Effects of the Oocyte Maturation Rate on Clinical Outcomes of Conventional IVF and ICSI in Female Patients Aged ≥ 38 Years

shuang liu (✉ liushuang929@yeah.net)

Shenyang Jinghua Hospital Joint Center of Human Reproduction and Genetics, Shenyang.

Hongjun Yu

Shenyang Jinghua Hospital

Baoshan Li

Shenyang Jinghua Hospital

Chunyi Li

Shenyang Jinghua Hospital

Dongkai Cheng

Shenyang Jinghua Hospital

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Abstract

Research Question: The average female reproductive potential peaks at age 25 and then begins to decline. As women are delaying childbearing, the prevalence of infertility has risen, leading to increasing demand for assisted reproductive technology (ART). Oocyte quality remains the most important issue during in vitro fertilization (IVF) cycles. This study investigated the effects of the oocyte maturation rate (OMR) on clinical outcomes of conventional IVF and intracytoplasmic sperm injection (ICSI) in female patients aged ≥ 38 years.

Design: A retrospective analysis of 6562 infertile patients who were treated with IVF/ICSI at the reproductive medicine center of our hospital from January 2011 to December 2017 was performed. According to the oocyte maturity (the ratio of the number of mature oocytes to the number of oocytes) on the day of egg collection, the patients were divided into three groups: group A (oocyte maturity $\leq 30\%$, $n=422$), and group B (oocyte maturity from 30-75%, $n=1290$), and group C (oocyte maturity $\geq 75\%$, $n=4850$). The patient age, years of infertility years, days of gonadotropin (Gn), Gn dosage, serum luteinizing hormone (LH), estradiol (E2), and progesterone (P) levels on the day of human chorionic Gn (HCG) injection, E2 levels per mature oocyte, E2 levels per oocyte, number of mature oocytes, oocyte recovery rate, fertilization rate, cleavage rate, excellent embryo rate, blastocyst formation rate, pregnancy rate, and live birth rate were compared among the three groups.

Results: Factors including age, years of infertility, number of eggs obtained, number of mature eggs, normal fertilization rate, cleavage rate, excellent embryo rate, blastocyst formation rate, pregnancy rate, and live birth rate were all found to be related to oocyte maturity on the day of HCG administration ($P < 0.05$).

Conclusions: There is a close relationship between oocyte maturity and embryo quality, and a low OMR may be related to a poor ovarian response or decreased sensitivity to Gn. Therefore, a low OMR may affect the fertilization and embryonic development potential in elderly patients undergoing IVF/ICSI, thus affecting the pregnancy rate.

Introduction

Since the early 2000s, there has been an increasing trend of women delaying childbearing^[1]. It is well established that female fertility declines with age and with DNA aberrations and genomic instability^[2-4]. The average female reproductive potential peaks at age 25 and then begins to decline. As women are delaying childbearing, the prevalence of infertility has risen, leading to increasing demand for assisted reproductive technology (ART). ART has remarkably advanced since its inception in 1978. ART, particularly in vitro fertilization (IVF), has been widely used for the treatment of infertility in humans^[5].

Oocyte quality remains the most important issue during IVF cycles^[6, 7]. The outcome of IVF is largely influenced by the developmental competence of oocytes^[8]. Oocyte maturation consists of nuclear and

cytoplasmic events that are required for the oocyte to acquire competency for fertilization and subsequent embryo development should fertilization occur. In addition, the maturity of retrieved oocytes is important for the success of IVF because mature oocytes are used for ART; the remaining immature oocytes are generally discarded.

Oocyte maturation is integral for a successful pregnancy^[9, 10]. Oocyte maturation involves the release of the primary meiotic arrest at prophase I and progression of the oocyte nucleus into meiotic divisions to produce a mature oocyte (egg) capable of being fertilized. In ART, controlled ovarian hyperstimulation (COH) is necessary to induce multiple follicular development to harvest numerous healthy mature oocytes. However, a lack of developmental synchronization causes unwanted immature oocytes to be harvested^[11]. This is directly related to oocyte fertilization and embryonic development, which leads to small or absent transferable embryos and may affect the clinical outcome.

