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Original Article

The influence of ovarian hyperstimulation drugs on morphometry and morphology of human oocytes in ICSI program

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ABSTRACT

Objective: To compare the influences of controlled ovarian hyperstimulation (COH) drugs using recombinant follicular stimulating hormone (rFSH) versus human menopausal gonadotropins (hMG) on morphometry and morphology of MII oocytes in ICSI cycles.

Materials and methods: In this prospective study, 363 MII oocytes from 50 ICSI cycles with male factor infertility were evaluated. The patients were divided into two groups according to the protocols of COH: I- rFSH and II- hMG. The immature oocytes were excluded from the study. All oocytes were categorized into four morphological groups of normal, and those with single, double, or multiple defects. The inclusive morphometrical criteria were: areas and diameters of oocyte, ooplasm, and zona pellucida (ZP). Also, circumferences of oocyte and ooplasm were assessed.

Results: The ZP area and ooplasm diameter for both normal and abnormal oocytes were significantly higher in group I (P: .05; P: .028, respectively) compared to group II (P: .023; P: .003, respectively). In abnormal oocytes, ooplasm diameter was higher in group I compared to group II. Furthermore, ooplasm area for abnormal oocytes was significantly higher in group I compared to group II. There was an increasing trend for number of mature oocytes, in abnormal oocytes, for group I (5.53 ± 3.1) in comparison with group II (4.4 ± 2.97; $P = .25$). The rate of oocytes with normal morphology was significantly higher in hMG, when compared to rFSH groups.

Conclusion: Morphometrical parameters were increased in rFSH group, but the normal morphology of oocytes were significantly enhanced in hMG group. Treatment with proper dosage of ovulation induction drugs may enhance the number of normal sized oocytes.

Introduction

Gonadotropin therapy plays an important role in ovarian stimulation for infertility treatments. It was introduced almost one century ago and yielded major advancements during last 25 years [1]. Recombinant follicle stimulating hormone (r-hFSH) and human menopausal gonadotrophin (hMG) are two gonadotrophin products primarily used for controlled ovarian hyperstimulation (COH) in assisted reproduction techniques (ART). FSH is a key gonadotropin hormone during the follicular phase and only low amounts of LH are needed in different stages of follicular development. Excessive levels of LH in the early or late follicular phase have adverse effects on fertilization, embryonic development, implantation, and pregnancy rates [2].

Although, both hMG and r-hFSH have been shown to be effective, a number of studies have further compared their safety and clinical effectiveness [3–8]. r-hFSH is free from urinary protein contaminants with less immunogenic potential than the urinary-derived medication and may be preferable from a safety standpoint [9]. hMG contains both FSH and LH activity extracted from the urine of post-menopausal women with low purity, as 95% of the proteins are contaminants. In addition, the urinary proteins may have negative effects on follicular recruitment and development [2]. Comparing the effectiveness of both compounds has been an object of large debate since 1990 [10–13]. Van Wely et al. reported that applying a long GnRH agonist protocol resulted in a higher clinical pregnancy rate for hMG compared to rFSH [5]. However,

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there was no evidence of a difference in rates of ongoing pregnancy or live birth between hMG and rFSH recipients [9]. Furthermore, no differences were found in gonadotropin doses used, oocytes retrieved, miscarriage or multiple pregnancy rates [5]. Also, a significant increase in live birth rate was found using hMG when compared to rFSH following a long down regulation protocol in ART cycles [6]. Al-Inany and colleagues showed the method of fertilization might influence the outcomes of patients receiving highly purified hMG or r-hFSH. They concluded that hMG achieved better pregnancy rates in IVF, but not in ICSI cycles compared to r-hFSH. However, most of these trials have focused on clinical parameters such as number of oocytes, dosage/duration of gonadotrophin used, fertilization and pregnancy rates.

