

Original Research

Effect of Stimulation Duration on Oocyte Maturation in Patients With Normal Ovarian Reserve Undergoing a Gonadotropin-Releasing Hormone Agonist Protocol: A Propensity Score Matched Study

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Abstract

Background: This study aimed to investigate the effect of gonadotropin (Gn) stimulation duration on the oocyte maturation rate in patients with normal ovarian reserve undergoing a gonadotropin-releasing hormone agonist (GnRHa) protocol. **Methods:** Data were retrospectively collected from patients with normal ovarian reserve who underwent a GnRHa protocol at the Reproductive Center of Changzhou Maternity and Child Health Care Hospital between January 1, 2017, and December 31, 2022. A total of 2840 cycles were included and categorized into three groups based on stimulation duration: <7 days, 7–10 days, and >10 days. **Results:** The results showed that patients with a Gn stimulation duration of ≥ 7 days exhibited significantly higher metaphase II (MII) oocyte rates, clinical pregnancy rates, and live birth rates compared with those with a stimulation duration of <7 days. Correlation and linear regression analyses identified Gn stimulation duration as an independent predictor of the MII oocyte rate. Curve fitting analysis demonstrated that when the stimulation duration was <7 days, the MII oocyte rate increased with longer Gn stimulation. **Conclusions:** For patients with normal ovarian reserve undergoing a GnRHa regimen, the optimal Gn stimulation duration is at least 7 days. Gn stimulation duration was positively correlated with the MII oocyte rate and independently predicted oocyte maturation. These findings provide valuable evidence for optimizing clinical management in this patient population.

Keywords: GnRH agonist regimen; metaphase II oocyte; gonadotropin stimulation duration; normal ovarian reserve; clinical outcomes

1. Introduction

With the continuous advancement of assisted reproductive technology, a variety of controlled ovulation stimulation protocols have been increasingly adopted in clinical practice. These include gonadotropin-releasing hormone agonist (GnRHa) protocols, GnRH antagonist protocols, and mild stimulation protocols [1,2]. Among these, the GnRHa protocol remains widely used, particularly in reproductive centers in China, due to its ability to promote synchronized follicular development and provide consistent treatment outcomes. The GnRHa protocol is primarily used in patients with normal ovarian reserve. Following a 14-day pituitary downregulation phase, an individualized starting dose of Gonadotropin (Gn) is selected based on the patient's clinical characteristics. Gn is then administered to stimulate follicular development following follicle recruitment, and oocyte retrieval is performed after ovulation triggering once the majority of follicles have reached maturity [3]. The optimal duration of Gn stimulation for maximizing oocyte maturation and improving clinical outcomes remains an important consideration in reproductive medicine.

Should high doses of Gn be administered to promote rapid follicular growth, or should lower doses be used to support more gradual follicular development?

The duration of Gn stimulation plays a crucial role in follicular growth, oocyte maturation, embryo development, and endometrial development. Insufficient Gn exposure may result in incomplete nuclear and cytoplasmic oocyte maturation [4,5], whereas prolonged exposure may lead to oocyte postmaturation and granulosa cell apoptosis [6]. Elevated levels of progesterone (P4) and estradiol (E2) may also adversely affect endometrial receptivity [7,8,9]. However, few studies have been conducted on the correlation between follicular phase duration and *in vitro* fertilization (IVF) outcomes [5,10,11], and the available findings have been inconsistent [12]. Previous study have reported no significant differences in clinical pregnancy rate among patients with varying Gn stimulation durations [13]. However, other studies have suggested that both prolonged stimulation (>13 days) or short stimulation (<6 days) may adversely affect clinical outcomes [11,14].

Previous studies have shown that the ovarian stimulation protocol is an independent factor influencing Gn stimu-



lation duration, with the GnRHa protocol generally requiring a longer stimulation period than the GnRH antagonist protocol [15]. Therefore, the present study aimed to investigate the effect of Gn stimulation duration on the oocyte maturation rate in patients with normal ovarian reserve undergoing GnRHa protocol.

2. Methods

2.1 Study Design

This study and its protocols were approved by the Ethics Committee of Changzhou Maternity and Child Health Care Hospital (Approval No.: 202403). Data for this retrospective analysis were collected from patients who underwent standard IVF treatment at the Reproductive Center of Changzhou Maternity and Child Health Care Hospital between January 1, 2017, and December 31, 2022. A total of 2840 treatment cycles were included, all of which employed the long GnRHa luteal protocol. Written informed consent was obtained from all participating couples. The study was conducted in accordance with the Declaration of Helsinki.

The inclusion criteria were as follows: (1) age < 38 years; (2) anti-Müllerian hormone (AMH) level >1.2 ng/mL; (3) antral follicle count (AFC) >5; and (4) retrieval of at least 5 oocytes. A total of 3145 treatment cycles were included.

The exclusion criteria were as follows: (1) chromosomal abnormalities in either partner (15); (2) ovarian cysts detected at the initiation of ovarian stimulation (23); (3) abnormal uterine cavity, ovarian insufficiency, or other systemic diseases (12); (4) body mass index (BMI) ≥ 30 kg/m² (120); and (5) polycystic ovary syndrome (PCOS) or endometriosis (135).

Patients were categorized into three groups based on the duration of ovarian stimulation: <7 days, 7–10 days, and >10 days. This stratification was performed to evaluate the effect of stimulation duration on IVF clinical outcomes.

