

EVALUATION OF THE MORPHOLOGY OF THE DAY-3 EMBRYO IN PATIENTS WITH POLYCYSTIC OVARY SYNDROME APPLIED THE GnRH ANTAGONIST PROTOCOL WITH GnRH AGONIST FOR TRIGGERING FINAL OOCYTE MATURATION

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SUMMARY

Objectives: To evaluate the day-3 embryo morphology in polycystic ovary syndrome (PCOS) patients utilized GnRH antagonist protocol along with GnRH agonist for triggering final oocyte maturation. **Subjects and methods:** A prospective, descriptive study was conducted on 140 patients with PCOS as defined by the Rotterdam 2003 criteria. Stimulation by GnRH antagonist protocol along with GnRH agonist for triggering final oocyte maturation. The evaluation of cleavage-stage embryos was based on the number of blastomeres, the uniformity of the blastomeres and the percentage of fragmentation. **Results:** The average number of embryos harvested was 8.21 ± 4.39 embryos. Most of the embryos had the number of blastomeres of 7 - 8 (71.4%). The embryos with equally-sized blastomeres accounted for higher rate (61.5%) than unequally-sized ones (31.5%). Embryos exhibiting fragmentation less than 10% accounted for the highest proportion (46.5%). There was no difference in the number of blastomeres, the uniformity of the blastomeres and the percentage of cytoplasmic fragmentation among phenotypic groups ($p < 0.05$). Good-quality embryos occupied the highest proportion (48%) whereas embryos of average and poor quality explained for the same percentage of 26%. **Conclusion:** The utilization of GnRH antagonist protocol along with GnRH agonist for triggering final oocyte maturation hardly influences on day-3 embryos in the patients with PCOS.

* **Keywords:** Day-3 embryo morphology; Polycystic ovary syndrome; GnRH antagonist protocol; In vitro fertilization.

INTRODUCTION

Polycystic ovary syndrome, a common disease, accounts for 8 - 13% among reproductive-aged women [1]. This syndrome negatively affects fertility, making up 80% of the causes of infertility due to anovulation, menstrual disorders, and pregnancy complications. In treatment of infertility by in vitro fertilization (IVF) for patients with PCOS, the utilization of the

ovarian stimulation with GnRH antagonist protocols has become increasingly popular. Moreover, according to some studies in the world, the GnRH antagonist protocol along with GnRH agonist for triggering oocyte maturation has many benefits such as: no ovarian cyst formation, shorter treatment duration, less medicine and particularly lower risk for ovarian hyperstimulation syndrome [3]. However,

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the endogenous LH surge induced by GnRH agonist has different characteristics from that one in the natural menstrual cycle as well as hCG. On the other hand, some researches show that in patients experiencing PCOS, there are many factors that directly and indirectly affect the oocytes and the embryos reducing their quality [4]. Therefore, this study aimed: *To evaluate the morphology of day-3 embryos using GnRH agonist.*

SUBJECTS AND METHODS

1. Subjects

140 patients diagnosed with PCOS underwent IVF with the GnRH antagonist protocol along with GnRH agonist from July 2017 to September 2019. The present study was conducted at the Military Institute of Clinical Embryology and Histology - Vietnam Military Medical Academy.

* *Inclusion criteria:* According to the Rotterdam criteria [4], patients have two of the three following characteristics: (1) Clinical hyperandrogenism and/or biochemical signs of hyperandrogenism (Hyperandrogenism - HA); (2) Ovulatory dysfunction (OD); and (3) Polycystic ovarian morphology (PCOM). The classified phenotypes include: phenotype A: HA + OD + PCOM; phenotype B: HA + OD; phenotype C: HA + PCOM; and phenotype D: OD + PCOM.

2. Research methods

* *Sample size:*

The sample size was calculated by the formula of expected proportion of a descriptive research. The pregnancy rate in patients with PCOS when utilizing

GnRH antagonist protocol according to Mokhtar S (2015) was 34% [5], 95%CI, tolerance was 8%. Therefore, the sample size was calculated $n = 134$ patients. Our selected samples included 140 patients.