In this study, 6540 infertile patients who received IVF/intracytoplasmic sperm injection (ICSI) treatment at our reproductive center from January 2011 to December 2017 were retrospectively analyzed to explore the predictive value of various monitoring indexes from the day of human chorionic gonadotropin (Gn) (HCG) injection on oocyte maturity and the effect of oocyte maturity on embryonic developmental potential and pregnancy outcome.

Materials And Methods

Data from IVF/ICSI cycles performed at the Reproductive Medicine Center of Shenyang Dongfang Jinghua Hospital from January 2011 to December 2017 were collected. This study was approved by the Institutional Review Board of Shenyang Dongfang Jinghua, and all patients provided informed consent.

Only cycles that included a maturity evaluation of oocytes were included. The exclusion criteria were as follows: in vitro maturation (IVM) cycles, preimplantation genetic diagnosis (PGD) cycles, female abnormal chromosomal karyotype, and patients younger than 38 years. The oocyte maturation rate (OMR) was calculated as the ratio of mature oocytes to retrieved oocytes in the same cycle. Patients were categorized into three groups: group A (oocyte maturity $\leq 30\%$, $n = 422$), group B (oocyte maturity from 30–75%, $n = 1290$), and group C (oocyte maturity $\geq 75\%$, $n = 4850$).

The ovulation induction regimen was performed as follows: all patients were treated with a long luteal phase regimen and subcutaneously injected with a Gn-releasing hormone agonist (0.25 mg of acetate; Merck Serono, Switzerland) in the middle luteal phase of the previous cycle (day 21 of menstruation) once a day starting on the 5th to 6th days of menstruation. After menstruation reached the downregulation standard, follicle-stimulating hormone (FSH, 5.5 μg (75 U); Merck Sherano, Switzerland) or human menopausal Gn (HMG, 75 U; Lizhu Pharmaceutical) was injected subcutaneously on the third day of menstruation. Regular B-mode ultrasound monitoring was performed, and serum luteinizing hormone (LH), estradiol (E2), and progesterone (P) levels were determined. According to follicular growth,

the dose of Gn was adjusted: when the follicular development was not obvious on the 5th and 7th days, an additional 75 U of HMG was injected intramuscularly every day.

Data collected included age, duration of infertility, number of previous assisted reproductive attempts, basal and gonadal hormone levels on the day of HCG injection, antral follicle count (AFC), and cycle outcomes. Patients were scheduled for oocyte retrieval 34–36 h after the HCG injection. Indications and techniques for oocyte aspiration, oocyte and embryo culture, insemination, ICSI, and embryo transfer were based on the routine procedures of the center. To confirm early pregnancy, serum HCG was measured 13 days after cleavage embryo transfer or 11 days after blastocyst transfer. An ultrasound scan was performed 30–35 days after embryo transfer. An intrauterine clinical pregnancy was defined as the presence of a gestational sac with fetal heart activity in the uterine cavity.

SPSS 17.0 statistical analysis software was used to analyze the data. Measurement data are expressed as percentages, and between-group comparisons were performed by analysis of variance and the chi-square test. Count data are expressed as the mean and standard deviation (statistical $\bar{x} \pm s$), and between-group comparisons were performed by the t-test.

Results

The basic conditions of the three groups are compared in Table 1. There was no significant difference in age or baseline FSH and anti-Mullerian hormone (AMH) levels among the three groups ($P > 0.05$). The duration of infertility, duration of Gn administration, and dosage of Gn in group C were less than those in group A, and the differences were statistically significant ($P < 0.05$).

On the day of HCG administration, the serum E2 levels were ranked as follows: group A > group B > group C; there were significant differences between each pair of groups ($P < 0.05$). On the day of HCG administration, there were no significant between-group differences in serum LH and P levels. There were no significant between-group differences in the E2 levels per mature oocyte ($P > 0.05$). There were no significant between-group differences in the E2 levels per oocyte ($P > 0.05$) (Table 2).

The number of oocytes retrieved, number of mature oocytes, and rate of oocytes recovered were ranked as follows: group C > group B > group A; there were significant between-group differences ($P < 0.05$) (Table 3).

