# **Factors Influencing the Pregnancy Outcome of Intrauterine Insemination and Follow-up Treatment**

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Background: Many factors were reported associated with the pregnancy rate of intrauterine insemination (IUI), which played key role is still debated. Aims: The aim of this study was to explore related clinical pregnancy outcome factor in IUI cycles of non-male factor. Settings and Design: The clinical data of 1232 IUI cycles in 690 couples experiencing infertility who attended the Reproductive Center of Jinling Hospital between July 2015 and November 2021 were retrospectively analysed. Materials and Methods: Female and male age, body mass index (BMI), anti-Müllerian hormone (AMH), male semen parameters before and after wash, endometrial thickness (EMT), artificial insemination timing and ovarian stimulation (OS) protocols were compared between the pregnant group and the non-pregnant group in order to explore any correlation. Statistical Analysis Used: Continuous variables were analysed using independent-samples t-test, and Chi-square test was used for comparison of measurement data between the two groups. P < 0.05 was considered statistical significance. Results: There were statistically significant differences in female AMH, EMT and duration of OS between the two groups. The AMH was higher in the pregnant group than in the non-pregnant group ( $P \le 0.01$ ), the stimulated days was significantly longer (P < 0.05) and EMT was significantly greater (P < 0.01) in the pregnant group than in the non-pregnant group. Further analysis showed that when patients with IUI had the following conditions: AMH > 4.5 ng/ml, EMT between 8 and 12 mm and letrozole + human menopausal gonadotropin stimulation with higher clinical pregnancy. However, there were no differences between the pregnant group and the non-pregnant group amongst the female and male age, BMI, hormones on baseline and day of human chorionic gonadotrophin, number of ovulated oocytes, sperm parameters before and after wash, treatment protocols and the timing of IUI (P > 0.05). Furthermore, there were 240 couples who not pregnant received one or more cycles of in vitro fertilisation/ intracytoplasmic sperm injection/ pre-implantation genetic technology treatment, and another 182 couples forgo follow-up treatment. Conclusion: The results of the

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present study demonstrate that the clinical IUI pregnancy rate is correlated with the factors of female AMH, EMT and OS protocol; more studies and samples are necessary to evaluate whether other factors affect pregnancy rate.

**KEYWORDS:** Anti-Müllerian hormone, clinical pregnancy rate, endometrial thickness, intrauterine insemination, ovarian stimulation

#### INTRODUCTION

**2** ntrauterine insemination (IUI) is the first-line treatment option for many infertile couples. Widely used indications for IUI are unexplained infertility (including mild endometriosis), male factor infertility and female cervical factor.<sup>[1]</sup> IUI is a simpler, safer and cheaper treatment protocol and with a lower complication rate when compared with *in vitro* fertilisation (IVF)/ intracytoplasmic sperm injection (ICSI).

The clinical pregnancy rate of IUI is lower than that of ICSI/IVF, many factors were reported associated with the pregnancy rate of IUI, such as female age, treatment protocol, the number of cycles and dominant pre-ovulatory follicles, endometrial thickness (EMT) and smoking.<sup>[2-5]</sup> However, no consistent reports of influencing factors on IUI pregnancy rates have been reported.

The present study evaluated the effect of the age, body mass index (BMI), duration of infertility, treatment protocol, endometrium, anti-Müllerian hormone (AMH), the duration of infertility, duration of stimulation, sperm parameters and insemination timing on pregnancy outcome in IUI cycle. The aim of this study of the data is to provide a reference for clinical decision-making.

### MATERIALS AND METHODS

#### Study population

The retrospective study was approved by the Jinling Hospital Research Ethics Board (Number: 2016NJKY-028). This study only involves the collection or study of existing data, documents and records, and these sources were publicly available and could not be used to identify subjects either directly or by subject-related identifiers, thus exempting informed consent, and this study adheres to the principles of Helsinki Declaration (2013). A total of 1232 IUI cycles in 690 couples experiencing infertility who attended the Reproductive Center of Jinling Hospital between July 2015 and November 2021 were included in the present study. Inclusion criteria: Follicular monitoring was performed in female patients until ovulation; at least one unobstructed fallopian tube; normal semen parameters or mild oligospermia.

## Ovarian stimulation and intrauterine insemination protocol

Natural cycles were performed in women with regular menstrual cycles; IUI was based on peak luteinising hormone, which was measured daily after the follicle reaches 16–18 mm in diameter.

