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Original Paper

The effect of GnRH analogues on oocyte morphology and subsequent embryo development following intra cytoplasmic sperm injection (ICSI)

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ABSTRACT

A competent, morphologically normal gamete (oocyte or sperm) is essential for successful fertilization, the development of a good-quality embryo, and implantation. Intracytoplasmic sperm injection (ICSI) allows direct microscopic assessment of gamete quality and the study of factors that may affect gamete quality and development. The aim of this study was to assess the effect of different gonadotropin-releasing hormone (GnRH) analogue protocols (antagonist vs agonist) on oocyte maturity, morphology, quality, and embryo development. Fifty-three subfertile women were included. Participant age ranged from 18–37 years and body mass index (BMI) from 18.9–24.9 kg/m². All underwent ICSI using GnRH antagonist and agonist protocols. Microscopic evaluation of oocyte and embryo quality was performed and compared between groups. Among all parameters studied (total retrieved oocytes; oocyte quality and morphology; embryo quality; fertilization and cleavage rates), only the total number of good-quality embryos was significantly higher and the total number of poor-quality embryos was significantly lower in the GnRH antagonist group. Thus, the GnRH antagonist significantly increases the rate of developing good-quality embryos and reduces the rate of poor-quality embryos, despite producing a nonsignificantly higher number of immature and morphologically abnormal oocytes, which appear to have no impact on embryo quality or subsequent development.

Keywords: *GnRH analogues, ICSI, Oocyte maturity, Oocyte morphology*

1 INTRODUCTION

The ovarian follicle and the oocyte within it consist of highly specialized cells that aid in activation of the embryonic genome [1]. The developmental competence of oocytes is usually acquired within the ovaries through oogenesis [2]. The oocyte is typically arrested at the diplotene stage of prophase in the first meiotic division until puberty [3]. Resumption of the first meiotic division and initiation of the second meiosis occur after the luteinizing hormone (LH) surge at ovulation. Following ovulation, the released oocyte remains arrested at metaphase of the second meiotic division until fertilization by a sperm [4]. Only an oocyte at metaphase II (MII) with the first polar body extruded into the perivitelline space is considered mature and can be used for intracytoplasmic sperm injection (ICSI) [5].

Assessment of oocyte quality includes both maturity and morphology, which must be evaluated together using an inverted microscope during the assisted reproduc-

tive cycle following controlled ovarian hyperstimulation (COS) [6]. A morphologically normal, mature oocyte of 120 µm should be round, with moderately clear, slightly granular cytoplasm and a single, non-fragmented first polar body extruded into a small, fragment-free perivitelline space. It is surrounded by a clear, translucent zona pellucida [7]. Significant morphological abnormalities are observed among oocytes and may affect developmental competence, embryo quality, and implantation potential [8]. A marked deviation from normal should be recorded as a dysmorphic oocyte [4]. Nuclear maturity is defined by reaching MII, whereas cytoplasmic maturity depends on features observed in the cytoplasm (e.g., vacuoles, granularity) and extracellulary (e.g., polar body shape/size, perivitelline space size or fragments) [2, 6, 9].

Some dysmorphic features may reflect physiological changes during maturation and have no significant effect on fertilization, embryo quality, or development [10]. Others have proposed that several factors predispose to these changes, including female age, body mass index (BMI), stimulation protocol, hormonal fluctuations during COS, premature LH surge, type and dose of the maturation trigger, and temperature and pH changes during laboratory handling [2, 11–15]. The intra-follicular microenvironment may also play a role; studies suggest that oocytes from women with polycystic ovary syndrome (PCOS) exhibit specific morphological features related to hormonal imbalance (elevated estradiol [E2], hyperandrogenemia, and excessive LH) [16, 17]. Some researchers argue that dysmorphic oocytes may impair fertilization with sperm, alter embryonic genome activation, and lead to poor-quality embryos with chromosomal defects, resulting in implantation failure after transfer [18–21]. Conversely, other reports consider these abnormalities to be normal variants with no impact on embryo quality, further development, or the embryonic genome [22]. This controversy regarding oocyte morphology and its effect on embryo quality and development underscores the need to study predisposing factors for oocyte dysmorphism so they can be mitigated where possible [23].

