Assisted reproduction in polycystic ovarian disease: A multicentric trial in India

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ABSTRACT

AIM: The aim of this study is to compare ovarian response, oocyte, embryo quality, ovarian hyperstimulation syndrome incidence, and pregnancy rates in polycystic ovary syndrome (PCOS) and non-PCOS group. **MATERIALS AND METHODS:** This was a prospective observational study on PCOS carried out in seven assisted reproduction centers in India between August 2008 and July 2010, as part of trial under the Indian Society of Assisted Reproduction. A total of 192 women (77 in the PCOS group and 115 in the non- PCOS group) undergoing *in vitro* fertilization/intracytoplasmic sperm injection were included. All women had long protocol and recombinant follicle-stimulating hormone stimulation. **ANALYSIS:** The mean number of follicles and oocytes was higher in PCOS group compared with non-PCOS, being 27.2 (±8.8) and 13.6 (±5.3); 15.9 (±6.3) and 10.9 (±6.2), respectively. The recovery rates of oocytes and mature oocytes per follicle were less in the PCOS group which was 64% and 61.1%, respectively as opposed to 80.3% and 74.5%, respectively in non-PCOS group. The total numbers of top-quality embryos were less in the PCOS group. **CONCLUSION:** In PCOS women though the number of follicles was more, recovery of mature oocytes, top-quality embryos was less. Pregnancy rates were comparable in both groups.

KEY WORDS: Fertilization response to ovulation induction, polycystic ovary syndrome

INTRODUCTION

The dynamics of fertilization, implantation, and conception eventually culminating into a successful pregnancy is a complex process.

Polycystic ovary syndrome (PCOS) is the commonest endocrine disorder in women and it accounts for approximately 80% of cases of anovulatory infertility.[1] PCOS affects 5%-10% of women of reproductive age.[2] The Rotterdam criteria for PCOS are the currently accepted criteria for diagnosis. The components of the criteria are as follows and those meeting two of the following are considered to have PCOS:

- Olio- and/or anovulation,
- Clinical and/or biochemical signs of hyperandrogenism,
- Polycystic ovaries (presence of 12 or more follicles in each Ovary, measuring 2 ± 9 mm in diameter, and/or increased ovarian volume more than 10 mL),
- And exclusion of other etiologies (congenital adrenal hyperplasias, androgen-secreting tumors, Cushing's syndrome).[3]

There is an arrest of antral follicle growth in patients of PCOS. The endocrine environment is characterized by hypersecretion of luteinizing hormone (LH), insulin, and androgens. LH stimulates steroidogenesis by granulose cells and causes arrest of follicle growth in the normal mature, preovulatory follicle.

AIMS AND OBJECTIVES

- To compare ovarian response, oocyte, and embryo quality in PCOS and non-PCOS group,
- To correlate body mass index (BMI) and basal LH levels with follicles and oocytes retrieved in all patients.

MATERIALS AND METHODS

Study design

This was a prospective observational study carried out at seven assisted reproduction

centers in India between August 2008 and July 2010. The study was done as part of trial under the Indian Society of Assisted Reproduction. All the centers were given the protocol and a proforma.

A total of 192 women (77 in the PCOS group and 115 in the non-PCOS group) undergoing *in vitro* fertilization (IVF)/ intracytoplasmic sperm injection were included in the study. All non-PCOS group were chosen after four cycles of failed intrauterine inseminations. Duration of infertility was between 5 and 7 years.

Inclusion criteria of patients are as follows:

1. All of the women who have had four cycles of failed intrauterine insemination were the non-PCOS group.
2. PCOS patients were selected on the basis of the Rotterdam criteria.

The diagnosis of PCOS was made on the basis of two out of the three of the following:

Oligo or anovulation, clinical or biochemical signs of hyperandrogenism, and polycystic ovaries on ultrasound or direct inspection.

Exclusion criteria of patients are as follows:

1. Those who have undergone any ovarian surgery,
2. Known diabetic,
3. Difficult to visualize/approach any of the ovaries,
4. Age > 35 years,
5. Male factor,
6. Clomiphene citrate resistance in previous ovulation induction.