Ovarian stimulation (OS) was performed for females with ovulation disorder, irregular menstruation and pregnancy failure after intercourse guided for 2–3 times. OS was started on days 3–5 of menstruation. Letrozole cycle (LE): letrozole, 2.5–5.0 mg/day for 5 days; clomiphene citrate cycle (CC): clomiphene, 50–100 mg/day for 5 days; LE + human menopausal gonadotropin cycle (LE + HMG): letrozole, 2.5–5.0 mg/day for 5 days followed by 75–150 IU of HMG that depending on patients response; CC + HMG cycle: clomiphene, 50– 100 mg/day for 5 days followed by 75–150 IU of HMG that depending on patients response; HMG cycle: 75– 150 IU/day of HMG for a variable duration depending on patients response.

The monitor of follicles and endometrium were done through transvaginal ultrasound (TVUS). IUI was cancelled if more than three dominant triggered with human follicles. Ovulation was chorionic gonadotrophin (HCG, 10,000 IU) or gonadotropin-releasing hormone agonist (GnRH-a, mg) when the mature follicle with 0.1 а diameter ≥18 mm. IUI was performed after 28-36 h of trigger injection, and TVUS was performed to check for ovulation and endometrium prior to IUI. Luteal phase support was provided with dydrogesterone 20 mg twice daily for 14 days after ovulation was determined.

#### Semen processing

Semen was collected via masturbation after an abstinence of 2-7 days and was prepared by double-density gradient centrifugation. In the case of abnormal liquefaction, the sperm was diluted with the same volume of culture medium and subjected to density gradient centrifugation. Next, 1.5 mL of 90% solution was pipetted into the tube and 1.5 mL of 45% solution was then slowly dripped on the top. Centrifuged the tube at 300 g for 20 min and removed the top two layers, then the semen was gently layered on top. As little of 90% solution as possible was used, the sperm pellet was transferred to a sterile conical tube with 5 mL of equilibrated G-IVF PLUS. The sperm sample was centrifuged at 300 g for 5 min, and the supernatant was discarded and washed sperm repeatedly. The sample volume for insemination was 0.5 mL. The motility, progressive sperm and concentration of semen sample were tested before and after washed.

#### **Outcome assessment**

Serum beta-HCG was carried out to determine pregnancy. Clinical pregnancy was defined as gestation sac ultrasound visibility by TVUS 1 month after IUI.

#### Statistical analysis

SPSS 21.0 (SPSS Inc., Chicago, IL, USA) software was used for statistical analysis; the measurement data were expressed as mean  $\pm$  standard deviation and evaluated using the independent-samples *t*-test. The Chi-square test was used for comparison of measurement data between the two groups. *P* <0.05 was considered statistical significance.

#### RESULTS

#### **Overall results**

The present study included a total of 1232 cycles in 690 couples. The characteristics of patients undergoing all cycles with IUI are presented in Table 1. The clinical pregnancy rate (CPR) was 21.75% (268/1232) and the biochemical pregnancy rate was 24.03% (296/1232). 22.3% of couples got clinical pregnancy in the first cycle, and the cumulative pregnancy rate increased gradually with the increase of the cycles [Figure 1a]. Of all pregnancies, 60.7% of patients achieved pregnancy during the first IUI cycle, and the cycle distribution is shown in Figure 1b.

#### **Sperm parameters**

The sperm parameters were analysed for the pregnant group and non-pregnant group. No significant differences were found of DNA fragmentation index, sperm volume, sperm concentration, vitality, progressive sperm motility, non-progressive rate, sperm count and abstinence days [Table 2]. Therefore, male factors had no effect on the clinical pregnancy rate in the present study.

Table 1: Characteristics of a patient undergoing all cycles with intrauterine insemination			
Variable	Mean±SD, <i>n</i> (%)		
Female, age (year)	28.66±3.45		
	22.52.2.02		

Female, BMI (kg/m <sup>2</sup> )	23.52±3.93
Male, age (year)	29.97±3.94
Male, BMI (kg/m <sup>2</sup> )	26.74±17.85
Infertility years	$2.63 \pm 2.26$
Primary infertility	867 (72.01)
Secondary infertility	337 (27.99)
Non-pregnant group	936 (75.97)
Pregnant group	268 (21.75)
Infertility diagnosis	
Unexplained	191 (15.5)
Male factor	320 (26)
Tubal factor	81 (6.6)
Ovulatory dysfunction	212 (17.2)
Combined factors	428 (34.7)