* *Research design:*

A prospective, descriptive study.

* *Research procedures:*

All of the patients underwent ovarian stimulation with GnRH antagonist protocol. FSH was used from day 2 of the menstrual cycle; commonly used FSH was α follitropin (Gonal-f; Merck Serono, Italy) or follitropin β (Puregon; Organon; Netherlands). The starting dose of FSH was usually 100 - 200 IU daily. On the day 6 of controlled ovarian stimulation cycles, GnRH antagonist was supplemented with commonly used products such as etotide (Merck Serono, Italy or Orgalutran (ganirelix) 0.25 mg Organon, Irish). Ultrasound monitoring of follicles and dose adjustment according to the patient's response were on days 6, 8 and 10 of controlled ovarian stimulation cycles. Continue to monitor the follicles until at least 2 follicular sizes ≥ 18 mm then GnRH agonist was used (commonly subcutaneously used Diphereline 0.2 mg) for triggering final oocyte maturation. Oocyte aspiration was performed, which was guided by transvaginal ultrasound after 35 hours of injection for final oocyte maturation. Fertilization was done through ICSI technique. The quality of embryos on day-3 was graded into 3 groups of good, average and poor levels. Good embryos include 7 - 8 uniformly-sized blastomeres and less than 10% of cytoplasmic fragmentation. Average-quality embryos

are the ones with less than 7 equally-sized blastomeres and 10 - 25% of cytoplasmic fragmentation. Comparisons among the phenotypic groups of polycystic ovary syndrome were also made.

RESULTS

1. Characteristics of the number of blastomeres of day-3 embryos

Table 1:

Number of blastomeres	Phenotype				Total n (%)	p(*)
	A n (%)	B n (%)	C n (%)	D n (%)		
≤ 4	21 (10.4)	10 (9.1)	16 (5.2)	42 (7.9)	89 (7.7)	0.08
5 - 6	22 (10.9)	17 (15.4)	53 (17.5)	61 (11.4)	153 (13.3)	0.42
7 - 8	134 (66.3)	76 (69.1)	217 (71.4)	393 (73.9)	820 (71.4)	0.39
≥ 9	25 (12.4)	7 (6.4)	19 (6.2)	36 (6.8)	87 (7.6)	0.33
Total	202	110	305	532	1,149	

(*): One-way ANOVA test.

2. Characteristics of the uniformity of the blastomeres

Table 2:

The uniformity of the blastomeres	Phenotype				Total n (%)	p(*)
	A n (%)	B n (%)	C n (%)	D n (%)		
Uniformity	124 (61.4)	69 (62.7)	186 (61.0)	328 (61.7)	707 (61.5)	0.51
Non-uniformity	78 (38.6)	41 (37.3)	119 (39.0)	204 (38.3)	442 (38.5)	0.76
Total	202	110	305	532	1,149	

3. Characteristics of the percentage of cytoplasmic fragmentation

Table 3:

Cytoplasmic fragmentation	Phenotype				Total n (%)	p(*)
	A n (%)	B n (%)	C n (%)	D n (%)		
≤ 10%	61 (30.2)	57 (51.8)	139 (45.6)	277 (52.1)	534 (46.5)	0.02
11 - 25%	94 (46.5)	33 (30.0)	116 (38.0)	187 (35.1)	430 (37.4)	0.47
> 25%	47 (23.3)	20 (18.2)	50 (16.4)	68 (12.8)	185 (16.1)	0.37
Total	202	110	305	532	1,149	

4. Classification of day-3 embryos

Table 4:

Classification	Phenotype, n (%)				Total n (%)	p(*)
	A	B	C	D		
Good	84 (41.6)	50 (45.5)	141 (46.2)	276 (51.9)	551 (48.0)	0.18
Average	53 (26.2)	28 (25.4)	89 (29.2)	129 (24.2)	299 (26.0)	0.98
Poor	65 (32.2)	32 (29.1)	75 (24.6)	127 (23.9)	299 (26.0)	0.32
Total	202	110	305	532	1,149	

DISCUSSION

Embryo morphology is the most widely used criterion for selecting the best embryo for transfer. The morphology of day-3 embryo is characterized by the number of blastomeres, the uniformity of the blastomeres and the percentage of cytoplasmic fragmentation.