Factors taken into criteria:

1. BMI was considered as normal: 20-25 kg/m^2, overweight: 26-30 kg/m^2, and obese: >30 kg/m^2
2. Hyperandrogenemia was defined as either the presence of hirsutism as per Ferriman and Gallwey and scoring. Score of more than equal to 8 was taken as abnormal and was considered for the study. A serum testosterone more than equal to 0.8 ng/dL was taken as a cut off to define hyperandrogenism
3. Basal LH value (IU/L) on day 2 of downregulation was divided into three groups as follows:
   - <1.5, 1.5, 1.6-3.0, >3
4. Transvaginal sonography diagnosis of PCOS was done when 12 or more subcapsular follicles 2-9 mm in diameter and/or increase in ovarian volume up to 10 cm^3 to diagnose PCO pattern.

In vitro fertilization protocol

The stimulation protocol involved downregulation with gonadotropin-releasing hormone (GnRH) analogue (Inj. Lupride 0.4 mL s.c.) Sun Pharmaceutical Ind. Ltd. Acme Plaza, Andheri-Kurla Road, Andheri (E) Mumbai-400059 (India) starting from the luteal phase of the previous cycle 1 week before the expected menses, followed by gonadotrophin stimulation with either Inj. Recagon 150 IU (MSD) Organon (Ireland) Ltd. P.O.Box 2857, Drynam Road Swords, Co. Dublin, Ireland or Inj. Gonal F 150 IU (Merck Serono) after ensuring adequate downregulation (Estradiol <30 pg/mL, endometrium <5 mm). The follicular development was monitored by transvaginal sonography along with serum estradiol levels. Follicular maturation was triggered by administration of Inj. human chronic gonadotropin (hCG) 10,000 IU, Inj. Ovitrelle (Merck Serono) Merck Serono S.p.A., Via delle Magnolie, 1570026 - Modugno (BA), Italy, when at least three follicles reached 18 mm in diameter. A total duration of recombinant FSH was an average of 9 days. Ultrasound-guided transvaginal oocyte retrieval was performed 35 h after the hCG administration. Embryo transfer was done 2 days later.

Grading of embryo quality

The Bolton classification, 1989, was used for grading the embryo quality. Three major components considered were blastomere regularity, speed of cleavage, and degree of fragmentation.[4] There are three grades, of which grade III embryos are the best quality embryos.

Data collection and outcome

The data including age, duration, and type of infertility, BMI, basal FSH, and LH level on day 2 following downregulation, number of follicles on the day of oocyte pick-up, number of mature oocytes, and number of grade 3 (top-quality) embryos were collected and compared between the two groups.

Statistical analysis

Results were expressed in mean and percentage. The statistical significance was carried out using the scientific package for social sciences (SPSS-2000). Statistical significance was determined using Mann-Whitney test, Z test for proportions, one-way analysis of variance (ANOVA), Chi-square, and independent *t*-test.

RESULTS AND OBSERVATION

The patient characteristics and profiles were matched in both the groups and the following observations were made. There were no significant differences in the age, BMI, and duration of infertility in the PCOS and non-PCOS groups.

Patients in both groups were less than 35 years of age. Patients above 35 years, those who underwent any ovarian surgery and known diabetics, were all excluded.

First objective was to compare the number of follicles obtained on the day of oocyte pick-up following stimulation. The independent *t*-test was used and a $P < 0.05$ was taken to be statistically significant.

As shown in Table 1, a total of 192 patients were analyzed of which 77 in the PCOS group and 115 in the non-PCOS group were studied. A mean of follicles of 27.2 (±8.8) in the PCOS group versus a mean of 13.6 (±5.3) follicles in the non-PCOS was observed on the day of hCG. $P < 0.001$ was statistically significant. On the day of oocyte pick-up, the total number of oocytes obtained was more in the PCOS group. A mean of 15.9 (±6.3) oocytes in the PCOS group versus a mean of 10.9 (±6.2) in the non-PCOS group was observed. $P < 0.001$ was statistically significant.

Although the total number of follicles obtained was more in the PCOS group, the oocytes obtained were significantly less as compared with the non-PCOS group. The Z test for proportion was applied and a $P < 0.001$ obtained was statistically significant between the two groups. Although the total number of oocytes obtained was more in the PCOS group, the mature oocytes obtained were significantly less as compared with the non-PCOS group. The Z test for proportion was applied and a $P< 0.001$ obtained was statistically significant between the two groups.

Figure 1 shows that the ratio between the number of grade 3 embryos and the total number of embryos was significantly higher in the non-PCOS group which implies that higher order of top-quality embryos were more in the non-PCOS group. Mann-Whitney test was applied and the *P* value obtained was significant. As shown in Table 2, the mean number of follicles did not differ in the three groups of BMI.