BMI=Body mass index, SD=Standard deviation

#### **Baseline characteristics**

There were no significant differences of female and male age, BMI, infertility years and baseline hormones between the pregnant group and the non-pregnant group. AMH was significantly higher in the pregnant group than the non-pregnant group (P < 0.01). Based on age, the patients were divided into three groups: aged 20-30 years, aged 31-34 years and aged >35 years. The level of AMH was significantly higher in pregnant group than non-pregnant with age 20-30, no differences of age 31–34 and  $\geq$ 35 groups [Table 3]. According to the value of AMH, three groups were divided: <1 ng/ml, 1-4.5 ng/ml and >4.5 ng/ml, the clinical pregnancy rate was significantly higher when AMH >4.5 ng/ml than 1–4.5 ng/ml (P < 0.01). Furthermore, of the three age groups, the downward trend in clinical pregnancy rate was along with increased female age, 23.3%, 20.58% and 15%, respectively [Table 4].

#### **Ovarian stimulation parameters**

The parameters of OS showed that there were no differences of HMG dose, hormones on HCG day, the number of dominant follicles on HCG day and ovulated oocytes between the pregnant group and the non-pregnant group during IUI cycle. However, the duration days of stimulation was longer  $(4.96 \pm 2.84 \text{ and } 4.48 \pm 3.03,$ respectively; P < 0.05) and the EMT on HCG day was thicker  $(9.31 \pm 1.97 \text{ mm} \text{ and } 8.89 \pm 1.93 \text{ mm},$ respectively; P < 0.01) in the pregnant group [Table 5]. Based on EMT, the patients were divided into three groups as <8 mm group, 8-12 mm group and >12 mm group. The CRP was 18.39%, 23.51% and 29.63% of the three groups which showed no difference. When the EMT was 8-12 mm, the endometrial was significantly increased in the pregnant group (P < 0.05). When the EMT was <8 mm or >12 mm, it did not affect clinical pregnancy.

#### **Ovarian stimulation protocol**

The present study included 72 natural cycles and 1132 ovulation cycles. There was no significant difference of clinical pregnancy rate between the pregnant group and the non-pregnant group. Based on the medication scheme, the ovulation induction cycles were divided into five groups: (1) group a: LE, (2) group b: CC, (3) group c: LE + HMG, (4) group d: CC + HMG and (5) group e: HMG. The clinical pregnancy rates amongst natural cycles and different stimulation protocol cycles are shown in Figure 2. When we performed the analysis of differences between subgroups, we found that different OS protocols related to the pregnancy outcome [Table 6]. The clinical pregnancy rate in group e was the highest when compared with the other groups; however, the sample

Table 2: Sperm parameters before and after wash for the pregnant group and non-pregnant group			
	Pregnant group	Non-pregnant group	P
DFI	16.22±11.06	15.91±11.06	NS
Before wash			
Volume (mL)	2.50±1.26	2.46±1.19	NS
Concentration (×10 <sup>6</sup> /mL)	62.09±34.84	63.09±34.09	NS
Vitality (%)	59.37±14.36	59.93±14.44	NS
Progressive sperm motility (%)	38.80±11.72	40.12±12.51	NS
Non-progressive rate (%)	11.17±28.15	38.05±39.59	NS
Sperm count (×10 <sup>6</sup> /mL)	86.75±58.89	90.74±72.41	NS
Abstinence days	3.00±1.66	3.02±1.72	NS
After wash			
Concentration (×10 <sup>6</sup> /mL)	47.66±28.56	49.83±31.88	NS
Vitality (%)	95.20±2.64	95.08±3.89	NS
Progressive sperm motility (%)	95.17±2.63	95.07±3.89	NS
Motile progressive sperm (×10 <sup>6</sup> )	22.75±13.72	23.77±15.37	NS

DFI=DNA fragmentation index, NS=Non-significant



Figure 1: IUI cycles and pregnancy outcomes. (a) The relationship between IUI cycles and cumulative clinical pregnancy rate, (b) The cycle distribution in the pregnancy group. IUI = Intrauterine insemination



**Figure 2:** The clinical pregnancy rate according to different treatment protocols. Data were presented as the ratio of pregnancy cycle/total cycle. LE = Letrozole, CC = Clomiphene citrate, HMG = Human menopausal gonadotropin

size was relatively small. The clinical pregnancy rate was significantly higher in group c than in groups b and d (group c/group b: P = 0.022; group c/group d: P = 0.017) [Table 6].

