0%)	0.268
	No	86(86.0%)	91(91.0%)	
Hypothyroidism	Yes	29(29.0%)	19(19.0%)	0.098
	No	71(71.0%)	81(81.0%)	
Diabetes Mellitus	Yes	3(3.0%)	1(1.0%)	0.614
	No	97(97.0%)	99(99.0%)	

**Table 2: Comparison between PCOS and non-PCOS group.**

Variables		PCOS (n=100)	Non-PCOS (n=100)	P-value
<b>Infertility variables</b>				
Type of infertility	Primary	86(86.0%)	90(90.0%)	0.384
	Secondary	14(14.0%)	10(10.0%)	
Period of infertility	<5 years	48(48.0%)	49(49.0%)	0.411
	5 to 10 years	38(38.0%)	31(31.0%)	
	>10 years	14(14.0%)	20(20.0%)	
<b>Clinical Pregnancy</b>				
Clinical Pregnancy	Yes	56(56.0%)	53(53.0%)	0.670
	No	44(44.0%)	47(47.0%)	
Clinical viable pregnancy	Yes	47(47.0%)	49(49.0%)	0.777
	No	53(53.0%)	51(51.0%)	
<b>Antag Injection</b>				
Need for double antag	Yes	40(40.0%)	2(2.0%)	<0.001*
	No	60(60.0%)	98(98.0%)	
Antag injection	<5	25(25.0%)	86(86.0%)	<0.001*
	>5	75(75.0%)	14(14.0%)	

\*highly Significant

The type and period of infertility showed no variation when PCOS was compared with non-PCOS women (Table 2). The rate of fertilization and cleavage, blastocyst conversion rate, frozen embryo rate in PCOS was like the non-PCOS subjects. Whereas significantly increased M2 conversion rate was noted in the non-PCOS subjects compared to PCOS (Table 3). The clinical pregnancy and clinical viable rate of pregnancy for the PCOS and Non-PCOS groups were found to be similar (Table 2). The need for two or more antag injection was more in the PCOS when compared to non PCO group (Table 2). The embryo quality was assessed in both the PCOS and Non-PCOS group. Majority of the embryos was found to be of good quality in both PCOS (86%) and non-PCOS (89%) while 14% and 11% were of average quality in PCOS and Non PCOS group respectively. 14% of the embryos were found to be of average quality with 86% good quality in PCOS and 11% average quality with 89% good quality in non PCOS.

**Table 3: Comparison of M2 conversion rate, fertilization rate, cleavage rate, blastocyst conversion rate and frozen embryo rate between PCOS and non-PCOS group**

Variables	PCOS (n=100)	Non-PCOS (n=100)	P- value
M2 conversion rate in % (Mean ± SD)	62.74±17.24	72.01±17.61	<0.001
Fertilization rate in % (Mean ± SD)	83.46±15.86	84.02±13.99	0.791
Cleavage rate in % (Mean ± SD)	74.29±20.14	70.46±19.43	0.173
Blastocyst conversion rate in % (Mean ± SD)	49.51±19.36	51.47±17.15	0.449
Frozen embryo rate in % (Mean ± SD)	39.37±16.26	43.30±15.95	0.086

## DISCUSSION

The pathophysiology of PCOS is multifactorial involving endocrine, metabolic, genetic, epigenetic, and environmental factors. Compared to normal ovulating women PCOS women have enhanced levels of serum LH, reduced or normal FSH and elevated LH to FSH ratios.

In the current study PCOS patients were categorized as cases while normal responders with male infertility or tubal factor associated infertility were categorized as controls. While enhanced weight and BMI was observed in the PCOS group compared to the non PCOS group, no difference was noted in the mean age and in proportion of hypothyroidism and DM in both groups. A positive correlation was observed between body mass index and the total dose needed in PCOS group which was found to be high. Our observation was in agreement with prior studies that showed that in more than fifty percentages of PCOS women were associated with higher BMI than average population.<sup>14</sup> Oocyte maturation and ovarian steroidogenesis was impaired in obese women which was already altered in PCOS women. Obesity also increases insulin level and cause changes in adipokines level which in turn impact oocyte developmental competence. Thus, obesity is linked to reduction in the quality of oocyte q and reduced fertilization rates which could account for the changes observed.<sup>15</sup>

