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Original Article

Decreased follicular progesterone level on follicle aspiration day results in better embryo formation in women with polycystic ovary syndrome

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Abstract: Aim: Polycystic ovary syndrome (PCOS) is associated with lower pregnancy and higher miscarriage rates in assisted reproductive technologies. Our study aimed to determine the effect of follicular progesterone on oocytes and embryo development in an antagonist protocol for PCOS patients. Methods: This prospective study recruited PCOS patients and healthy controls who underwent controlled ovarian stimulation using an antagonist protocol in which fluid from a single primary follicle was collected and tested. Data were analysed and embryo outcomes were assessed. Results: (1) On trigger day, PCOS patients showed higher plasma level of luteinizing hormone than controls, while on follicle aspiration day, PCOS patients showed significantly lower follicular progesterone level than controls; however, both groups obtained similar oocyte outcomes, except for significantly lower viable embryo rates in PCOS patients. (2) A PCOS subgroup with a relatively lower follicular progesterone level ($P \leq 1.8 \times 10^4$ ng/mL) on aspiration day showed a significantly higher viable embryo rate compared to PCOS patients with a higher follicular progesterone level ($P > 1.8 \times 10^4$ ng/mL) and non-PCOS controls; in addition, the blastocyst formation rate was significantly higher in the subgroup with lower follicular progesterone compared to that in healthy controls. PCOS patients with higher follicular progesterone exhibited levels similar to those in non-PCOS patients but the viable embryo rate was dramatically lower. Conclusion: PCOS patients showed lower progesterone levels in primary follicles. However, the reduction of follicular progesterone level on aspiration day in PCOS patients was associated with better early embryo development, and therefore might imply a potential protective mechanism for oocyte development while not affecting oocyte maturation in PCOS.

Keywords: Polycystic ovary syndrome, follicular progesterone, embryo quality, in-vitro fertilization

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common causes of infertility, with multiple associated endocrine disorders and ovulation failure. With the development of assisted reproductive technologies (ART), ovulatory problems are mostly solved. However, PCOS is still associated with lower pregnancy rates and higher risk of spontaneous abortions with ART [1-3]. While the altered endometrial environment in PCOS patients undergoing in-vitro fertilization (IVF) has been reported by many authors [4-7], whether embryonic factors play a role remains uncertain. Changes that occur in the intrafollicular environment where oocytes develop and the mechanisms by which aberrations affect the quality of oocytes and embryos are intricate. Bellver et al. found that the metabolomic profile of D3 embryo spent culture medium was similar in obese women with or without PCOS [8]. Other authors discovered that granulosa cells from PCOS patients cultured in vitro exhibit decreased progesterone (P4) production [9]. However, there are few reports on whether the follicular P4 level in vivo is decreased in PCOS patients and how the P4 alteration affects the fate of oocytes. The aim of this study was to compare the follicular P4 level in vivo on aspiration day in PCOS patients and non-PCOS controls undergoing in-vitro-fertilization (IVF) procedures, and to study the associations between follicular P4 level and early embryo formation.

Materials and methods

Patients and controls

All subjects were suitable candidates for IVF treatment, with a history of no more than two prior attempts. Informed consent was obtained for participation. The local ethics committee authorized the experiments. Infertility factors were determined using hysterosalpingography, laparoscopy, etc. Patients were diagnosed with PCOS according to the Rotterdam criteria [10]. Age- and weight-matched women were recruited as controls. Controls had regular menstruation (cycle period 28-35 d), with no hyperandrogenism. Infertility was mainly due to a male factor, fallopian tube obstruction, or other undefined reason. Controls also included healthy and fertile couples undergoing IVF with preimplantation genetic testing for chromosomal mutations. Women with moderate to severe endometriosis or pelvic surgical histories that might influence ovarian function were excluded.