#### **Follow-up**

There were 422 cases without pregnancy after IUI treatment, 240 of them had follow-up of IVF, ICSI or PGT treatment and 182 did not receive follow-up treatment. Of the 240 patients who received subsequent antiretroviral therapy (ART) treatment, 219 (91.25%) achieved clinical pregnancy. It was worth noting that 10 cases underwent PGT-assisted pregnancy due to chromosomal problems [Table 7].

#### DISCUSSION

Previous studies have indicated that factors such as female age, male age, the timing and frequency of IUI, OS protocol and the post-wash total motile sperm count of semen were related to the clinical pregnancy rate of IUI treatment.<sup>[1-6]</sup> We explore the effects on the pregnancy outcome of IUI for couples; the results revealed that AMH, duration days of stimulation, OS protocols, EMT and types had significant differences on pregnancy outcome. What's more, there were no significant differences of male factor,

Table 3: Basic characteristics of the study population for the pregnant group and non-pregnant group			
	Pregnant group	Non-pregnant group	P
Female, age (year)	28.32±3.32	28.76±3.48	NS
Female, BMI	23.66±3.79	23.48±3.97	NS
Male, age (year)	29.65±3.72	30.05±4.00	NS
Male, BMI	26.35±16.28	26.85±18.29	NS
Infertility years	2.51±1.43	2.67±2.45	NS
Baseline hormones			
FSH (IU/L)	7.11±1.92	7.19±1.99	NS
LH (IU/L)	$7.97 \pm 9.97$	7.33±8.29	NS
PRL (mIU/L)	311.20±173.90	302.01±155.42	NS
E2 (pmol/L)	173.60±195.53	175.15±81.91	NS
T (pmol/L)	$1.66{\pm}0.70$	$1.64{\pm}0.71$	NS
P (nmol/L)	2.08±1.45	2.24±2.54	NS
AMH (ng/mL)	$6.00{\pm}3.69$	5.22±3.46**	0.001519
Subjects in AMH categories by age			
20-30	6.32±3.71	5.62±3.61*	0.017
31-34	5.20±3.67	4.36±2.90	NS
≥35	3.84±1.83	3.77±2.57	NS

\*P<0.05, \*\*P<0.01. NS=Non-significant, BMI=Body mass index, FSH=Follicle-stimulating hormone, LH=Luteinising hormone, PRL=Prolactin, E2=Oestradiol, AMH=Anti-Mullerian hormone, T=Testosterone, P=Progesterone

Table 4: The clinical pregnancy rate according to anti-Mullerian hormone and female age						
		AMH (ng/mL)			Female age (years)	
	<1.0 (a)	1-4.5 (b)	>4.5 (c)	20-30 (d)	31-34 (e)	≥35 (f)
Pregnant group, n (%)	2 (16.67)	106 (18.4)	160 (25.97)	202 (23.3)	57 (20.58)	9 (15)
Non-pregnant group, n (%)	10 (83.33)	470 (81.6)	456 (74.03)	665 (76.7)	220 (79.42)	51 (85)
$\chi^2$		10.08			2.821	
P		0.006			0.244	

Pairwise comparison (χ<sup>2</sup> [*P*]). *P*<0.05=b: c 9.843 (0.002). *P*>0.05=a: b 0.024 (0.878), a: c 0.533 (0.465), d: e 0.888 (0.346), d: f 2.198 (0.138), e: f 0.974 (0.324). AMH=Anti-Mullerian hormone

Table 5: Stimulation parameters of the study population for the pregnant group and non-pregnant group				
	Pregnant group	Non-pregnant group	Р	
Total HMG dose (U)	422.16±283.59	389.78±327.47	NS	
Duration of HMG stimulation (days)	$4.96{\pm}2.84$	4.48±3.03*	0.019848	
LE + HMG	$5.64{\pm}1.90$	5.27±2.11	NS	
CC + HMG	5.67±2.73	5.21±2.71	NS	
HMG	$6.55 \pm 2.98$	5.52±3.67	NS	
Hormones on HCG day				
FSH (IU/L)	9.59±3.69	9.26±3.63	NS	
LH (IU/L)	$17.38 \pm 16.10$	15.96±14.63	NS	
E2 (pmol/L)	2545.84±2071.68	2768.04±2634.18	NS	
P (nmol/L)	3.33±2.75	3.36±4.49	NS	
Number of follicles on HCG day	$2.18 \pm 1.26$	2.10±1.24	NS	
EMT on HCG day (mm)	9.31±1.97	8.89±1.93**	0.002288	
<8	$6.76{\pm}0.65$	7.81±1.67	NS	
8-12	9.68±1.24	9.45±1.24	0.022093	
>12	13.91±1.23	14.00±1.15	NS	