2.2 Ovarian Stimulation

This study employed a flexible GnRHa protocol. The procedure was implemented as follows: patients received a daily injection of 0.1 mg triptorelin (Decapeptyl, Ipsen, Paris, France) for 21 days, beginning on day 3 of the menstrual cycle to achieve pituitary downregulation. Following downregulation, the initial Gn dose, ranging from 100 to 225 IU, was determined based on patient age, BMI, and AMH levels.

Ovarian stimulation was performed using recombinant follicle-stimulating hormone (FSH) (rFSH; Gonal-f®, Merck-Serono, Geneva, Switzerland). Follicular development was monitored via transvaginal ultrasonography and serum hormone measurements, including E2, P4, and luteinizing hormone (LH). The Gn dose was adjusted according to the hormonal profile and follicular growth. Final oocyte maturation was triggered when at least three fol-

licles reached a mean diameter of ≥ 18 mm and hormonal levels were considered appropriate. The trigger consisted of a combination of 5000 IU urinary human chorionic gonadotropin (hCG) and 250 µg recombinant hCG. Oocyte retrieval was performed 36–38 hours after hCG administration.

2.3 IVF and Embryo Culture

Retrieved oocytes were fertilized by either conventional IVF or intracytoplasmic sperm injection (ICSI), depending on semen parameters. Cleavage-stage embryos were evaluated according to previously established criteria [16], focusing on the number and regularity of blastomeres and the degree of fragmentation. Embryos classified as grade I or II were considered top-quality embryos. Blastocysts were assessed using the Gardner scoring system [17], which evaluates the degree of expansion, as well as the quality of the inner cell mass and trophectoderm. Based on embryo quality and patient's clinical status, a single high-quality blastocyst was selected for transfer on day 5 or 6, when clinically appropriate.

2.4 Hormone Analysis

Serum samples were collected on the morning of day 3 of the menstrual cycle and on the day of hCG administration. Serum levels of FSH, LH, E2, testosterone (T), and P4 were routinely measured using commercially available chemiluminescence immunoassay kits (Abbott Biologicals B.V., Olst, Overijssel, the Netherlands). The lower limits of detection were 0.06 mIU/mL FSH; 0.09 mIU/mL LH; 10 pg/mL E2; and 0.1 ng/mL P4. The intra- and inter-assay coefficients of variation (CVs) were 3.8% and 6.4% for LH, 21.0% and 12.0% for E2, and 9.6% and 11.2% for P4, respectively.

2.5 Statistical Analysis

Statistical analyses were performed using SPSS software version 26.0 (IBM, Armonk, NY, USA). The normality of continuous variables was assessed using the Kolmogorov-Smirnov test. Normally distributed continuous variables were expressed as the mean $\pm$ standard deviation (SD) and between-group comparisons were performed using Student's *t*-test. Continuous variables with a non-normal distribution or unequal variances were expressed as the median and interquartile range (IQR; P25, P75), and group comparisons were performed using the Mann-Whitney U test. Categorical variables were compared using the chi-square test. Fisher's exact test was used when the expected count was <5 or the total sample size was <40. All statistical tests were two-sided, and a *p*-value < 0.05 was considered statistically significant.

For comparisons among the three groups, the Kruskal-Wallis test was used for non-normally distributed data, followed by Dunn's test for pairwise comparisons. One-way analysis of variance (ANOVA) was used for normally dis-

tributed data, followed by the LSD-*t* test for pairwise comparisons. The chi-square test was used for categorical data, and the chi-square segmentation method was applied for pairwise comparisons.

Propensity score matching (PSM) was performed to balance baseline characteristics among the three groups (<7 days, 7–10 days, and >10 days). Propensity scores were estimated using the logistic regression models, with group assignment as the dependent variable and the following factors as independent variables: female age, AMH level, BMI, duration and type of infertility, infertility factors, LH and E2 levels on stimulation initiation, and the initial and total Gn dosages. Nearest neighbor matching was applied at 1:1:1 ratio with a caliper width of 0.05 SD of the logit of the propensity score. Generalized propensity scores were estimated simultaneously for the three groups using multiple logistic regression, followed by multivariate matching algorithm to achieve 1:1:1 matching. The caliper width was set at 0.05 times the logit SD of the propensity score. After matching, covariate balance was assessed by comparing the SDs of the independent variables among the groups, with an SD difference of <0.1 indicating adequate balance [18]. Generalized linear regression analysis was then employed to investigate the dose-response relationship between the duration of Gn stimulation and the rates of metaphase II (MII) oocytes, clinical pregnancy, and live birth.

3. Results

3.1 Comparison of Baseline Characteristics Among the Three Groups Before and After PSM

Patients were categorized into three groups according to the duration of Gn stimulation: <7 days, 7–10 days, and >10 days. PSM was performed using 1:1:1 nearest neighbor matching with a caliper width of 0.05. Prior to PSM, statistically significant differences were observed among the three groups in age, AMH level, E2 level at stimulation initiation day, initial Gn dose, and total Gn dose ($p < 0.05$). After PSM, 104 patients were successfully matched in each group. Following matching, no significant differences were observed among the three groups in age, BMI, duration of infertility, type of infertility, infertility factors, AMH level, LH and E2 levels at stimulation initiation, initial Gn dose, or total Gn dose ($p > 0.05$), as detailed in Table 1. Before PSM, the standardized mean differences (SMDs) for age, AMH level, E2 level at stimulation initiation, initial Gn dose, and total Gn dose exceeded 0.1. After PSM, the SMD values for all baseline variables were all less than 0.1, indicating satisfactory covariate balance following PSM (Table 1 and Fig. 1).