Treatment protocol

Both PCOS patients and controls underwent controlled ovarian hyperstimulation using an antagonist protocol alone. When at least 2 follicles reached 18 mm in diameter, blood samples were taken to test plasma luteinizing hormone (LH), oestrogen (E2), and P4 levels; based on the results, human chorionic gonadotropin (HCG) was administered to trigger ovulation. After 36 hours, oocytes were retrieved. The fluid from the first primary follicle (≥18 mm in diameter) on one side of the ovary was carefully collected after oocytes were retrieved. Only the midstream 1-2 mL was collected to avoid dilution by the needle washing fluid and blood contamination near the end of the stream. The follicular sample was centrifuged and the clear fluid samples were stored at -80°C for later analysis. The pellet was suspended with 1 mL phosphate-buffered saline and a haemocytometer was used to ensure no blood cells were present. Otherwise, samples were discarded.

Notably, some PCOS patients received a dual trigger with combined HCG 2,000 IU and a gonadotropin-releasing hormone (GnRH)-agonist (GnRH-a) 200 μg to avoid ovarian hyperstimulation syndrome in light of the shorter LH surge duration with a GnRH-a trigger [11-13]. Since the dual trigger can achieve a maturation effect similar to that using a standard HCG trigger, and since our test point was early in the luteal follicular stage, we assumed that the dual trigger would not interfere with our study aim. We compared the constituent ratio of the 2 triggering methods in our study groups.

Definition of rates

Oocyte maturation rate was calculated as the ratio of mature oocytes to harvested oocytes. Normal fertilization rate referred to the ratio of normally fertilized eggs with two pronuclei (2PN) to all fertilized eggs. Cleavage rate referred to the ratio of cleavage embryos to fertilized oocytes. A viable cleavage embryo was assessed according to the Society for Assisted Reproductive Technology grading standard [14], and the Blastocyst Quality Score [15] was used for blastocyst grading. The viable embryo rate specifically referred to the ratio of good quality cleavage embryos to all 2PN-derived cleavage embryos.

Statistical analyses

All statistical analyses were performed using SPSS version 20.0 (SPSS for Windows, version 20.0, USA). Continuous data were presented as means ± standard deviations. Student's t-test was used to compare clinical and biochemical characteristics. Constituent ratio differences in male factor infertility and triggering methods were compared using chi-square analysis, with Fisher's exact test for expected frequencies less than 5. A value of $P<0.05$ was considered statistically significant.

Results

A total of 53 women met the inclusion criteria for the study, with 28 PCOS patients and 25 controls. There were no significant differences between PCOS patients and controls in terms of clinical and biochemical characteristics, except that the PCOS group exhibited significantly higher plasma levels of basal LH and trigger day LH. Strikingly, PCOS patients had significantly lower levels of follicular P4 on the day of oocyte retrieval. However, PCOS patients had similar outcomes in terms of numbers and rates of oocytes, fertilization, and embryo formation, without significant statistical differences, as shown in **Table 1**.

Table 1. Clinical characteristics and oocyte outcomes in PCOS patients and controls

Variable	PCOS group n=28 (52.8%)	Control group n=25 (47.2%)	*P* value
Age of women (years)	29.1±0.60	28.3±0.7	0.85
BMI in women (kg/m^2)	22.8±0.6	21.3±0.6	0.08
Age of men (years)	31.3±0.8	30.9±0.8	0.73
Sperm factor	8	10	0.40
Non-sperm factor	20	15	
Basal FSH (IU/L)	4.73±0.20	5.22±0.24	0.12
Basal LH (IU/L)	7.17±0.76	3.85±0.34	0.00
Initiation day FSH (IU/L)	4.56±0.27	4.87±0.24	0.41
Initiation day LH (IU/L)	5.53±0.68	3.48±0.37	0.01
Initiation day E2 (pg/L)	34.3±2.6	31.9±3.1	0.56
Initial dose of Gn (IU)	148.66±4.83	171.50±10.32	0.05
Duration of Gn (days)	9.39±0.35	9.12±0.37	0.59
Total dose of Gn (IU)	1287.2±57.8	1490.8±90.6	0.06
HCG-day plasma P4 (ng/mL)	0.7±0.1	0.8±0.1	0.23
HCG-day plasma E2 (pg/L)	3349.4±272.4	2710.1±226.2	0.08
HCG-day plasma LH (IU/L)	1.80±0.30	1.42±0.18	0.29
Endometrium (mm)	11.2±0.4	11.4±0.5	0.80
HCG trigger	22	23	0.26
Dural trigger	6	2	
Follicular P4 (ng/mL)	21926.7±1722.2	27290.5±1981.1	0.05
Number of oocytes	17±1	14±1	0.10
Maturation rate (%)	95.01±2.18	84.38±4.93	0.10
Non-ICSI normal fertilization rate (%)	58.94±4.46	62.12±5.18	0.68
ICSI normal fertilization rate (%)	85.58±3.53	80.63±3.00	0.13
Cleavage rate (%)	98.11±0.83	94.26±2.39	0.06
Viable embryo rate (%)	43.09±3.64	55.86±4.34	0.03
Blastocyst formation rate (%)	60.22±5.31	53.30±5.88	0.39
Embryos preserved	6±1	5±1	0.25