HMG=Human menopausal gonadotropin, LE=Letrozole, CC=Clomiphene citrate, HCG=Human chorionic gonadotrophin, FSH=Follicle-stimulating hormone, EMT=Endometrial thickness, LH=Luteinising hormone, E2=Oestradiol, NS=Non-significant, P=Progesterone. \**P*<0.05, \*\**P*<0.01

including male age and BMI and sperm parameters in each group. Therefore, the factors affecting the pregnancy rate in this study were mainly female factors.

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#### Anti-Müllerian hormone and age

There was no agreement about whether AMH affects pregnancy rates. A previous study indicated that AMH

Table 6: The clinical pregnancy rate according to treatment protocol							
	Treatment protocol			(	Ovarian stimulatio	on cycle	
	Natural cycle	Ovarian stimulation cycle	LE (a)	CC (b)	LE + HMG (c)	CC + HMG (d)	HMG (e)
Pregnant group, n (%)	18 (25)	250 (22.08)	9 (21.43)	9 (12.85)	142 (25.27)	79 (18.81)	11 (28.95)
Non-pregnant group, $n$ (%)	54 (75)	882 (77.92)	33 (78.57)	61 (87.15)	420 (74.73)	341 (81.19)	27 (71.05)
$\chi^2$		0.332			10.44		
Р		0.56			0.034		

Pairwise comparison ( $\chi^2$  [*P*]). *P*<0.05=b: c 5.272 (0.022), b: e 4.226 (0.04), c: d 5.747 (0.017). *P*>0.05=a: e 0.602 (0.438), c: e 0.254 (0.614), a: b 1.43 (0.232), d: e 2.268 (0.132), b: d 1.443 (0.23), a: c 0.307 (0.579), a: d 0.170 (0.68). HMG=Human menopausal gonadotropin, LE=Letrozole, CC=Clomiphene citrate

Table 7: The follow-up treatments and clinical outcome	es
of non-pregnant cases	

of non-pregnant cases				
Following	Pregnant	Non-pregnant		
treatment	group, <i>n</i> (%)	group, <i>n</i> (%)		
IVF (n=199)	183 (76.25)	16 (6.67)		
ICSI (n=30)	27 (11.25)	3 (1.25)		
PGD ( <i>n</i> =11)	9 (3.75)	2 (0.83)		
Total	219 (91.25)	21 (8.75)		

IVF=*In vitro* fertilisation, ICSI=Intracytoplasmic sperm injection, PGD=Pre-implantation genetic diagnosis

levels were positively correlated with CPR at the first attempt and cumulative clinical pregnancy rate of IUI, and found a threshold of 1.8 ng/ml and 2.3 ng/ ml allowing to discriminate women according to their chances of success,<sup>[7,8]</sup> other reported that AMH was not associated with pregnancy in 348 donor sperm inseminations cycles<sup>[9]</sup> and poorly affects cumulative live birth rate after IUI cycles.<sup>[10]</sup> We showed that the AMH value of the pregnant group was significantly higher than that of the non-pregnant group, and it was found that the clinical pregnancy rate was the highest when AMH >4.5 ng/ml. AMH and age are closely linked;<sup>[11,12]</sup> the value was always negatively related to age. Tarasconi and Lyttle et al. showed that low AMH was linked, independently of age, to increased pregnancy loss.<sup>[13,14]</sup> Here, we found that AMH value of patients in the age 20-30 was significantly related with CRP. The average age of the women was 28.67 years, and over 95% of patients were younger than 35 years in this study. There was no difference of clinical pregnancy rate according to female age but showed decreased clinical pregnancy rate when female age increased. It may be due to the fact that young patients not only have a better ovarian response but also have better oocyte quality and better embryos, resulting in a higher clinical pregnancy rate while aged patients have relatively more aneuploid embryos.<sup>[15]</sup>