3.2 Comparison of Laboratory and Clinical Outcomes Among the Three Groups After PSM

After PSM, the numbers of retrieved oocytes, mature oocytes, normally fertilized oocytes rate, and cleavage-

stage embryos rate were significantly higher in the <7 days group than in the 7–10 days and >10 days groups ($p < 0.05$). No statistically significant differences were observed between 7–10 days and >10 days groups in the numbers of retrieved oocytes, mature oocytes, normally fertilized oocytes, or cleavage-stage oocytes (all $p > 0.05$). Conversely, the rates of MII oocytes, normal fertilization, cleavage, high-quality cleavage embryos, blastocyst formation, high-quality blastocysts, available embryos, clinical pregnancy, and live birth were significantly lower in the <7 days group than in the other two groups ($p < 0.05$). No significant differences were observed in these outcomes between the 7–10 days and >10 days groups (all $p > 0.05$), as detailed in Table 2.

3.3 Correlation Between Gn Stimulation Duration and MII Oocyte Rate

The correlation analysis results showed that the duration of Gn stimulation was significantly positively correlated with the MII oocyte rate ($r = 0.20$, $p < 0.001$), clinical pregnancy rate ($r = 0.11$, $p = 0.003$), and live birth rate ($r = 0.12$, $p = 0.016$), as shown in Table 3 and Fig. 2.

3.4 Multiple Linear Regression Analysis of the MII Oocyte Rate

Multiple linear regression analysis was performed with the metaphase (MII) oocyte rate as the dependent outcome variable and the duration of Gn stimulation as the independent variable. After adjusting for potential confounding factors, including age, BMI, infertility duration, infertility type, infertility factors, AMH level, and LH and E2 at stimulation day, initial Gn dose, and total Gn dosage, the duration of Gn stimulation remained an independent predictor of the MII oocyte rate ($p = 0.019$), as shown in Table 4.

3.5 Curve Fitting Analysis of Gn Stimulation Duration and the MII Oocyte Rate

Curve fitting analysis of Gn stimulation duration and the MII oocyte rate revealed that a sigmoidal model provided a better fit than a linear regression model ($R^2 = 0.488$, $F = 296.012$, $p < 0.001$ vs. $R^2 = 0.400$, $F = 206.890$, $p < 0.001$). The analysis revealed that when the duration of Gn stimulation was <7 days, the MII oocyte rate increased with increasing Gn stimulation duration. However, when the duration of Gn stimulation was ≥ 7 days, further increases in stimulation duration were not associated with a significant increase in the MII oocyte rate. In addition, generalized linear regression analysis showed that the duration of Gn stimulation was significantly associated with both clinical pregnancy rate (odds ratio [OR] = 1.133 [1.015, 1.157], $p < 0.001$) and the live birth rate (OR = 1.082 [1.004, 1.132], $p < 0.01$), as shown in Figs. 2,3.

Table 1. Baseline characteristics of three patient groups before and after PSM.

Variables	Before PSM				After PSM			
	Gn <7 days (n = 183)	7 days ≤ Gn ≤ 10 days (n = 2344)	Gn >10 days (n = 313)	<i>p</i> -value	Gn <7 days (n = 104)	7 days ≤ Gn ≤ 10 days (n = 104)	Gn >10 days (n = 104)	<i>p</i> -value
Age (years)	33.530 ± 2.010	32.010 ± 1.930	34.510 ± 2.170	<0.001	32.130 ± 1.750	32.410 ± 1.690	32.140 ± 1.720	0.975
BMI (kg/m ²)	22.140 ± 3.220	22.310 ± 2.780	22.500 ± 2.920	0.654	22.280 ± 2.520	22.390 ± 2.710	22.400 ± 2.470	0.813
Duration of infertility (years)	2.200 (1.200, 5.500)	2.100 (1.100, 5.300)	2.200 (1.400, 5.800)	0.773	2.200 (1.300, 5.800)	2.200 (1.000, 5.400)	2.100 (1.100, 5.700)	0.642
Infertility type (%)	/	/	/	0.996	/	/	/	0.856
Primary infertility	104 (56.830)	1338 (57.080)	178 (56.870)	/	59 (56.730)	55 (52.880)	57 (54.810)	/
Secondary infertility	79 (43.170)	1006 (42.920)	135 (43.130)	/	45 (43.270)	49 (47.120)	47 (45.190)	/
Diagnosis (%)	/	/	/	0.813	/	/	/	0.890
Tubal factor	86 (46.990)	1067 (45.520)	138 (44.090)	/	49 (47.120)	46 (44.230)	47 (45.190)	/
Ovulatory dysfunction	47 (25.683)	678 (28.920)	83 (26.518)		26 (25.000)	24 (23.077)	22 (21.154)	
Uterine factors	23 (12.568)	347 (14.804)	45 (14.377)		16 (15.385)	17 (16.346)	14 (13.462)	
Others	27 (14.754)	252 (10.751)	47 (15.016)	/	13 (12.500)	17 (16.346)	21 (20.192)	/
AMH (ng/mL)	4.180 ± 0.710	3.130 ± 0.470	1.903 ± 0.500	<0.001	2.740 ± 0.420	2.580 ± 0.370	2.660 ± 0.400	0.775
LH level on Gn starting day (mIU/mL)	1.550 (1.140, 2.110)	1.560 (1.170, 2.910)	1.580 (1.160, 2.030)	0.905	1.520 (1.120, 2.030)	1.500 (1.070, 2.420)	1.510 (1.110, 2.130)	0.878
E2 level on Gn starting day (ng/L)	26.260 (21.340, 33.220)	19.070 (11.890, 26.490)	16.970 (8.090, 22.030)	<0.001	21.440 (18.170, 25.630)	20.320 (16.770, 26.720)	19.880 (17.140, 25.130)	0.402
Gn starting dose (IU)	120.000 (105.500, 167.500)	150.000 (112.500, 187.500)	150.000 (120.500, 197.500)	<0.001	150.000 (105.000, 175.500)	150.500 (110.500, 170.000)	150.500 (110.000, 175.500)	0.274
Total Gn (IU)	1047 (942, 1162)	1601 (1274, 1905)	1921 (1433, 2105)	<0.001	1574 (1223, 1812)	1581 (1238, 1825)	1601 (1283, 1895)	0.628