The normal fertilization rate in group C was higher than those in group A and group B ($P < 0.05$). There was no significant difference in the normal fertilization rate between groups B and C ($P > 0.05$), and the cleavage rate was ranked as follows: group C > group B > group A ($P < 0.05$). The excellent embryo rate was ranked as follows: group C > group B > group A; there were significant between-group differences ($P < 0.05$). The blastocyst formation rate was ranked as follows: group C > group B > group A; there were statistically significant differences ($P < 0.05$). The pregnancy rate in groups B and C was higher than that in group A ($P < 0.05$). There was no significant difference in the pregnancy rate between groups C and B

($P > 0.05$). The live birth rates in groups B and C were significantly higher than that in group A ($P < 0.05$). There was no significant difference between groups B and C ($P > 0.05$) (Table 4).

Table 1
General between-group data comparison

Group	A	B	C
Cycle numbers	422	1290	4850
Age, years	42.71 $\pm$ 2.95	42.34 $\pm$ 2.31	42.06 $\pm$ 6.36
Duration of infertility, years	9.47 $\pm$ 6.74	8.14 $\pm$ 6.32	7.92 $\pm$ 6.33
Basal FSH, IU/L	6.73 $\pm$ 3.62	6.25 $\pm$ 7.53	6.36 $\pm$ 5.44
Basal AMH, ng/mL	3.18 $\pm$ 1.14	3.45 $\pm$ 2.03	3.24 $\pm$ 1.54
Dosage of Gn, IU	3364.34 $\pm$ 1124.91 Δ	2766.33 $\pm$ 1079.18	2721.41 $\pm$ 975.44
Duration of Gn, days	13.69 $\pm$ 3.07 Δ	12.33 $\pm$ 2.47	12.22 $\pm$ 1.76
Note: Δ Compared with groups B and C, $P < 0.05$.			

Table 2
Between-group comparison of daily HCG hormone levels

Group	A	B	C
E2 on HCG administration day, pmol/L	1056.56 $\pm$ 133.83 Δ	6778.32 $\pm$ 600.33	7331.4 $\pm$ 578.69
LH on HCG administration day, U/L	0.95 $\pm$ 0.92	0.96 $\pm$ 0.70	0.95 $\pm$ 0.84
P on HCG administration day, nmol/L	3.57 $\pm$ 0.80	3.56 $\pm$ 0.76	3.59 $\pm$ 0.81
E2 per mature oocyte	996.44 $\pm$ 98.77	998.43 $\pm$ 100.89	1017.45 $\pm$ 95.33
E2 per oocyte	1021.21 $\pm$ 101.53	1034.41 $\pm$ 105.27	1043.44 $\pm$ 97.35
Note: Δ Compared with groups B and C, $P < 0.05$.			

Table 3
Number of oocytes harvested from the three groups

Group	A	B	C
Number of oocytes retrieved	3.24 $\pm$ 2.62 Δ	6.72 $\pm$ 5.10	5.00 $\pm$ 4.75*
Number of mature oocytes	1.59 $\pm$ 0.99 Δ	4.56 $\pm$ 4.16	4.09 $\pm$ 3.45*
Rate of oocyte recovery	83.03(944/1137) Δ	90.55(8414/9292) *	93.35(24975/26754)
Note: Δ Compared with groups B and C, $P < 0.05$; *Compared with groups A and C, $P < 0.05$.			

Table 4
Between-group comparison of embryonic development and clinical outcomes

Group	A	B	C
Normal fertilization rate	74.58 (44/59)	89.63 (3986/4447)	90.25 [△] (17492/19381)
Cleavage rate	95.45 (42/44)	96.96 (3865/3986)*	98.15 (17169/17492) [△]
High-quality embryo rate	42.86 (18/42)	58.73 (2270/3865)*	60.94 (10462/17169) [△]
Blastocyst formation rate	0 (0/2)	55.41 (901/1626)*	56.64 (1058/1868) [△]
Clinical pregnancy rate	17.39 (4/23)	23.95 (223/931) [#]	24.93 (831/3336) [#]
Live birth rate	4.35 (1/23)	10.31 (96/931) [#]	10.55 (352/3336) [#]
Note: [△] Compared with groups A and B, $P < 0.05$; *Compared with groups A and C, $P < 0.05$. [#] Compared with group A, $P < 0.05$.			