The number of follicles and oocytes was compared in different groups of LH obtained on the second day of menses following downregulation. One-way ANOVA test was used and there was no statistical significance obtained [Table 3]. Since the study was done with downregulation protocol, sporadic ovulation was not seen due to strict dosage schedule.

The two groups were compared to see for the incidence of ovarian hyperstimulation syndrome which was significantly found to be higher in the PCOS group. The Chi-square test was used and a *P* value of 0.008 obtained was statistically significant [Table 4]. The pregnancy rates were comparable in both the groups as evident in Table 4. Chi-square test was used and the *P* value obtained was insignificant.

DISCUSSION

PCOS patients have posed significant challenges when treated for their infertility with IVF and embryo transfer.

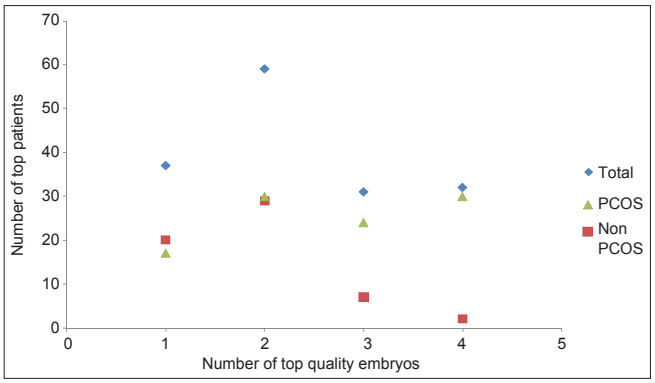

Figure 1: Scatter diagram showing distribution of top-quality embryos

Table 1: Follicles, oocytes number, and recovery

	PCOS (*n*=77)	Non-PCOS (*n*=115)	*P* value
Mean number of follicles	27.2 (±8.8)	13.6 (±5.3)	<0.001
Mean number of oocytes	15.9 (±6.3)	10.9 (±6.2)	<0.001
Total number of follicles	1894	1268	
Total number of oocytes	1227	1019	
Recovery rate (%)	64.7	80.3	*P*<0.001
Total number of mature oocytes	750	759	
Recovery rate (%)	61.1	74.5	*P*<0.001

PCOS=Polycystic ovary syndrome

Table 2: Mean number of follicles and body mass index (*N*=192)

BMI (kg/m^2)	20-25 (*n*=109)	26-30 (*n*=66)	31-35 (*n*=17)
Mean number of follicles	16.59 (±8.3)	17.04 (±8.2)	15.9 (±8.0)
P value	*P*>0.05		

BMI=Body mass index

Table 3: Mean number of follicles and luteinizing hormone (*N*=192)

LH (IU/L)	<1.5 (*n*=85)	1.6-3.0 (*n*=56)	>3.0 (*n*=51)	*P* value
Mean number of follicles	18.54 (±7.2)	18.53 (±7.4)	20.56 (±8.1)	>0.05
Mean number of oocytes	12.74 (±5.2)	12.84 (±5.1)	13.3 (±5.5)	>0.05

LH=Luteinizing hormone

Table 4: Ovarian hyperstimulation syndrome and pregnancy

Group	PCOS (*n*=77)		Non-PCOS (*n*=115)		*P* value
	n	%	*n*	%	
OHSS (*n*=21)	12	15.5	9	7.8	0.008
Pregnancy (*n*=59)	23	29.8	36	31.3	>0.05

OHSS=Ovarian hyperstimulation syndrome; PCOS=Polycystic ovary syndrome

In our study, we found that the mean number of oocytes retrieved on the day of OPU in the PCOS group was 15.9 (±6.3), whereas in the non-PCOS group was 10.9 (±6.2) the *P* value of which was less than 0.001 and was found to

be significant. However, the recovery rate of mature oocytes was 64.7% in the PCOS group and 80% in the non-PCOS group ($P < 0.001$ which was found to be significant). These findings were in agreement with those of the study on 112 patients with comparable age, BMI, and duration of infertility.[5]

The embryo quality to a great extent depends on the quality of oocyte from which it was obtained. Embryos with higher cell numbers, regular appearing cells, and little or no fragmentation have a higher overall chance of implantation. We have used Bolton's criteria according to which, grade 3 embryos are the top-quality embryos. In our study, we compared the grade 3 embryos with respect to the total embryos in each group and found that in the PCOS group, the ratio of grade 3 embryos and total embryos was significantly less in the PCOS group. Similarly in the study of 112 patients,[5] the embryo quality was scored using a criteria similar to Bolton's criteria; however, a cumulative embryo score was taken as opposed to individual score in our study. The cumulative embryo score was better for the PCOS group probably due to a higher mean number of embryos. In our study, the total number of embryos obtained was relatively more in the non-PCOS group as mature oocytes obtained were higher.