PSM, propensity score matching; BMI, body mass index; Gn, gonadotropin; AMH, anti-Müllerian hormone; E2, estradiol; LH, luteinizing hormone.

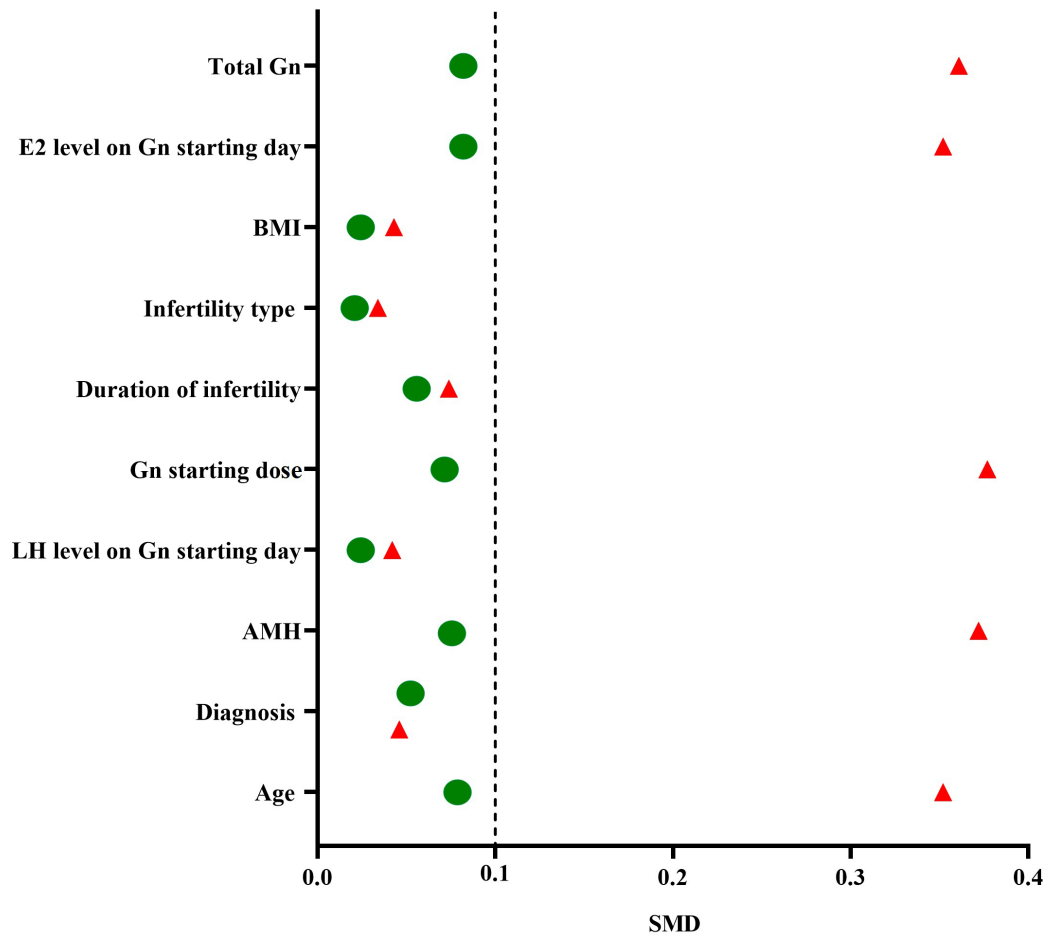


Fig. 1. Love plot of SMD before and after PSM. The x-axis represents the SMD values, and the y-axis represents the baseline covariates. Red symbols represent SMD values for each covariate before PSM, whereas green dots indicate the SMD after PSM. PSM, propensity score matching; BMI, body mass index; Gn, gonadotropin; AMH, anti-Müllerian hormone; E2, estradiol; LH, luteinizing hormone; SMD, standardized mean difference.