There are few studies [14–16] examining the effects of different gonadotrophin preparations on oocyte morphometrical and morphological quality, despite their importance on the outcome of ART cycles. It was shown that oocyte morphology is an important prognostic factor in the success of ART program [17–19]. Studies on different species have elucidated the relation between oocyte diameter and competence for maturation and embryo development. Griffin and colleagues (2006) showed that oocyte diameter can be used as a marker for oocyte maturity or meiotic competence [20]. From their controlled study, Bao Obata et al. (2000) found that the capacity for cleavage after maturation and IVF is related to the increase in oocyte age and diameter [21]. Ng et al. (2001) found no significant difference in the percentage of MII oocytes between the hMG and rFSH groups (86.9% versus 87.4%, respectively). Their findings showed that the proportions of normal and abnormal morphology of oocytes were similarly distributed in two groups. In addition, the zona thickness as well as the diameters of both oocyte and ooplasm were comparable between the aforementioned groups [22]. Michelmann et al. (1995) also found no significant differences between morphometrical parameters, including ooplasm, ZP, and perivitelline space (PVS) of fertilized and unfertilized oocytes [23]. However, in one of the largest meta-analysis published to date, the effects of ovarian stimulation drugs on oocyte quality has not evaluated [9]. Up to date there is no references regarding the effects of oocyte morphology and morphometry on ART outcomes and no study to compare the effects of stimulation drugs on oocyte parameters in a clinical program. So, the purpose was to investigate the effect of different ovarian stimulation drugs on both morphometrical and morphological aspects of oocytes aspirated from patients with male factor infertility in ICSI setting.

Materials and methods

Patient selection

In this prospective study, a total of 50 consecutive ICSI cycles with male factor infertility were included. The range of female patients' age was from 20 to 40 years old. Patients received the initial dosage of 150 IU/day of COH drugs on the 2nd day of menstrual cycle. Subsequent applied dosages were adjusted according to the estradiol level and sonographic results on the 8th day of the cycle. The patients were divided into two groups according to the protocols of COH: Group I (hMG; Merioanal, IBSA, Lugano, Switzerland) and Group II (rFSH; Gonal-F, Serono, Rome, Italy). When serum E2 concentration exceeded 1000 pg/ml, and at least 2 follicles $\geq$18 mm in diameter were recorded by ultrasound, 10,000 IU human chorionic gonadotropin (hCG; Serono, Rome, Italy) was administered to induce ovulation. After 36 h, oocytes were collected by transvaginal ultrasound guidance. This research study was approved by ethics committee of our institution. This study was approved by the ethics committee of the Yazd Research and Clinical Center for Infertility. Written informed consent was obtained from all patients.

Oocyte evaluation

A total of 359 MII oocytes were morphologically and morphmetrically evaluated from their photomicrographs taken using inverted microscope (Nikon, Japan) equipped with Cornus software (Research instruments Ltd Co, UK). Measurements comprised of the diameters of whole oocyte (μm), ooplasm (μm), width (thickness) of ZP (μm), peripheral and areas of whole oocyte, ooplasm, and ZP (μm^2). Several measurements for different morphometrical parameters, were measured and the average of the measurements was recorded (Fig. 1). The oocytes were denuded from cumulus cells using 80 IU hyaluronidase/ml (Sigma Chemical Co., USA), also with the mechanical aid of drawn pasture pipettes. Immature oocytes were excluded from study. The rates of immature oocytes were recorded, with no further assessments.

The oocyte abnormalities were irregular shaped oocyte, cytoplasmic granulation, dark ooplasm, vacuolation, smooth endoplasmic reticulum (SER), refractile bodies (RF), bull's eye, wide PVS, ZP abnormality, and fragmented polar body (PB) (Fig. 2). All oocytes were categorized into four morphological groups of normal, and the ones with single, double, and multiple defects. The inclusive morphometrical criteria were diameters of oocyte, ooplasm, and ZP; areas of oocyte, ooplasm, and ZP. Also, circumferences of both oocyte, and ooplasm were assessed as well.

Statistical analysis

All data were expressed as mean ± standard deviation. Shapiro–Wilk test was applied for evaluating the distribution of data. Comparisons between groups were performed using student's *t*-test or chi-square, and the Mann–Whitney *U* test. P value of <.05 was considered statistically significant.

Results

Oocyte characteristics

The comparisons between oocyte's morphometrical parameters are shown in Tables 1 and 2. The results indicated that the mean of ZP area for both normal and abnormal oocytes between groups I and II was marginally significant (1992.20 ± 201.50, 1848.23 ± 338.6; P = .05), (9670.06 ± 1710.58, 10,100 ± 9456.78; P: .023), respectively. In addition, the same was happened for ooplasm diameter; (57.56 ± 4.6, 54.73 ± 5.06; P: .028), (118.24 ± 96.34, 110.87 ± 11.29; P: .003), respectively.