Obesity and PCOS are common diagnoses among infertility patients, accounting for a significant proportion of women seeking IVF embryo transfer treatment. Obesity is defined as ≥30 kg/m^2. As compared to normal weight patients, it is believed that obese patients stimulate poorly during ovulation induction with Intra uterine insemination (IUI) and IVF embryo transfer cycles. A study[6] conducted on 372 patients found mean number of follicles and oocytes retrieved were not related to BMI in accordance with our study. Despite consensus agreement on the adverse effects of extremes of BMI on chances of spontaneous conception, the affect of extremes of BMI on IVF success is controversial. A study on 333 patients has failed to find any effect of BMI on IVF pregnancy rate,[7] whereas another study on 180 patients demonstrated a negative effect only when the women were in the obese category.[8]

High tonic LH concentrations are one of the hallmarks of PCOS. High LH has been claimed to decrease oocyte quality and cause a higher miscarriage rate. This high LH concentration is thought to be detrimental to oocyte maturation and quality and can be the cause of the lower conception rate and higher abortion rate in these patients. Therefore, pituitary suppression could be helpful in these patients. In a study conducted on 100 patients with PCOS,[9] it was found that elevated levels of LH had a higher rate of miscarriage than the women with PCOS and normal LH levels. However, in a prospective randomized controlled trial,[10] it was seen that there was no advantage of LH suppression in PCOS patients, since despite the use of GnRH analogue treatment the incidence of clinical abortions was significantly higher in women with PCOS compared with controls. The pregnancy rate was in the same range. In our study, LH on day 2 of menses following downregulation was comparable in both groups. No significant association was noted between the number of follicles and oocytes and different groups of LH.

In a prospective, randomized, double-blind study,[11] the pretreatment effect of metformin in PCOS patients undergoing IVF was studied. It was observed that folliculogenesis was better in those who received metformin. Hyperinsulinemia and insulin resistance are present in the majority of women with PCOS. Hence, efforts have focused on improving insulin sensitivity through diet and lifestyle modification, weight reduction, and insulin-sensitizing drugs.

A meta-analysis of nine studies[12] compared conventional IVF outcomes in PCOS patients with matched controls. The analysis reported significantly more oocytes per oocyte retrieval in PCOS group when compared with controls as in our study, but higher fertilization rate in the control group which resulted in an equal number of fertilized oocytes in both studied groups.

In a study done on 314 patients,[12] PCOS patients had more oocytes retrieved as compared with non-PCOS. Although there were a higher number of immature oocytes in PCOS patients, they also noticed higher number of mature oocytes, unlike our study, with comparable fertilization rate in both studied groups.

SUMMARY AND LIMITATIONS

Summary

1. The total number of follicles obtained in the PCOS group were higher than in the non-PCOS group which was a mean of 27.2 (±8.8) in the PCOS group and 13.6 (±5.3) in the non-PCOS group.
2. The total number of oocytes obtained in the PCOS group was higher than in the non- PCOS group and was 15.9 (±6.3) in the PCOS group and 10.9 (±6.2) in the non-PCOS group.
3. Though the number of follicles was more in PCOS group, the recovery rate of oocytes and mature oocytes per follicle were less in the PCOS group, which was 64% and 61.1%, respectively in PCOS group as opposed to 80.3% and 74.5%, respectively in non-PCOS group.
4. The total number of top-quality embryos per total embryos was less in the PCOS group. The ratio between the number of grade 3 embryos and the total number of

embryos in each group was calculated. It was 0.28 in the PCOS group as opposed to 0.5 in the non-PCOS group which was statistically significant.

5. There was no statistical significant difference in the number of follicles and oocytes recovered in different BMI groups and different LH groups.

Limitations

1. In the present study, BMI and LH did not correlate with the number of oocytes retrieved which probably was due to the smaller number of patients.
2. Other causes of anovulation in the PCOS group were not studied like hyperprolactinemia or thyroid disorders which if corrected would enhance ovulation.
3. The effect of insulin resistance in the group of PCOS patients has not been studied with respect to oocyte and embryo quality. Insulin resistance results in increased intrafollicular androgen production and increased LH production both of which have deleterious effects on the oocyte.

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