4. Discussion

Our results demonstrated that patients with a Gn stimulation duration of more than 7 days presented significantly greater MII oocyte, normal fertilization, high-quality embryo, blastocyst formation, clinical pregnancy, and live birth rates than those with a stimulation duration of less than 7 days. Correlation and multiple linear regression analyses revealed that the duration of Gn stimulation was correlated with and an independent influencing factor of the MII oocyte rate. Curve fitting analysis showed that the MII rate increased with increasing Gn stimulation duration when stimulation lasted <7 days. However, when the duration of Gn stimulation was ≥ 7 days, further increases in stimulation duration were not associated with a significant improvement in the MII oocyte rate. It is well established that the duration of ovarian stimulation has a significant impact on oocyte maturation, embryo quality, and endometrial receptivity, with both excessively short and prolonged stimulation durations adversely affecting these outcomes

[4,12]. An insufficient stimulation period may not provide adequate time for optimal oocyte and endometrial development, thereby compromising clinical outcomes. Consistent with this, a previous study in normal responders reported that the oocyte maturation rate was significantly lower in the 6-day stimulation group than in other groups and increased progressively with stimulation duration (≤ 6 days: 67%; 7–8 days: 80%; 9–10 days: 85%; and ≥ 11 days: 87%; $p = 0.02$) [12]. Conversely, another study indicated that women with a short stimulation duration of 6–8 days had comparable ongoing pregnancy rates to those of women with longer stimulation durations, regardless of whether ovarian stimulation was performed with corifollitropin alfa or daily recombinant FSH [19]. In the present study, Gn stimulation duration was identified as an independent predictor of the MII oocyte rate, and a stimulation duration exceeding 7 days was associated with improved IVF clinical outcomes. Regarding prolonged stimulation, previous research has associated extended stimulation duration with

Table 2. Comparison of laboratory and clinical outcomes among the three groups after PSM.

Variables	Gn <7 days (n = 104)	7 days ≤ Gn ≤ 10 days (n = 104)	Gn >10 days (n = 104)	p-value
No. of oocytes retrieved [M (P25, P75), n]	17.500 (15.000, 20.500)	11.000 (9.500, 16.000)#	9.500 (6.000, 11.500)#	<0.001
MII oocytes [M (P25, P75), n]	11.000 (7.500, 13.000)	10.500 (8.000, 14.500)	8.500 (7.000, 10.500)#	0.038
MII oocyte rate (%)	75.310 ± 5.530	94.710 ± 4.280#	93.940 ± 3.760#	<0.001
2PN oocytes [M (P25, P75), n]	10.500 (8.500, 12.000)	10.000 (7.500, 13.500)	8.000 (7.500, 10.000)#	0.041
2PN oocyte rate, n (%)	72.450 ± 4.760	92.550 ± 4.220#	92.970 ± 4.530#	<0.001
Cleavage embryo number [M (P25, P75), n]	10.000 (8.000, 11.500)	10.000 (7.000, 13.000)	7.500 (6.500, 9.500)#	0.028
Cleavage embryo rate, (%)	73.780 ± 5.120	94.610 ± 4.660#	94.010 ± 2.870#	<0.001
High-quality cleavage embryo rate, n (%)	41.5200 ± 2.88	62.450 ± 3.620#	61.180 ± 3.440#	<0.001
Blastocyst formation [M (P25, P75), n]	5.00 (3.00, 8.00)	5.500 (3.500, 8.500)	4.500 (2.500, 6.500)	0.058
Blastocyst formation rate (%)	48.470 ± 3.550	65.770 ± 4.870#	64.210 ± 3.970#	<0.001
High-quality blastocyst formation rate (%)	22.420 ± 2.310	41.720 ± 3.210#	40.970 ± 3.520#	<0.001
Available embryo rate (%)	60.220 ± 4.970	84.510 ± 5.650#	83.490 ± 4.970#	<0.001
Clinical pregnancy rate [n (%)]	42 (40.380)	59 (56.730)#	56 (53.846)#	0.042
Miscarriage rate [n (%)]	11 (26.190)	11 (18.640)	9 (16.070)	0.444
Live birth rate [n (%)]	31 (29.810)	48 (46.150)#	47 (45.190)#	0.026
Endometrial thickness [M (P25, P75), mm]	8.500 (6.700, 10.000)	9.00 (6.100, 10.500)	9.20 (6.300, 10.200)	0.864

Compared with Gn <7 days group, the difference was statistically significant.

PSM, propensity score matching; 2PN, two pronucleus; MII, metaphase II.

increased blastomere asymmetry and fragmentation, as well as an elevated risk of endometrial maturation [3]. Chuang et al. [3] reported that a Gn stimulation duration of ≥13 days was an independent negative predictor of assisted reproductive technology (ART) success [11]. In contrast, our study revealed no significant decline in the MII oocyte maturation rate or clinical outcomes when the Gn stimulation duration reached ≥10 days. This discrepancy may be attributed to the inclusion of only patients with normal ovarian reserve in our study, resulting in a minimal proportion of poor responders.

The duration of ovarian stimulation is closely correlated with ovarian response. Patients with a high ovarian response typically exhibit faster follicular development and therefore require a shorter stimulation duration [7]. Conversely, patients with a low ovarian response often experience slower follicular growth and require prolonged stimulation. In summary, when uniform criteria for triggering final oocyte maturation are applied, ovarian response is intrinsically related to the duration of ovarian stimulation [6]. Our results revealed that patients with a Gn stimulation duration of <7 days presented with elevated AMH levels and higher E2 levels on the initiation day. Despite receiving a comparable initial Gn dose, this group yielded significantly more retrieved oocytes than the other groups. However, their MII oocyte maturation rate was significantly lower.