Gonadotropin-releasing hormone (GnRH) antagonists and agonists are used during assisted reproductive cycles to achieve pituitary down-regulation through suppression of follicle-stimulating hormone (FSH) and LH, allowing clinicians to control endogenous gonadotropin secretion, prevent premature LH surge and spontaneous ovulation, and time oocyte retrieval for ICSI. By inhibiting endogenous FSH and LH and administering exogenous FSH with or without LH according to age, infertility etiology, prior response, or prior ICSI outcomes, oocyte quality and morphology, embryo quality, and subsequent development may be affected; however, the exact effects remain incompletely defined [12].

2 MATERIALS AND METHODS

This retrospective cohort study included couples randomly selected from the fertility consultation clinic at Al-Sadr Medical City IVF Center, Al-Najaf Al-Ashraf, Iraq. It comprised 53 subfertile women who underwent controlled ovarian hyperstimulation (COS) and intracytoplasmic sperm injection (ICSI) between June 2022 and December 2022. Patients were divided by protocol into two groups: group 1, agonist (n = 12), and group 2, antagonist (n = 41). Included women were normal ovulators, aged 18–37 years, with body mass index (BMI) 18.9–24.9 kg/m². Women with polycystic ovary syndrome (PCOS), endometriosis, hypogonadism, hypothyroidism, advanced age ($>$ 37 years), BMI $>$25 kg/m², or chronic diseases were not included. Male partners had normal semen analysis; men with severe semen impairment or using frozen sperm were not included.

Pituitary down-regulation was achieved with either a gonadotropin-releasing hormone (GnRH) agonist (Decapeptyle 0.1 mg; Serona) using a short protocol from day 2 of the menstrual cycle or a GnRH antagonist (Cetrotide 0.25 mg; Serona) from day 6 (fixed protocol). Ovarian stimulation used recombinant follicle-stimulating hormone (r-FSH; Gonal-F 75 ×2 IU; Merck), monitored by serial transvaginal ultrasound (TVUS) and hormonal assays. Final oocyte maturation was triggered with recombinant human chorionic gonadotropin (hCG; Ovitrelle 250 µg ×2) after achieving an adequate number (7–14) of follicles $\geq$ 18 mm. Oocytes were retrieved transvaginally.

Noninvasive assessment of oocyte maturity and morphology was performed with an inverted microscope at 400× magnification. Oocytes that had completed meiosis I and reached metaphase II (MII) were considered mature. Fertilization was assessed 18 hours later by the presence of two pronuclei and two polar bodies (zygote). Oocyte morphology was also evaluated for four abnormalities: cytoplasmic vacuoles, cytoplasmic granularity, abnormal polar body, and perivitelline space (PVS) abnormalities.

Embryos were graded by a standard system considering blastomere number, shape, size equality, mono- or multinucleation, and degree of fragmentation. Good-quality embryos (grades I and II) met the criteria of four

cells at 48 hours or eight cells at 55 hours, mononucleation, and ¡15% fragmentation. All others were classified as poor-quality embryos (grades III and IV).

Data were analyzed using SPSS version 24. Mean ± SD was calculated for continuous variables and percentages for categorical variables. Group differences were assessed with the t-test and chi-square test, respectively. A p-value ≤ 0.05 was considered statistically significant.

3 RESULTS

The mean age of women in the two groups was 28.7 ± 3.03 years, and the mean BMI was 23.57 ± 1.89 kg/m². Table 1 presents the mean total gonadotropin dose, type of infertility, and duration of infertility for both groups and shows no significant differences between them, although women in the agonist group required descriptively higher gonadotropin doses for stimulation.

Table 1 Total gonadotropin dose, duration, and type of infertility in the agonist and antagonist groups.

Studied parameter	Agonist n=12	Antagonist n=41	P-value
Total dose of Gonadotropin (iu/l)	1864.58±1011.71	1604.26±441.44	0.20
Duration of infertility (years)	8.66± 4.11	6.87 ±3.13	0.11
Type of infertility			
Primary	11	29	0.13
Secondary	1	12	
Total	12	41	

Note. Significant difference at P-value of ≤ 0.05.

Table 2 reports the mean total number of retrieved oocytes, their maturity, and the total number of morphologically abnormal oocytes. Although the differences were not statistically significant, the GnRH antagonist group had higher totals for retrieved oocytes, mature oocytes, and morphologically abnormal oocytes than the agonist group. For individual morphological abnormalities, both groups produced comparable numbers of affected oocytes, with no significant variation between them.