The mean of ooplasm area for abnormal oocytes between groups I and II was also significant; (1095.277 ± 801.963, 1188.328 ± 1345.9568; P: .004); respectively (Table 1). There was an increasing trend for number of mature oocytes, in abnormal oocytes, for group I (5.53 ± 3.1) in comparison with group II (4.4 ± 2.97) (P = .25). The rate of oocytes with single, double, and multiple abnormalities in groups I and II is shown in Table 3. Our data showed that multiple oocyte defects were more common in both groups.

Discussion

The purpose of COH is producing more mature oocytes available for IVF with less side effects. The drug used for COH may have impact(s) on oocyte quality including oocyte morphology and morphometry. The role on oocyte quality on ART outcome has been proved. According to Coticchio et al. (2004), developmental fate of

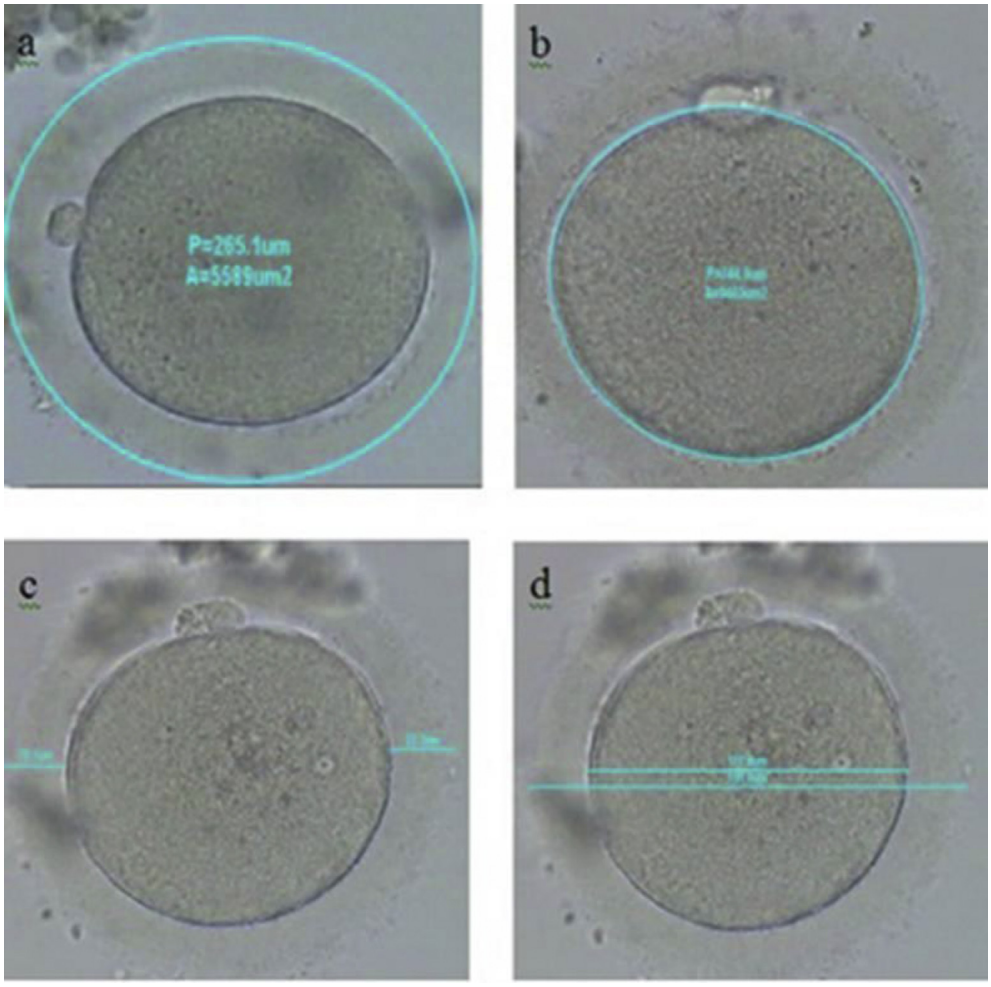

Fig. 1. Dimensions measured during morphometry evaluation of oocytes (200× magnification). a: peripheral and area of oocyte, b: ooplasm peripheral and area, c: ZP thickness, d: oocyte and ooplasm diameter.

the embryo is principally dictated by the mature oocytes [24]. In this regard, the relationship between PB morphology and 70 consecutive ICSI outcomes was investigated by Ebner et al. (2000). They observed a significant correlation between non-fragmented Pb morphology and high fertilization rate and embryo quality [25]. Several studies have evaluated the relation between oocyte diameter and the capacity for maturation and embryo development in different animal species and human [20]. Griffen et al. carried out a comparative analysis of follicle and oocyte diameter in rats, mice, pigs, and humans. Their conclusion was that the relation between oocyte diameter and degree of maturation is species-specific and should not be generalized to other animal models [20]. Nazari et al. [26] evaluated maturation capacity, morphology and morphometric parameters of human immature oocytes and concluded that morphometric parameters can not be applied as prognosis factor in oocyte maturation outcome in IVM program.