We therefore hypothesize that, in patients with elevated AMH levels, reducing the daily Gn dose may prolong the stimulation duration beyond 7 days, thereby allowing sufficient time for both nuclear and cytoplasmic oocyte maturation. Adequate follicular development and oocyte maturation involve complex, tightly coordinated processes. The acquisition of oocyte developmental competence requires synchronized nuclear and cytoplasmic maturation [20,21]. Nuclear maturation involves germinal vesicle breakdown, resumption of meiosis, extrusion of the first polar body, and subsequent arrest at the MII stage [14,22]. Cytoplasmic maturation, although more difficult to assess, is crucial for oocyte developmental competence. Abnormalities in this process often manifest later as impaired embryo cleavage or implantation failure [14,23]. Consistent with this concept, our findings demonstrated that embryonic developmental potential and clinical outcomes were poorer in the <7 days group compared with the ≥7 days groups. This discrepancy likely reflects the fact that MII oocyte assessment is based solely on the extrusion of the first polar body, which does not necessarily indicate that cytoplasmic maturation has occurred synchronously with nuclear maturation.

Correlation analysis, multivariate linear regression analysis, and curve fitting consistently revealed that the duration of Gn stimulation was significantly positively correlated with the MII oocyte rate, clinical pregnancy rate,

Table 3. Correlation analysis between Gn stimulation duration and the MII oocyte rate.

Variables	Gn days (n)		2PN oocytes rate, n (%)		Cleavage rate (%)		High-quality cleavage embryos rate, n (%)		Blastocyst formation rate (%)		High-quality blastocyst formation rate (%)		Available embryo rate (%)	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p
MIH oocytes rate (%)	0.200	<0.001	0.250	<0.001	0.160	<0.001	0.120	0.007	0.150	0.001	0.230	0.001	0.280	<0.001
Clinical pregnancy rate (%)	0.110	0.003	0.01	0.750	0.100	0.003	0.080	0.010	0.110	0.026	0.130	0.001	0.02	0.002
Live birth rate (%)	0.120	0.016	0.08	0.078	0.070	0.110	0.070	0.089	0.080	0.057	0.160	<0.001	0.130	0.002

Table 4. Multiple linear regression analysis of MII oocyte rate.

Variables	95% CI	SD	<i>t</i>	Beta (95% CI)	<i>p</i>
Age (years)	−0.121	0.014	−3.629	−0.121 (−0.080, −0.025)	<0.001
BMI (kg/m ²)	−0.187	0.017	−9.582	−0.187 (−0.194, −0.128)	<0.001
Duration of infertility (years)	−0.041	0.144	−2.222	−0.041 (−0.603, −0.038)	0.038
Diagnosis	−0.002	0.039	−0.051	−0.002 (−0.079, 0.075)	0.960
AMH (ng/mL)	−0.023	0.068	−0.676	−0.023 (−0.179, 0.087)	0.499
LH level on Gn starting day (mIU/mL)	0.018	0.016	0.566	0.018 (−0.022, 0.04)	0.572
E2 level on Gn starting day (ng/L)	−0.064	0.039	−1.102	−0.064 (−0.118, 0.033)	0.271
Gn starting dose (IU)	−0.012	0.154	−0.643	−0.012 (−0.401, 0.203)	0.520
Total Gn (IU)	−0.031	0.126	−1.616	−0.031 (−0.451, 0.044)	0.106
Gn days (n)	0.077	0.009	2.347	0.077 (0.004, 0.040)	0.019