Table 3 presents the total number of embryos, embryo quality, and fertilization and cleavage rates. The GnRH agonist group showed a significantly decreased number of good-quality embryos ($p \leq 0.05$), while the number of poor-quality embryos was highly significantly higher ($p \leq 0.01$). No significant differences were observed in fertilization or cleavage rates between the two groups.

4 DISCUSSION

Among the factors that can affect oocyte quality (maturity and morphology), two protocols are commonly used in assisted reproduction: GnRH antagonist and GnRH agonist protocols [11, 12]. Most comparative studies have evaluated efficacy (response to stimulation, pregnancy rate) and safety (e.g., ovarian hyperstimulation syndrome [OHSS]) and concluded that GnRH antagonists are safer and as effective as GnRH agonists [5,7,24]. However, less is known about their effects on oocyte maturity, oocyte morphology, and embryo quality.

The present study showed that the GnRH antagonist protocol produced more oocytes with a lower total gonadotropin dose; these oocytes had a higher maturation rate but a greater likelihood of morphological abnormality than those from the GnRH agonist protocol. Several studies have reported no significant differences in oocyte morphology or maturity between antagonist and agonist protocols [11, 25], while another reported that the antagonist may improve oocyte maturity and morphology [12].

Regarding embryo quality, the current study found a significantly higher number of good-quality embryos in the antagonist group, and a highly significant reduction in poor-quality embryos compared with the agonist group. Similar findings suggest that GnRH antagonists may improve embryo quality, blastocyst quality, and euploidy rates [26]. By contrast, no significant differences in embryo quality were reported by Hassan MF and by Al-Yasiry et al. [24, 27]. These observations indicate that morphologically abnormal oocytes did not have a significant impact on embryo quality or subsequent embryonic development, consistent with Abood AH and Hassan MF [2] and a recent meta-analysis by Bartolacci et al. that examined diverse oocyte morphological abnormalities [10, 22, 27]. Thus, GnRH antagonists may be preferred over GnRH agonists for yielding more mature oocytes with a lower exogenous gonadotropin dose and a higher rate of good-quality embryos.

5 CONCLUSION

Pituitary down-regulation with a GnRH antagonist significantly increased the number of good-quality embryos and reduced the number of poor-quality embryos, despite a nonsignificant increase in immature and morphologically abnormal oocytes, which appeared to have no effect on embryo quality or subsequent development.

Table 2 Total oocytes, oocyte maturity, and morphologically abnormal oocytes.

Studied parameter	Agonist n=12	Antagonist n=41	P-value
Total number of oocytes	8.90±4.43	13.65±8.03	0.06
Total number of mature oocytes	7.27±4.12	11.51±7.66	0.08
Total number of immature oocytes	1.45±1.43	2.35±2.91	0.33
Total number of morphologically abnormal oocytes	1.72±3.63	2.27±5.08	0.76
Total number of oocytes with cytoplasmic granularity	0.81±2.71	0.50±1.39	0.59
Total number of oocytes with cytoplasmic vacuoles	0.00±0.00	0.42±1.70	0.41
Total number of oocytes with abnormal polar body	0.18±0.40	0.60±2.71	0.61
Total number of oocytes with abnormal PVS	0.72±2.41	0.75±3.31	0.98

Note. Significant difference at P-value of $\leq$0.05.

Table 3 Total oocytes, oocyte maturity, and morphologically abnormal oocytes.

Studied parameter	Agonist n=12	Antagonist n=41	P-value
Total number of embryos	5.00±3.26	7.15±4.55	0.16
Total number of good quality embryos	3.90±2.55	6.93±4.46	0.04*
Total number of bad quality embryos	1.10±1.91	0.23±0.57	0.01**
Fertilization rate	66.44±21.78	72.88±21.93	0.39
Cleavage rate	88.88±30.22	95.23±17.27	0.36

Note. Significant difference at P-value of $\leq$ 0.05.

DECLARATIONS

Conflict of interest

The authors declare no competing interests.

Consent to publish

All authors consent to the publication of this work. Written informed consent for publication was obtained from the participants.

Ethical approval

The study was approved by the Medical Research Bioethical Committee/College of Medicine/Kerbala university No.11,30-3-2022. Verbal consent was obtained from the patients to be included in the study.

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