Discussion

During ART, cumulus cells are considered to be of vital importance to the success of oocyte maturation^[12]. Oocyte maturation is a key step in IVF because it affects the quality of oocytes, which in turn affects embryonic development, fetal development, and even the health of offspring^[13]. Beall et al. suggested the concept of "oocyte maturation failure syndrome" in 2010^[14]. This concept indicates that most immature oocytes are reproduced. Due to the decrease in the number of mature oocytes, the success rate of IVF is reduced. Bar-Ami reported that the success rate of IVF was significantly reduced concomitant with an increase in the percentage of mature oocytes^[15]. Moreover, advanced maternal age is a known cause of degradation of oocyte quality and a poor IVF outcome^[9, 16].

According to our clinical experience in this study, we described a decrease in the OMR as less than 30%. Using this definition, the incidence of a decreased OMR was 6.43% (422/6562) among the cycles included, which was slightly higher than that reported by Lin et al. (1.3%)^[11].

By comparing the clinical outcomes among the three groups, the fertilization rate, blastocyst formation rate, and high-quality embryo rate were found to decrease with the decrease in the OMR. In particular, the decline in the blastocyst formation rate was particularly evident. When the OMR was less than 75%, the cleavage rate, blastocyst formation rate, clinical pregnancy rate, and live birth rate decreased accordingly. Our results were consistent with those of Bar-Ami et al^[15]. Thus, oocyte maturity is one of the main factors affecting embryonic developmental potential.

The univariate analysis revealed that some candidate variables such as age, duration of infertility, dosage of Gn, duration of Gn treatment, and number of oocytes retrieved were factors affecting the incidence of a

decreased OMR. Together with older age, a longer duration of infertility, and poorer quality of oocytes, these factors indicate more severe infertility.

COH is a particularly important link in the whole process of assisted reproduction^[17]. The effect of ovulation induction directly affects the quality of the oocytes obtained, which then affects the clinical outcome of the whole assisted pregnancy therapy. COH includes the processes of follicular recruitment, development, and ovulation, which are usually not completely synchronized^[18]. Only by overcoming the factors of nonsynchronization is it possible to obtain high-quality oocytes of the same quality as the dominant follicles in the natural cycle.

There are mature and immature oocytes in human ovaries, and most of them are in the germinal vesicle (GV) stage^[19]. Under the stimulation of ovulation-stimulating factors such as hormones, germinal vesicle breakdown (GVBD) occurs, and the first polar body (Pb1) of GVBD is excreted. Pb1 then enters the MII stage and becomes a mature oocyte. The process from the GV stage to the MII stage is "oocyte maturation". Oocyte maturation involves nuclear and cytoplasmic maturation, which includes a series of complex and orderly nuclear and cytoplasmic events. Nuclear maturation includes GV rupture and meiosis in which the chromosomes are assembled on the spindle and move to the periphery of the cell to complete the first meiosis. A set of homologous chromosomes surrounded by a small amount of cytoplasm is excreted as Pb1, and another set of genetic material is left in the cytoplasm and enters the metaphase of the second meiosis. Finally, the oocytes are excreted from the follicles. Cytoplasmic maturation involves many events such as organelle reorganization, increased Ca^{2+} storage, and increased antioxidant, mRNA and protein storage, and cytoplasmic maturity is closely related to subsequent embryonic development^[20]. Oocyte maturation usually refers to nuclear maturation; however, in the process of COH, exogenous Gn can lead to abnormal metabolism, decreased adenosine triphosphate (ATP) content, abnormal transcription, and asynchronous nuclear/cytoplasmic maturation^[21]. Only oocytes that have undergone cytoplasmic maturation can synchronize blastomere development, promote embryonic development and become high-quality embryos. Therefore, in a cycle with a low OMR, the fertilization rate and excellent embryo rate of mature oocytes are relatively low, which is most likely due to nonsynchronization of the nucleus and cytoplasm. Only oocytes with simultaneous nuclear and cytoplasmic maturation have good potential for fertilization and embryonic development. The whole process of follicular development is affected by ovulation-inducing drugs. High doses of Gn may affect the meiosis, developmental potential, and epigenetic modification of oocytes.

In the process of COH, considerable attention should be paid to the influence of a low OMR on clinical outcomes. Through the analysis of past medical history and the determination of serum hormone levels, the dosage of Gn and timing of HCG injection should be evaluated, and the clinical medication regimen should be adjusted promptly to reduce the occurrence of a low OMR. It is particularly important to obtain high-quality oocytes with synchronous development to obtain a better clinical outcome.