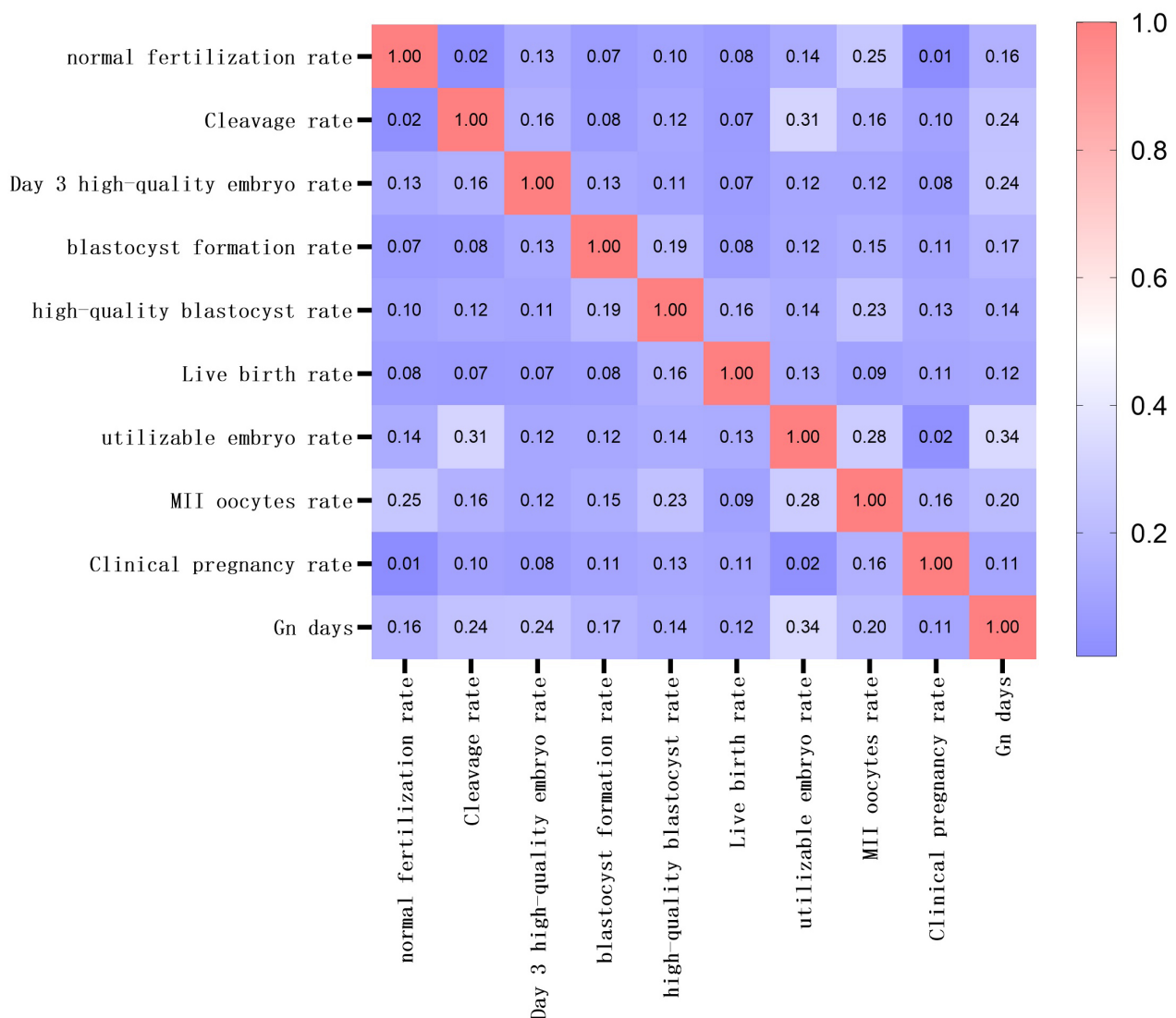


Fig. 2. Correlation analysis between the duration of Gn and MII oocyte rate. Heatmap showing the magnitude of Pearson correlation coefficients. The color gradient represents the strength of correlation; all correlation values are positive. The numbers in squares represent the correlation coefficients (*r*). The horizontal and vertical axes represent each correlation factor. The color gradient from blue to red indicates the degree of correlation, ranging from low to high. MII, metaphase II; Gn, gonadotropin.

and live birth rate and was an independent predictor of the MII oocyte rate. The MII oocyte rate increased significantly

with increasing Gn stimulation duration; however, no further significant improvement was observed once the dura-