#### **Ovarian stimulation**

CC, LE and gonadotropin (HMG) are applied for treating ovulatory dysfunction and infertility during IUI cycles.<sup>[1,6]</sup> In previous studies, some studies suggested

that there was no difference in clinical pregnancy rates between natural cycles and OS cycles;[4,16,17] however, other studies suggested that OS can improve clinical pregnancy rates than natural cycle.<sup>[2,18,19]</sup> In addition, several studies have demonstrated that cycles with HMG are associated with better clinical outcomes than cycles with CC and LE.<sup>[20-23]</sup> The data in the present study indicated that there were 72 cycles (5.8%) experienced natural cycle and showed no significance of clinical pregnancy rate compared with OS cycle, which mainly accounted for the small sample size. We also found that the average number of dominant follicles were 1.01 and 2.18 in natural cycles and OS cycles, and the number of ovulated follicle were 1 and 1.69. The relatively reduced ovulated oocytes during OS may be related to more polycystic ovary syndrome or ovulation disorder cases, which lead to a relatively low clinical pregnancy rate.

Furthermore, it was found that the clinical pregnancy rate in the LE + HMG group was significantly higher than the CC group and CC + HMG group, the CC group had the lowest clinical pregnancy rate. Wang et al. showed similar results that CC group with the lowest clinical pregnancy rate compared with other OS protocols.<sup>[24]</sup> Dinelli et al. also considered the single CC use unable to improve the clinical pregnancy rate in unexplained subfertility.[25] This may be associated with anti-oestrogen effect on the endometrium and single-follicle development when CC was used alone. The follicle number can be appropriately increased by combining CC with HMG by increasing the oestrogen level and EMT and improving the clinical pregnancy rate. Our result showed that the clinical pregnancy rate of the CC + HMG group was higher than the CC group; however, there is no statistical difference. HMG is a commonly used gonadotropin in clinical practice, and the days of HMG stimulated was higher in the pregnant group than the non-pregnant group; however, subgroup analysis showed that there was no significant difference in the days of HMG stimulation amongst the LE + HMG, CC + HMG and HMG groups. What's

more, the HMG group had the highest clinical pregnancy rate in the present study; however, a previous study has shown that the clinical pregnancy rate in the LE + HMG group is higher than that in the HMG group,<sup>[24]</sup> which is inconsistent with the results of this study, which may be related to the relatively small sample size of this study.

#### **Endometrial thickness**

EMT and classification can be used as an index to evaluate endometrial receptivity. A large number of studies have shown that the pregnancy outcome is affected by the EMT on the day of IUI performance,<sup>[5,26]</sup> and the clinical pregnancy rate was highest when EMT was between 8 and 12 mm.<sup>[16]</sup> One retrospective research that assessed 1065 IUI cycles indicated that when the EMT was <7 mm or more than 14 mm, the clinical pregnancy rate was lower than the thickness between 7 and 14 mm.<sup>[27]</sup> We also found that clinical pregnancy rates were lowest when EMT was < 8mm. What' more, it seems that EMT >12 mm with the highest clinical pregnancy rate, but its sample size was relatively small and more data validation is needed. The better thickness of the endometrium was 8-12mm, and significantly thicker in the pregnant group. What' more, it seems that EMT >12 mm with the highest clinical pregnancy rate, but its sample size was relatively small and more data validation is needed. We further analyzed the effect of endometrial type on the clinical pregnancy rate, and the clinical pregnancy rate of endometrial type A was higher than that of type B and type C. Therefore, A type with a thickness between 8 and 12 mm can achieve a higher clinical pregnancy rate for IUI patients.

For the non-pregnant patients, further treatments including IVF and ICSI were performed and a better clinical outcome can be obtained in the present study. A previous study indicated that IUI might be a better alternative than IVF as the first-line treatment of unexplained infertility.<sup>[28]</sup> As Bahadur et al. found that IUI success rates are much closer to IVF than previously reported, more cost-effective in delivering one live birth, and associated with lower risk of complications for maternal and neonatal complications. It is prudent to offer IUI before IVF nationally.<sup>[29,30]</sup> Therefore, couples who have not become pregnant after two IUI cycles will be advised to opt for ART treatment.