Eppig and Schroeder also demonstrated in rats that successive stages of meiotic maturation and embryogenesis were related to oocyte age and size [27]. Also, Durinzi et al. [28] used oocytes from women who were undergoing surgical procedures for non-ovarian disorders, demonstrated that the ability of human oocytes from non-stimulated cycles to resume meiosis and complete IVM was correlated with oocyte diameter at the time retrieval [28]. Thus, the maturation potential of immature oocytes must be related to the level of growth reached by oocytes inside the follicles before the detachment provoked by ovulation [28]. In a recent study, Cavilla et al. [29] used IVM to evaluate oocytes from women with polycystic ovary syndrome and demonstrated that larger oocytes had a better probability of maturation. Garside et al. (1997) noted that changes in zona thickness correlated with the number of blastomeres, grade, fragmentation, age and were more evident in embryos transferred from cycles resulting in successful pregnancies. Therefore, ZP measurements should be included in the overall assessment of embryo quality, since this information may be useful in the selection of optimal embryos for uterine transfer [30]. Furthermore, it has reported that oocyte abnormalities such as vacuolation and refractile bodies were found to correlate with lower fertilization and cleavage rates [25,31].

Claudia Valeri et al., [2011] evaluated the correlation between oocyte morphometry parameters and woman's age and concluded that there was association in human oocytes between zona pellucida and spindle birefringence and a decrease of oocyte size and ZP thickness as a function of women's age [16]. Rashidi et al. compared the effect of HMG and r-hFSH on oocyte quality and concluded that there were no significant differences between rate of metaphase II oocytes between these two groups [32].

Our results demonstrated that there were no differences in morphological patterns between rFSH and hMG groups while Our data showed that multiple oocyte defects were more common in both groups and significantly was higher in rFSH group. This