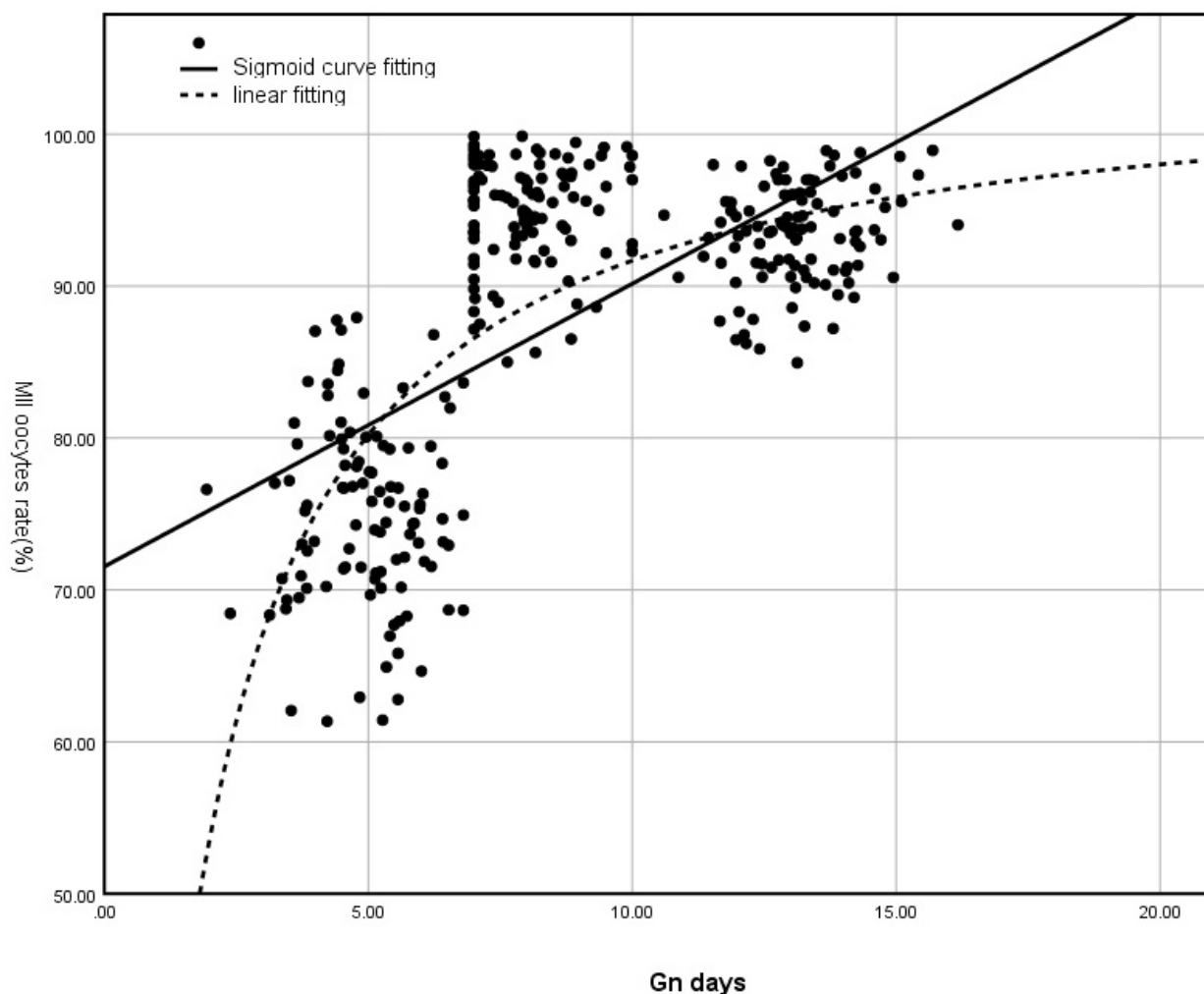


Fig. 3. Curve fitting of Gn usage time and MII oocyte rate. The x-axis represents the number of Gn days, the y-axis represents the MII oocyte rate, and the dashed line in the figure is the linear regression fitting line for Gn days and MII oocyte rate. The dashed lines represent the fitting lines for Gn days and MII oocyte rate using a Sigmoid curve. Gn, gonadotropin; MII, metaphase II.

tion was >7 days. These findings confirmed the use of 7 days as a key indicator of Gn stimulation, and it is necessary to use the GnRHa regimen to promote ovulation for 7 days for patients with a normal ovarian reserve. Consistent with our findings, Mardešić et al. [19] reported that women with a short duration of stimulation of only 6–8 days achieved pregnancy rates comparable to those of women with longer stimulation durations. Previous studies have reported that a stimulation duration of less than 6 days is associated with poorer IVF outcomes, consistent with our results, although those studies included more heterogeneous patient populations with respect to ovarian reserve and ovarian stimulation protocols [12]. Furthermore, unlike our study, which focused primarily on the MII oocyte maturation rate as the primary outcome, most previous studies used clinical outcome as the main research index.

This study has several notable advantages. First, the use of both multivariate linear regression analysis and PSM in this retrospective study strengthened the validity of the

findings by controlling for potential confounding factors that could have influenced the clinical outcomes, such as the total Gn dose, the initial Gn dose, and the levels of LH and E2 at the stimulation initiation. This approach allowed the specific effect of Gn stimulation duration on follicular development during ovarian stimulation to be evaluated independently of other influential factors. Collectively, these methodological strengths increase the rigor and internal validity of the study, providing valuable evidence for the relationship between Gn stimulation duration and the MII oocyte maturation rate. Second, we selected the MII oocyte maturation rate as the primary research outcome, whereas most previous studies have focused mainly on clinical outcomes, such as the clinical pregnancy rate and live birth rate. The principal objective of optimizing ovarian stimulation protocols is to maximize the yield of mature oocytes, as this is a critical determinant of IVF success [24,25]. Many factors affect the clinical outcome, including oocyte quality as well as sperm quality and endometrial factors. Third,

the type of ovarian stimulation protocol may also independently influence the duration of stimulation regardless of the ovarian reserve. Previous studies have suggested that the GnRH antagonist protocols typically require a shorter stimulation duration than GnRHa protocols [15]. A possible explanation is that the GnRH antagonist protocol does not require pituitary downregulation before ovarian stimulation. However, in the present study, all enrolled patients received the same GnRHa protocol, allowing the effect of different Gn stimulation durations to be evaluated within a uniform stimulation protocol.

Limitations

However, this study has several limitations that should be acknowledged. First, the retrospective nature of the study may have introduced imbalances in baseline patient clinical characteristics. To mitigate this, multivariable regression analysis and PSM were utilized to minimize the potential effects of confounders. Second, this was a single-center study with a relatively small sample size, which may limit the generalizability of the findings. Further validation with subgroup analysis and a larger multicenter sample is warranted to validate these results. Third, the relatively short follow-up period prevented the assessment of maternal and neonatal outcomes and the long-term health of offspring. Future studies with extended follow-up periods are needed to provide a more comprehensive evaluation of these outcomes. Finally, although GnRH antagonist protocols are popular worldwide, our center continues to focus on conventional GnRHa protocols, with relatively limited use of antagonist protocols. Consequently, the applicability of our findings to GnRH antagonist cycles remains to be established. Despite these limitations, this study still provides valuable insights into the effect of the Gn stimulation duration on clinical outcomes in patients with normal ovarian reserve undergoing the conventional long GnRHa protocol.

5. Conclusions

In conclusion, patients with normal ovarian reserve undergoing a GnRHa protocol should receive at least 7 days of Gn stimulation. The duration of Gn stimulation was correlated with and independently predicted the MII oocyte rate. These findings contribute to optimizing clinical strategies and improving outcomes for normal-ovarian-reserve patients undergoing GnRHa treatment.

Availability of Data and Materials

The data used during the current study are available from the corresponding author on reasonable request.

Author Contributions

CY, YW and LC conceived and designed this study. YW, CZ, LZ, JJ and HY collected and analyzed the data. CY, YW and CZ interpreted the data. YW, CZ, LZ, JJ

and HY drafted the manuscript. CY and LC reviewed and edited the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

(1) The study and the protocols used were approved by the Ethics Committee of the Changzhou Maternity and Child Health Care Hospital (202403). Written informed consent was obtained from the infertile couples participating in the study. The study was conducted according to the Declaration of Helsinki for medical research. (2) This study was a retrospective study with data from the reproductive center case system, and no clinical trial registration was required.

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Conflicts of Interest

The authors declare no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/CEOG48905>.

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