The patients in group A may have had a relatively small number of mature and retrieved oocytes due to the longer time of administration of Gn and the larger dosage of Gn, which eventually affect the

fertilization rate, cleavage rate, and clinical pregnancy outcome.

With the increase in oocyte maturity, the number of oocytes obtained and the number of mature eggs increased in the three groups (group A: 2.24 ± 2.62 and 0.59 ± 0.99 , respectively; group B: 5.00 ± 4.75 and 4.09 ± 3.45 , respectively; and group C: 6.72 ± 5.10 and 4.56 ± 4.16 , respectively). The number of Gn days in group A was significantly higher than those in group B and group C (group A: 13.69 ± 3.07 d vs. group B: 12.33 ± 2.47 vs. group C: 12.22 ± 1.76 d) ($P < 0.05$). Furthermore, the dosage of Gn in group A was significantly higher than those in group B and group C (group A: 3364.34 ± 1124.91 IU vs. group B: 2766.33 ± 1079.185 IU vs. group C: 2721.41 ± 975.44 IU) ($P < 0.05$). In the natural cycle, the development of a single dominant follicle can induce the serum E2 level to reach approximately 1098 pmol/L. In COH, exogenous Gn stimulates multiple follicles to develop simultaneously and then produces a corresponding multiple of serum E2. With the increase in the number of eggs obtained and mature eggs, the serum E2 levels increased correspondingly among the three groups, but there was no significant difference in the E2 levels per mature follicle or the number of eggs obtained among the three groups. This finding shows that the standard of HCG injection in the three groups was the same. The reasons may be as follows: (1) This finding may be related to the process of ovulation induction, such as excessive inhibition of the pituitary-ovary axis leading to decreased sensitivity of the ovary to Gn, a low ovarian response, uneven follicular development, increased dosage of Gn and decreased oocyte quality. (2) The timing of HCG injection may also account for this finding: if thing time is too early, the granulosa cells have insufficient LH receptors and an insufficient response to HCG, resulting in a small amount of cumulus close to the follicular wall that is not easy to suction, few recovered oocytes, and mostly immature oocytes; thus, the fertilization rate decreases, which can also promote follicular atresia. If HCG is injected too late, the follicles are overmature, the oocytes in the mature follicles are lost, and immature eggs are obtained from the small follicles. (3) Finally, this finding may be caused by a poor ovarian reaction or polycystic ovary syndrome (PCOS). Sachs et al.'s research suggests that patients with high responses tend to produce more immature eggs^[22]. In group A, there may have been a poor ovarian response, decreased sensitivity to Gn, or uneven follicular development leading to a low number of collected and mature eggs on the day of HCG injection. If the timing of HCG administration is delayed, the dosage of Gn and the days of Gn use may be increased.

In this study, 6540 elderly infertile patients who were treated with IVF/ICSI at our reproductive center were selected as subjects to explore the clinical factors affecting oocyte maturity on the day of HCG administration and the effect of oocyte maturity on embryonic developmental potential and pregnancy outcome. The results showed that there were significant differences in patient age, years of infertility, number of eggs obtained, number of mature eggs, normal fertilization rate, cleavage rate, excellent embryo rate, blastocyst formation rate, pregnancy rate and live birth rate ($P < 0.05$).

Conclusions

There is a close relationship between oocyte maturity and embryo quality, and a low OMR may be related to a poor ovarian response or a decreased sensitivity to Gn. Therefore, a low OMR may affect the

fertilization and embryonic development potential among elderly patients undergoing IVF/ICSI, thus affecting the pregnancy rate.

Abbreviations

Gn

Gonadotropin; IVF:In vitro fertilization; P:progesterone; HCG:human chorionic gonadotropin; ART:Assisted reproductive technology; COH:Controlled ovarian hyperstimulation; AFC:Antral follicle count; FSH:Follicle-Stimulating Hormone; AMH:Anti Mullerian Hormone; Pb1:The first polar body

Declarations

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SL took part in the performed data analysis and prepared the first draft of the manuscript; SL and HJY contributed to study design, data analysis and preparation of the manuscript; BSL was involved in embryological experiments and contributed to study design; CYL contributed to study design and were involved in critical discussions; DKC contributed to the concept, design and preparation of the manuscript and was involved in patient enrolment and management. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Shenyang Jinghua Hospital (#2019011). The clinical trial registration ID on the Chinese Clinical. All the participants provided written consent to participate in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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