PCOS, polycystic ovarian syndrome.

We further analysed how P4 reduction could affect IVF outcomes in the PCOS group. We divided PCOS patients into 2 groups according to follicular P4 level, with a cut-off value of 1.8×10^4 ng/mL, which was the tertile of follicular P4. PCOS patients with lower follicular P4 levels ($P\leq1.8\times10^4$ ng/mL) were in group L. PCOS patients with higher follicular levels ($P>1.8\times10^4$ ng/mL) were in group H. Clinical data were compared between the 2 subgroups and between subgroups and healthy controls. When compared with non-PCOS controls, the PCOS-L subgroup exhibited significantly higher basal LH and HCG-day plasma LH; however, follicular P4 level was significantly lower, and resulted in a significantly higher viable embryo rate. In a comparison between the PCOS-H group and non-PCOS controls, we found that the PCOS-H group showed significantly higher basal, initial, and HCG-day plasma LH, but both the follicular P4 level and viable embryo rates were similar in these 2 groups. When we compared PCOS-H and PCOS-L groups, basal LH was similar, but the PCOS-L group had significantly lower initial and HCG-day plasma LH levels compared to those in the PCOS-H group; the follicular P4 was higher in the PCOS-H group, but the PCOS-L group achieved significantly higher viable embryo rates and higher embryo formation rates. Data are summarized in **Table 2**. Therefore, the plasma LH and follicular P4 level on oocyte aspiration day displayed a more complex association that was not in accordance with the stimulatory effect of LH on plasma P4 level.

Discussion

The disadvantages of IVF for PCOS patients remain controversial. PCOS is still associated with lower pregnancy rates and a higher risk of spontaneous abortion with ART. PCOS increases the risk of various pregnancy-related and neonatal complications [16]. Uterine dysfunction with an excess of oestrogen and an inadequacy of P4 response is a key factor [4]. Whether PCOS patients have worse IVF outcomes because of oocyte- or embryo-derived factors remains unclear. In a review article, Sermondade et al. stated that PCOS patients had alterations in oocyte and embryo quality but had IVF outcomes similar to those in healthy controls [17]. On the other hand, Kdous et al. found that patients with PCOS showed better global oocyte and embryo quality. Liu et al. reported better fertilization and cleavage rates

Table 2. Clinical characteristics and oocyte outcomes in L and H subgroups in PCOS patients and in healthy controls