Other studies reported factors of age, sperm parameters, BMI, obesity, smoking status, artificial insemination timing, insemination frequency were correlated with clinical pregnancy rate.<sup>[6,31]</sup> However, these factors have been inconsistent across studies, and in the present study, none of these factors were associated with clinical pregnancy rate. Nonetheless, this study has some limitations and biases that should be taken into account

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that the main limitation of the study was its retrospective design. What's more, the small sample size was also one of the limitations of this study, which may lead to some analysis results to be confirmed by large samples. For example, there may be other factors that influence clinical pregnancy rates in IUI. The study population includes mainly those with favourable prognosis and hence the findings may not be generalised, an expanded sample size and prospective study would help confirm this regularity in future investigations.

#### CONCLUSION

This study analysed clinical data of patients with clinical pregnancy. We found that the optimal EMT was 8-12 mm in both natural cycle and ovulation induction cycle. LE + HMG regimen could be preferred for clinical selection which obtains the highest clinical pregnancy rate. Most patients achieve clinical pregnancy in the first cycle, for patients who have not become pregnant after two or more cycles of IUI treatment, further ART treatment including IVF or ICSI may be a better choice to shorten the time to pregnancy. For patients who have not become pregnant after two or more cycles of IUI treatment, further ART treatment including IVF or ICSI may be a better choice to shorten the time to pregnancy. Therefore, for patients undergoing IUI treatment, attention should be paid to the OS protocol and the EMT, and it is necessary to combine the patient's age and ovarian reserve for comprehensive treatment.

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#### **Conflicts of interest**

There are no conflicts of interest.

#### Data availability statement

The data set used in the current study is available (tick the appropriate option and fill the information).

 Available on request from (contact name/email id) Xuan Huang/huangxuan1670@163.com

#### REFERENCES

- 1. Cohlen B, Bijkerk A, Van der Poel S, Ombelet W. IUI: Review and systematic assessment of the evidence that supports global recommendations. Hum Reprod Update 2018;24:300-19.
- Xu YY, Wang HY, Qiao J, Liu P, Chen XN, Ma CH, et al. Analysis of clinical factors affecting pregnancy rate of intrauterine insemination. Beijing Da Xue Xue Bao Yi Xue Ban 2013;45:887-91.

- Yildirim G, Turkgeldi LS, Koroglu N, Sebileguler, Mervetalmac. Predictive factors for pregnancy outcome following controlled ovarian stimulation and intrauterine insemination. J Pak Med Assoc 2017;67:422-7.
- Guan H, Tang H, Pan L, Song H, Tang L. Pregnancy predictors in unexplained infertility after intrauterine insemination. J Gynecol Obstet Hum Reprod 2021;50:102071.
- Masrour MJ, Yoonesi L, Aerabsheibani H. The effect of endometrial thickness and endometrial blood flow on pregnancy outcome in intrauterine insemination cycles. J Family Med Prim Care 2019;8:2845-9.
- Starosta A, Gordon CE, Hornstein MD. Predictive factors for intrauterine insemination outcomes: A review. Fertil Res Pract 2020;6:23.
- 7. Li HW, Yeung WS, Lau EY, Ho PC, Ng EH. Evaluating the performance of serum antimullerian hormone concentration in predicting the live birth rate of controlled ovarian stimulation and intrauterine insemination. Fertil Steril 2010;94:2177-81.
- Moro F, Tropea A, Scarinci E, Leoncini E, Boccia S, Federico A, et al. Anti-Müllerian hormone concentrations and antral follicle counts for the prediction of pregnancy outcomes after intrauterine insemination. Int J Gynaecol Obstet 2016;133:64-8.
- González-Foruria I, Martínez F, Rodríguez-Purata J, Ballester M, Alonso-Mosquera V, Buxaderas R, *et al.* Can anti-Müllerian hormone predict success outcomes in donor sperm inseminations? Gynecol Endocrinol 2019;35:40-3.
- Moreau J, Gatimel N, Simon C, Cohade C, Lesourd F, Parinaud J, et al. Age-specific anti-Mullerian hormone (AMH) levels poorly affects cumulative live birth rate after intra-uterine insemination. Eur J Obstet Gynecol Reprod Biol X 2019;3:100043.
- Kotlyar AM, Seifer DB. Ethnicity/race and age-specific variations of serum AMH in women-a review. Front Endocrinol (Lausanne) 2020;11:593216.
- Bleil ME, Gregorich SE, Adler NE, Sternfeld B, Rosen MP, Cedars MI. Race/ethnic disparities in reproductive age: An examination of ovarian reserve estimates across four race/ ethnic groups of healthy, regularly cycling women. Fertil Steril 2014;101:199-207.
- Lyttle Schumacher BM, Jukic AM, Steiner AZ. Antimüllerian hormone as a risk factor for miscarriage in naturally conceived pregnancies. Fertil Steril 2018;109:1065-71.e1.
- Tarasconi B, Tadros T, Ayoubi JM, Belloc S, de Ziegler D, Fanchin R. Serum antimüllerian hormone levels are independently related to miscarriage rates after *in vitro* fertilization-embryo transfer. Fertil Steril 2017;108:518-24.
- 15. La Marca A, Minasi MG, Sighinolfi G, Greco P, Argento C, Grisendi V, *et al.* Female age, serum antimüllerian hormone level, and number of oocytes affect the rate and number of euploid blastocysts in *in vitro* fertilization/intracytoplasmic sperm injection cycles. Fertil Steril 2017;108:777-83.e2.
- Zhang K, Shi Y, Wang E, Wang L, Hu Q, Dai Y, *et al.* Ovarian stimulated cycle: Not a better alternative for women without ovulation disorder in intrauterine insemination. Oncotarget 2017;8:100773-80.