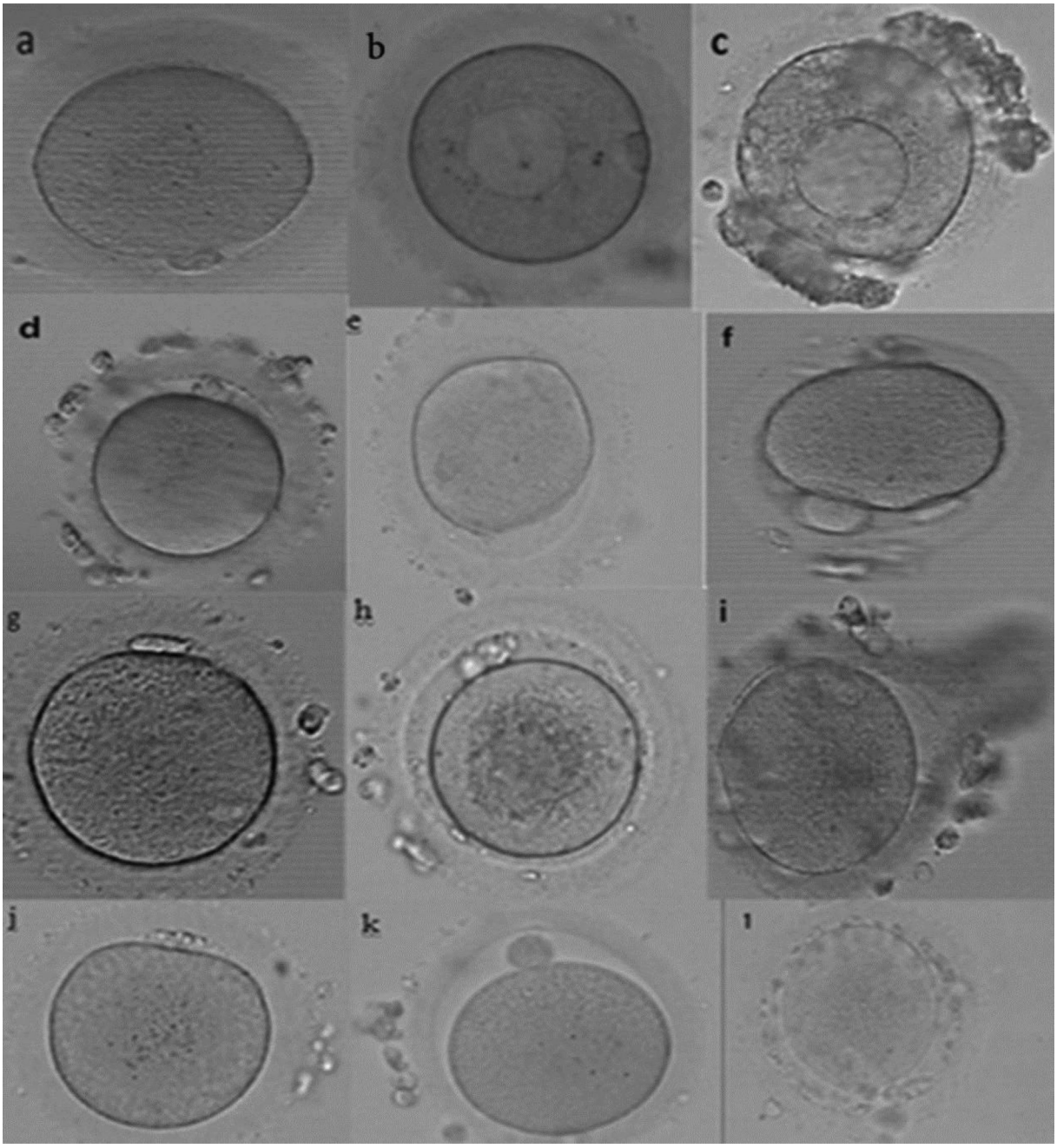

Fig. 2. Different oocyte morphological criteria: normal, single and multiple abnormalities (200× magnification). a) normal, b) smooth endoplasmic reticulum cluster, c) vacuole, d) fragmented polar body, e) irregular shape, f) huge polar body, g) general granulation, h) central granulation, fragmented polar body, wide PVS and debris in PVS i) irregular ZP and dark cytoplasm j) fragmented polar body and refractile body, k) wide PVS, irregular ZP and refractile body l) wide PVS, debris in PVS and fragmented polar body.

outcome is similar to study of Ng et al. (2001) that showed the proportions of normal and abnormal morphology of ZP, oocyte and PB were similarly distributed in two groups [22]. Thibault and associates (1997) suggested that this could be related to a progesterone and estradiol deficiency which is closely connected to maturation of the nucleus and cytoplasm [33]. Also, Ng et al. (2001) noted that the hormonal supply in their study seemed to be optimal which was manifested in a lower percentage of cytoplasmic anomalies [22]. In addition, Van Blerkom and Henry (1992) noticed chromosomal disorder for up to 40% of all eggs collected which could be another reason for oocyte morphological anomalies [34]. Imthurn et al. [15] examined the effect of stimulation with highly purified FSH compared to hMG on oocyte morphology and concluded that short-term FSH highly purified treatment protocol synchronizes oocyte maturation better than stimulation with HMG and higher proportion of oocytes in the FSH highly purified group were nuclear mature than in the HMG group.

Furthermore, we realized that ooplasm morphometric parameters were significantly changed in rFSH group. In contrast to our data, Ng et al. (2001) found that the ZP thickness and the diameters of oocyte and ooplasm were comparable between rFSH and hMG groups [22]. According to Otoi et al., canine oocytes acquire meiotic competence when they reach a diameter of more than 120 μm, although the ability of sperm penetration does not depend on

Table 1
Comparisons of morphometrical parameters of abnormal oocytes in different groups.

Parameters	Group I (rFSH)	Group II (HMG)	P
Number of oocytes	162	92	
Oocyte area	2199.621 ± 340.174	21,788.98 ± 2128.54	.056
Oocyte peripheral	522.64 ± 58.51	522.61 ± 25.16	.066
Oocyte diameter (μm)	166.61 ± 10.7	165.31 ± 8.09	.61
ZP Area	9670.06 ± 1710.58	10,100 ± 9456.78	.023
ZP thickness (μm)	19.90 ± 9.62	18.23 ± 2.82	.094
Ooplasm area	1095.277 ± 801.963	1188.328 ± 1345.956	.004
Ooplasm peripheral	421.15 ± 754.32	460.68 ± 1018.15	.003
Ooplasm diameter (μm)	118.24 ± 96.34	110.87 ± 11.29	.003

Mean ± SD.