Variable	PCOS-L n=10 (35.7%)	PCOS-H n=18 (64.3%)	*P* value (L vs. H)	*P* value (L vs. control)	*P* value (H vs. control)
Age of women (years)	29.2±1.1	29.8±0.8	0.67	0.64	0.22
BMI in women (kg/m^2)	22.1±1.0	23.0±0.9	0.52	0.31	0.07
Age of men (years)	32.4±1.5	31.2±1.2	0.55	0.81	0.53
Sperm factor	3	5	1.00	0.71	0.52
Non-sperm factor	7	13			
Basal FSH (IU/L)	4.83±0.36	4.76±0.29	0.90	0.15	0.34
Basal LH (IU/L)	7.57±1.31	7.29±1.17	0.88	0.00	0.00
Initiation day FSH (IU/L)	4.11±0.56	4.28±0.32	0.35	0.25	0.36
Initiation day LH (IU/L)	4.38±0.93	5.03±0.66	0.35	0.10	0.04
Initiation day E2 (pg/L)	26.8±3.6	35.6±5.2	0.23	0.65	0.32
Initial dose of Gn (IU)	152.8±11.4	143.8±6.8	0.72	0.16	0.86
Duration of Gn (days)	9.6±0.5	9.8±0.7	0.87	0.95	1.00
Total dose of Gn (IU)	1356.4±124.7	1341.5±99.4	0.94	0.07	0.51
HCG-day plasma P (ng/mL)	0.66±0.14	0.74±0.08	0.49	0.10	0.37
HCG-day plasma E2 (pg/L)	3273.0	3608.7	0.45	0.24	0.16
HCG-day plasma LH (IU/L)	1.13±0.12	2.69±0.36	0.00	0.01	0.08
Endometrium (mm)	11.0±0.8	12.1±0.8	0.43	0.42	0.97
HCG trigger	9	13	1.00	0.30	0.36
Dual trigger	3	3			
Follicular P4 (ng/mL)	12953.7±1099.8	27310.5±1364.1	0.00	0.00	0.40
Number of oocytes	19.1±2.8	15.4±1.6	0.07	0.01	0.56
Maturation rate (%)	94.2±2.9	99.1±0.8	0.16	0.45	0.08
Non-ICSI normal fertilization rate (%)	53.69±5.20	67.45±7.96	0.17	0.04	0.59
ICSI normal fertilization rate (%)	80.45±10.45	87.22±5.24	0.55	0.91	0.12
Cleavage rate (%)	98.21±1.12	97.56±1.36	0.75	0.04	0.31
Viable embryo rate (%)	54.55±6.69	36.89±3.90	0.02	0.33	0.01
Blastocyst formation rate (%)	67.54±5.17	52.96±8.99	0.25	0.04	0.78
Embryos preserved	8±1	6±1	0.12	0.08	0.66

PCOS, Polycystic Ovarian Syndrome. PCOS-L, PCOS patients with lower follicular P4 levels (P≤1.8×10^4 ng/mL). PCOS-H, PCOS patients with higher follicular P4 levels (P≤1.8×10^4 ng/mL).

in PCOS patients and concluded that ART can improve the quality of cumulus-oocytes [18]. The follicular biochemical environment that involves granulosa cells and follicular fluid are key targets. Hamori et al. discovered that granulosa cells from PCOS patients cultured in vitro showed decreased P4 production [9]. Artimani et al. found that granulosa cell P4 receptor subtype levels were significantly decreased in PCOS patients [3]. Wissing et al. discovered several cycle-related gene and pathway alterations in PCOS corona radiata cells, yet failed to find any detectable embryological defects [4].

Our data showed that PCOS patients had 2-fold higher basal LH levels, and significantly higher trigger-day LH levels than controls. Plasma P4 levels were similar. However, PCOS patients exhibited significantly lower follicular P4 levels while achieving comparable oocyte and embryo outcomes, which is in accordance with published data cited above [17]. These results indicate inconsistency between intrafollicular and plasma P4 levels; moreover, the role of LH in the stimulation of P4 secretion might also be affected, and requires further study.

When it comes to P4 and PCOS, some studies revealed impaired P4 secretion in granulosa cells recovered from PCOS patients when cultured in vitro [9]. However, few have revealed the true effect in vivo and the impact on IVF outcomes. The association between plasma P4 levels on follicle aspiration day with IVF outcomes in PCOS patients has been studied [19, 20]. Slightly better pregnancy rates were reported in PCOS patients with higher P4 levels, but the differences were not significant.