- The number of blastomeres:

Embryos from 7 to 8 blastomeres occupied the majority (71.4%). Our results are similar to Duong DH et al's finding in an evaluation on a 1,323 day-3 embryos. He found that the number of blastomeres of 7 - 8 was the highest (48%) [2]. The study by Holte J et al (2007) also confirmed the important role of the number of blastomeres in relation to the successful transfer on day-3 embryos. He also showed that 7 or 8 blastomeres increases the rate of blastocyst implantation, clinical pregnancy and the live birth rate [7].

- The uniformity of the blastomeres:

In our study, this results were similar to Holte J et al's finding (2007) in which the ratio of embryos with equally-sized blastomeres accounted for 60% [7]. Duong DH et al (2016) found that the

number of day-3 embryos with blastomeres made up 42.4% [2] which was lower than in our study. Holte J et al also confirmed the important role of the embryos with equal blastomeres that significantly increases pregnancy rates in comparison with embryos transfer for unequal blastomeres [7]. Another study by Duong DH et al examined the impact of quality of embryos on day 3 in relation to their ability to form blastocysts. He found that blastocysts formation rate of day-3 embryos with uniform blastomeres was 67.4%, while this rate of uneven blastomeres was only 25.4% [2].

- The rate of cytoplasmic fragmentation: The degree of cytoplasmic fragmentation is assessed in almost all scoring systems of embryos. Cytoplasmic fragmentation in our study less than 10% constituted the majority (46.5%), the number of embryos with cytoplasmic fragmentation degree of 11 - 25% accounted for 37.4%, the degree of fragmentation more than 25% accounted for 16%. This result was similar to Duong DH et al's finding [2]. According to a research evaluating the impacts of fragmentation on the formation of embryonic cyst, when degree of fragmentation increases,

there will be fewer embryos undergoing compaction, cavitation and become normal blastocysts. In addition, fragmentation influences the spatial distribution of blastomeres during differentiation, so the author suggests that in order to increase pregnancy rates, embryos with high degree of fragmentation should not be transferred [8]. In the research by Seok-Gi et al (2018) the removal of embryo fragmentation out of day-2 embryos significantly improves the developmental potential and the pregnancy rate of these embryos.

** Evaluation of day 3 embryo classified by morphology:* Our study evaluates embryo quality based on morphological characteristics with 3 levels: good, average and poor. The results show that good-quality embryos was the highest (48%), whereas the quality of average and poor embryos were equal (26%). According to Duong DH et al, the percentage of good-quality embryos was 28.3%, 43.4% was average embryos and 28.3% the poor embryos [6]. The research by Singh N et al (2014) comparing GnRH antagonist and long GnRH agonist protocol in patients with PCOS showed that the average number of good embryos was 74.1% for antagonist protocol compared to 81.1% in long GnRH agonist protocol; however, the difference is not statistically significant [9]. The research by Sigala et al (2015) included 194 patients classified into 2 groups. The experimental group consisted of 97 patients with polycystic ovarian morphology (PCOM) and a control group (97 patients without PCOM). The average number of embryos harvested in

the experimental group was significantly higher than the control group (6.3 embryos compared to 4.7 embryos), but the incidence of day-3 good embryo was equivalent between the two groups [10]. Thus, it can be said that in our study, the rate of good quality day-3 embryos was rather high and similar to other authors' finding, which shows that the group of patients with PCOS or PCOM utilizing GnRH antagonist protocol have the same or better embryos in comparison with patients using other ovarian stimulation protocols.

CONCLUSION

The morphological study of 1,149 day-3 embryos in 140 patients with PCOS, we reached conclusion that the utilization of GnRH antagonist protocol along with GnRH agonist for triggering final oocyte maturation hardly influences on day-3 embryos in the patients with PCOS.

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