Table 2
Comparisons of morphometrical parameters of normal oocytes in different groups.

Parameters	Group I (rFSH)	Group II (HMG)	P
Number of oocytes	50	55	
Oocyte area $(\mu m)^2$	4573.066 ± 255.25	4424.16 ± 324.54	.053
Oocyte peripheral (μm)	245.46 ± 5.39	244.96 ± 6.002	.736
Oocyte diameter (μm)	75.30 ± 3.85	74.73 ± 5.17	.632
ZP area $(\mu m)^2$	1992.20 ± 201.50	1848.23 ± 338.60	.05
ZP thickness (μm)	8.16 ± .530	8.30 ± .59	.34
Ooplasm area $(\mu m)^2$	2557.26 ± 45.62	2574.26 ± 53.54	.191
Ooplasm peripheral (μm)	176.80 ± 6.09	174.73 ± 6.24	.20
Ooplasm diameter (μm)	57.56 ± 4.67	54.73 ± 5.06	.028

Mean ± SD.

Table 3
Comparisons of morphological parameters of oocytes in different groups.

Parameters	Group I (rFSH)	Group II (HMG)	P
Number of oocytes	212	147	–
Normal oocyte[a]	23.58	37.41	.000
Single defect[a]	7.9	5.6	.783
Double defects[a]	19	13.5	.347
Multiple defects[a]	34.9	16.34	.006

[a] The data are presented as percentage.

oocyte diameter [35]. Durinzi et al. evaluated 49 oocytes that were obtained from six young women for diameter and degree of oocyte maturation. Only one-third of the oocytes with a diameter of over 105 μm were able to resume meiosis or to mature. Evaluation of oocyte diameter in the final stage of maturation revealed that among oocytes in GV stage, 60% with a diameter between 116 and 125 μm were MII. It was also revealed that among oocytes in the GV stage, those with a diameter between 116 and 125 μm progressed more rapidly to extrusion of the first polar corpuscle [28]. These results support the idea that human oocytes present a size-dependent latency to progress in the division process until complete maturation. Cavilla et al. evaluated via IVM, a total of 86 oocytes obtained from women with polycystic ovary syndrome. The diameter of the 28 oocytes that survived and progressed in IVM ranged from 103 to 121 μm. Of these, 10 progressed to fertilization after ICSI. They concluded that oocytes that were larger when collected for IVM had a greater probability to mature [29]. In the same study, 20 mature oocytes retrieved from 17 young women, and the mean oocyte diameter (MOD) after the removal of cumulus cells ranged from 112 to 119 μm. In contrast to the results for IVM, no significant relation was observed between MOD and probability of fertilization or embryo cleavage. Wolf et al. (1995) found that fertilization rate was significantly lower for the smaller oocytes (less than 108 μm diameter) compared with the larger oocytes, In fact, insufficient development of the ooplasm may contribute to fertilization failure, particularly when sperm with functional defects are used [36]. Westergaard and co-workers (2007) reported a correlation between the number of granulose cells (GC) in a follicle in relation to the oocyte diameter. Oocyte diameter showed a biphasic increase in relation to the number of GCs present in the follicle. In follicles with <30 GC, the oocyte diameter increased from 31 to 37 μm; whereas, almost no increase in oocyte diameter was observed in follicle containing between 30 and 70 GC. The oocyte started to enlarge further when follicles acquired more than 70 GC per follicle. Moreover, the mean number of mature oocytes were higher in rFSH group versus hMG group. However, the rate of immature oocytes was higher in hMG group versus rFSH group [37].

Competence for meiotic resumption is believed to be acquired during the phase of oocyte growth, when the process of synthesis and storage of proteins, as well as the ribosomal and heterogeneous RNA take place. This events lead to nuclear maturation and cytoplasmic modifications, which is necessary for development of the future embryos [18].

This outcome is similar to study of Ng et al. (2001) that showed the proportions of normal and abnormal morphology of ZP, oocyte and PB were similarly distributed in two groups [22].

In conclusion, rFSH and hMG have very little effects on oocyte morphometry.

However, this study shown that Morphometrical parameters were increased in rFSH group, but the normal morphology of oocytes were significantly enhanced in hMG group.

However, combination of mentioned drugs regarding the increasing ooplasm morphometrical parameters within normal range, also, increasing the number of mature oocytes, is more practical in prognosis during ICSI cycles than using alone.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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