However, these authors investigated the extrafollicular levels of plasma P4, which may not directly and effectively reflect the intrafollicular environment in the presence of the blood-follicle barrier. A definite association between intrafollicular and plasma P4 levels is not necessarily present. Moreover, plasma P4 is released by all follicles, and is influenced by overall follicle numbers. According to our data, the follicular P4 level in single dominant follicle measures about 10 μg/mL, which is much higher than the plasma level. A direct investigation of the intrafollicular environment is essential to determine what is best for the development of each and every oocyte and embryo. Indeed, Foong et al. also found that follicular P4 level was decreased in PCOS patients undergoing long protocol stimulation [21]. However, the follicular P4 level they reported was dramatically lower than ours. We speculate this may be due to our larger target follicle diameter and strict prevention of blood contamination. The phenomenon of follicular P4 reduction in PCOS patients was verified in our research limited to the GnRH-antagonist protocol. No data have been reported for the association between follicular P4 levels and IVF outcomes in PCOS patients.

To elucidate this, we divided PCOS patients into 2 subgroups according to follicular P4 levels: PCOS-H and PCOS-L. Both H and L groups had higher basal LH levels than those in non-PCOS controls; however, while follicular P4 levels were significantly different, they achieved significantly different viable embryo rates. The PCOS-H group showed higher follicular P4 levels, comparable to those in non-PCOS controls, and the viable embryo rates in the PCOS-H group were significantly lower than in the PCOS-L group, while similar to those in non-PCOS controls. When the follicular P4 level was comparable in PCOS and non-PCOS patients, PCOS patients exhibited worse embryo development; however, when follicular P4 was decreased, the viable embryo rate increased. We also found significantly higher blastocyst formation rates in the PCOS group with lower follicular P4 compared to healthy controls.

According to these results, and considering the correlation between the significantly decreased level of follicular P4 and similar oocyte and embryo quality in PCOS patients compared to controls, it seems tempting to speculate that within a proper range and to a certain degree, follicular P4 reduction may act as a protective factor for development of oocytes against damage caused by other harmful elements in PCOS, such as unusually high pituitary LH production. Moreover, it appeared that the lower the plasma LH level on HCG-day, the better the embryo outcome. This requires clinical study for confirmation.

P4 plays a role in suppressing follicle growth in the rat follicular phase [22]. Chaffkin et al. found that a high dose of P4 added to early granulosa-lutein cell culture medium obtained from IVF patients inhibit cell growth [23]. Jesel demonstrated that P4 in the early oestrous cycle inhibited follicle growth in pigs [24]. On the other hand, a number of studies reported that P4 could inhibit apoptosis of HCG-induced early granulosa-lutein cells in both rat and human ovaries [25, 26]. P4 is vital to maintain corpus luteal function [27]. Based on the evidence cited above, we assume that the mechanism responsible for suppression of P4 secretion could develop in the follicular phase to protect follicle growth. We hypothesize that the suppression of P4 secretion in the early follicular stage fails to recover from previous inhibitory action to maintain luteal function, which creates a dilemma in PCOS physiology. Given the seemingly contradictory roles of P4 in granulosa cells, P4 may indeed function differently in different stages of the menstrual cycle, and deserves further investigation.

In our study, PCOS patients showed decreased follicular P4 levels on aspiration day. As noted above, the P4 secretion ability is impaired [9]. Therefore, we strongly agree with the hypothesis that impaired IVF pregnancy outcomes in PCOS patients could mainly result from dysfunctional corpus luteal function [9], rather than because of impaired oocyte or embryo quality, because embryo development is comparable in PCOS and non-PCOS patients (**Table 1**). Regarding the higher viable embryo rates in the PCOS group with lower P4 levels, there seems to be a paradox in terms of subfertility in women with PCOS, such that P4 may be decreased to catch up with follicular growth, while failing to maintain normal corpus luteal function. We recommend mechanical and functional studies to further clarify this phenomenon.

As nearly half of our PCOS patients had their embryos frozen without a subsequent fresh embryo transfer, and even completed frozen embryo transfer cycles, long-term studies with more samples are required to determine the impact of a P4 decrease in PCOS on ultimate pregnancy outcomes.

In conclusion, we found that the follicular P4 level was decreased in PCOS patients compared to that in non-PCOS controls in IVF using antagonist protocols. PCOS patients with lower follicular P4 levels have higher viable embryo rates. Additional studies are needed to confirm this finding and to identify regulatory mechanisms.

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Disclosure of conflict of interest

None.

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