- De Brucker M, Haentjens P, Evenepoel J, Devroey P, Collins J, Tournaye H. Cumulative delivery rates in different age groups after artificial insemination with donor sperm. Hum Reprod 2009;24:1891-9.
- Xia M, Zheng J, Lei YL, Liu J, Sun H, Wen XF. Analysis of the related factors influencing intrauterine insemination pregnancy rate[J]. J Practical Med 2013;29:2945-7.
- Gomez R, Schorsch M, Steetskamp J, Hahn T, Heidner K, Seufert R, *et al.* The effect of ovarian stimulation on the outcome of intrauterine insemination. Arch Gynecol Obstet 2014;289:181-5.
- Diamond MP, Legro RS, Coutifaris C, Alvero R, Robinson RD, Casson P, *et al.* Letrozole, gonadotropin, or clomiphene for unexplained infertility. N Engl J Med 2015;373:1230-40.
- Zolton JR, Lindner PG, Terry N, DeCherney AH, Hill MJ. Gonadotropins versus oral ovarian stimulation agents for unexplained infertility: A systematic review and meta-analysis. Fertil Steril 2020;113:417-25.e1.
- 22. Guzick DS, Sullivan MW, Adamson GD, Cedars MI, Falk RJ, Peterson EP, *et al.* Efficacy of treatment for unexplained infertility. Fertil Steril 1998;70:207-13.
- Diamond MP, Legro RS, Coutifaris C, Alvero R, Zhang H. Letrozole, gonadotropin, or clomiphene for unexplained infertility[J]. Obstet Gynecol Surv 2015;70:757-8.
- Wang X, Zhang Y, Sun HL, Wang LT, Li XF, Wang F, Wang YL, Li QC. Factors Affecting Artificial Insemination Pregnancy Outcome. Int J Gen Med 2021;14:3961-9.
- Dinelli L, Courbière B, Achard V, Jouve E, Deveze C, Gnisci A, *et al.* Prognosis factors of pregnancy after intrauterine insemination with the husband's sperm: Conclusions of an analysis of 2019 cycles. Fertil Steril 2014;101:994-1000.
- Verma A, Mulchandani R, Lauria N, Verma K, Himani S. Endometrial thickness and pregnancy outcome in IUI cycles. Journal of Evolution of Medical and Dental Sciences 2013;2278-4802.
- Liu Y, Ye XY, Chan C. The association between endometrial thickness and pregnancy outcome in gonadotropin-stimulated intrauterine insemination cycles. Reprod Biol Endocrinol 2019;17:14.
- Homburg R. IUI is a better alternative than IVF as the first-line treatment of unexplained infertility. Reprod Biomed Online 2022;45:1-3.
- Bahadur G, Homburg R, Bosmans JE, Huirne JAF, Hinstridge P, Jayaprakasan K, *et al.* Observational retrospective study of UK national success, risks and costs for 319,105 IVF/ICSI and 30,669 IUI treatment cycles. BMJ Open 2020;10:e034566.
- Bahadur G, Homburg R, Al-Habib A, Muneer AJ. Overuse of IVF risks public funding. BMJ 2021;5735.
- Jansen CH, Elisen MG, Leenstra CW, Kaaijk EM, van Stralen KJ, Verhoeve HR. Longer time interval between semen processing and intrauterine insemination does not affect pregnancy outcome. Fertil Steril 2017;108:764-9.