There was no risk of OHSS observed in PCOS as well as non PCOS group. Meta-analysis findings demonstrated a lower risk of OHSS in *in-vitro* fertilization cycles using GnRH antagonist while compared to cycles using gonadotrophin releasing hormone agonist as part of the treatment protocol for controlled ovarian hyper stimulation. Thus, when compared to agonist protocol, GnRH antagonist trigger protocol was effective in preventing OHSS.<sup>16</sup> The differential action of GnRH antagonist at both pituitary and ovarian receptors could be responsible for reduced risk of OHSS.<sup>17</sup>

The total quantity of oocytes retrieved was more in the PCOS group. Similar observation was made by Dor *et al.* who noted a profound increase in oocytes retrieved per cycle in PCOS when compared with tubal factor infertility group, but lower fertilization rates were noted in their study unlike ours where no difference in fertilization rate was observed among the two groups. Additionally, no variation was observed between the PCOS and non-PCOS groups in terms of fertilization rate, blastocyst formation rate, or number of useable embryos. Also, no variations were noted in the clinical viable pregnancy rate among PCOS and non PCOS group. These findings were reflected by Roshan *et al.* who showed that the quality of oocytes, the quantity and quality of embryos showed no significant variation among PCOS and non

PCOS patients. The antagonist protocol was known to help in the reduction of OHSS incidence rates without compromising pregnancy outcomes.<sup>18</sup> Fernandez *et al.* showed that oocyte quantity obtained from PCOS women were higher, but no change was observed in the number of high-quality oocytes obtained.<sup>19</sup> According to Nikbakht *et al.* findings there was a statistically significant difference in the quantity of oocytes extracted, but our study's results demonstrate that there was no significant difference in the quantity and quality of embryos.<sup>20</sup> In a case control, retrospective study while comparing PCOS and Non PCOS women, though the quantity of oocytes in PCOS patients were significantly more, no changes were noted in the quality of oocytes.<sup>20</sup> In a prospective cohort study done among PCOS patients in comparison with controls with male factor infertility, no difference was noted in the oocyte quality, embryo, and pregnancy rates between PCOS patients and control subjects.<sup>21</sup> In comparison with the non-PCOS group the M2 conversion rate was reduced in the PCOS group even though the oocyte retrieval was higher with no change in viable pregnancy. Esinler *et al.* showed that number of good grade embryos were increased in PCOS group in comparison to the control.<sup>22</sup>

The capacity of an oocyte to successfully go through meiosis, fertilise, and form an embryo along with fruitful pregnancy is known as oocyte competence. According to recent research, the follicular microenvironment's dysfunction, and a different synchronisation among the cumulus cells of the growing follicle and the oocyte may both affect oocyte competence.<sup>23,24</sup> In various retrospective studies it has been found that a similar rate of oocytes in the metaphase stage was observed in the PCOS and control group while the number of embryos were higher in PCOS group. The high quality of embryo was maintained in both the groups.<sup>22,25,26</sup> A meta-analysis by Heijnen *et al.* states the same results.<sup>27</sup>

The salient findings between PCOS and Non-PCOS Group after OHSS was increase in egg production and decreased M2 conversion. All other factors like blastocyst formation, pregnancy etc were the same between PCOS and Non-PCOS group. In PCOS patients as there is a follicular growth disturbance with modification in follicular microenvironment, detrimental effects on oocyte quality may be observed. The oocyte competency and pregnancy rates appear to be compromised by the confluence of a metabolic condition and PCOM. To enable a better understanding of the precise molecular mechanisms involved in the oocyte competence according to each phenotype of PCOS, prospective studies with internationally accepted diagnostic criteria are required.

## CONCLUSION

In our study we found that oocytes retrieved from PCOS group was significantly more in number when compared to non-PCOS group as it was said in previous studies but M2 conversion rate is almost same in both groups. Fertilisation rate, cleavage rate, blastocyst conversion rate was same in both groups. The percentage of top-quality embryos also remained the same. However, because of a greater number of oocyte retrieval PCOS patient had more set of backup embryos when compared to non PCOS group. Regarding clinical pregnancy rate it was same in both groups. As observed in previous studies the total dosage needed during the period of stimulation is higher in obese patients compared to patients with normal BMI. In our study compared to controls PCOS patients are found to have high BMI. It can be concluded that antagonist protocol would be the better protocol for PCOS women in reducing the risk of OHSS without compromising embryo quality or fertilisation rate or clinical pregnancy rate.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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