The Interval Between Insemination and Ovulation Predicts Outcome After Intrauterine Insemination With Donor Sperm (IUI-D)

XIN MU
Northwest women's and children's hospital https://orcid.org/0000-0003-0413-9045
Hui Wang
northwest women's and children's hospital
Pei-jun Liu
The First Affiliated Hospital of Chongqing Medical University
Juan-zi Shi (shijuanzi@126.com)
Northwest women's and children's hospital

Research

Keywords: I-O interval, intrauterine insemination, donor sperm, clinical pregnancy rate, live birth rate, prognostic factor

Posted Date: October 8th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-84808/v1

Version of Record: A version of this preprint was published at International Journal of Gynecology & Obstetrics on March 24th, 2021. See the published version at https://doi.org/10.1002/ijgo.13641.

Abstract

Objective

To identify whether the time interval from insemination to ovulation (I-O interval) affects outcome after intrauterine insemination with donor sperm (IUI-D).

Design

Retrospective study.

Setting

A single public medical center.

Patient(s)

1,165 couples, for 2,091 IUI-D cycles.

Main Outcome Measure

Live birth rate (LBR)

Results

Multiple predictors were identified for LBR. The I-O interval was the predictor for LBR. An I-O interval ≥19 hours significantly decreased CPR (odds ratio [OR], 95% confidence interval [CI] =0.285, 0.171-0.475) and LBR (OR, 95%CI =0.322, 0.189-0.549). The presence of at least two follicles ≥18mm on ovulation day significantly increased the LBR (OR, 95%CI =1.274, 1.012-1.602). Women aged 35 years and older had a significant decreased LBR (OR, 95% CI =0.607, 0.377-0.976).

Conclusion(s)

The I-O interval, a new prognostic factor, combination with the women's age and number of mature follicle, can predict the outcome after IUI-D. IUI-D is best performed within 19 hours of I-O interval for a higher probability of clinical pregnancy and live birth.

Introduction

Infertility is becoming a serious health problem in developed countries. It is estimated that about 8%-32% of all married couples of reproductive age suffer from infertility and sterility worldwide [1,2]. With respect to males, approximately 5%-10% of infertility is due to azoospermia [3]. Intrauterine insemination with donor sperm (IUI-D), is mainly used for couples who have severe semen deficiencies or azoospermia, lesbian couples and single women [4]. For these women, IUI-D could be a better choice because of its

simplicity, low cost and fewer complications compared to other assistant reproductive technologies (ARTs) and has been widely accepted as a first-line treatment for achieving pregnancy [5] .

Although the technique of IUI-D has been improving, the success rate of IUI-D is still low and unsatisfactory [6]. Many factors have been verified to influence the outcome of IUI-D, including the women's age [7,8], an extreme body mass index, decreased ovarian reserve, primary infertility, unilateral tubal occlusion, pelvic adhesions and mild endometriosis [9-13], insemination times in a cycle [14] and semen factors (volume, motility and morphology of donor's semen) [15-17].

The timing of insemination is a key factor affecting the outcome after IUI-D [18]. A proper interval from insemination to ovulation (I-O interval) may increase the chance of pregnancy [19]. Several studies have investigated the effects of different insemination time on the pregnancy rate (PR) but have reported inconsistent conclusions [20-22]. According to the newest global recommendation, a single IUI for an ovarian stimulation cycle can be performed any time between 24-40h after HCG injection and the IUI for a natural cycle should be performed 1day after the increase in LH [23]. However, these studies did not include an important covariate, the ovulation time, which is key to determining IUI timing and can potentially affect the IUI outcome. The error in the predicted ovulation time could be a confounding factor affecting the clinical outcome after IUI-D. Moreover, in the IUI-D cycle, the processed spermatozoa are injected directly into uterus cavity, which means that the time for successful pregnancy in the IUI-D cycle is shorter than that for natural conception[24] . It is plausible that the insemination time should be scheduled as close as possible to ovulation to obtain a higher PR. In the present study, we retrospectively analyzed 2,091IUI-D cycles from 1,165 couples and used the relative exact ovulation time to calculate the I-O interval to determine whether short I-O interval would contribute to better IUI-D outcomes.

Materials And Methods

Patient and study design

We retrospectively analyzed the IUI-D cycle performed in the reproductive medicine center of Northwest Women's And Children's Hospital, China, from January 2014 to December 2016. The data were collected from the medical records of couples. The study protocol was approved by the Ethics Committee for the Clinical Application of Human Assisted Reproductive Technology of Northwest Women's and Children's Hospital.

In our center, IUI-D is performed for male factor infertility (azoospermia or severe oligospermia). Before treatment, all women underwent a detailed history and physical examination. All women had a salpingography to confirm at least single tubal patency. Both the natural cycle and stimulated cycle were included. The recorded parameters were mainly related to the woman, including age, duration of infertility, pregnancy history, number of attempts, tubal patency, Cos protocol, endometrium thickness and types, number and diameter of mature follicles on the day of insemination, I-O interval and donor semen quality.

IUI-D Procedure and I-O Interval Evaluation

Transvaginal ultrasound and serum LH and E_2 tests were performed to monitor ovulation. For natural cycles, the ultrasound check started on the eighth day of the cycle. For ovulation stimulation cycles, the test started on the fifth day of the cycle. The COS cycle was stimulated by clomiphene citrate, gonadotropins or clomiphene citrate plus gonadotropins. The initial dose was 50 mg/day for clomiphene citrate (CC) (days 5–9) or 75 IU/day for gonadotropins and was modified according to the ovarian response. The ovulation trigger was given with an injection of 10,000 IU HCG when the follicle was ≥18 mm but the serum LH was <35 IU/L.

An ultrasound check was carried out between 8:00 a.m. and 9:00 a.m. every one to five days depending on the growth speed of the follicle. When the leading follicle was larger than 14 mm, the patients started the test for urinary LH; if the test was positive or the leading follicle was larger than 18 mm, serum estrogen (E_2) and LH were quantified (except for those who refused blood tests). If the serum LH was ≥35 IU/L (defined as a spontaneous LH rise), E_2 and LH were retested three hours later. If the serum LH was <35 IU/L, we continued to test serum LH and E2 the next day for natural cycles, but for stimulating cycles, we administered an HCG injection when the follicle was ≥18 mm. Meanwhile, we increased the frequency of the ultrasound test (Figure 1).

According to the change in E_2 and LH, the HCG injection time and the ultrasound results, the insemination was arranged at 4:00 p.m. or 9:00 a.m. Thus, the I-O interval could be identified according to multiple ultrasound checks and the time of insemination (Table 1). In total, there were three types of I-O intervals: ≥ +19 h, -1 h ~ +19 h, and -19 h ~ -1 h (a minus value means that sperm was delivered after ovulation; Table 1).

Processing of Donor Sperm

The donor sperm samples were supplied by Shaanxi Province Human Sperm Bank and guaranteed by the National Health and Family Planning Commission (NHFPC) of the People's Republic of China. Sperm donors were screened strictly in accordance with NHFPC standards. Generally, eligible sperm had a minimum concentration of 60×10^6 per ml, progressive motility of 60%, and normal morphology of 4%. A proper match between patients and donors in racial and ethnic features, as well as blood type, were guaranteed. Before IUI, the frozen sperm sample was thawed fully and then centrifuged at 300g for 20 minutes, using a two-step discontinuous density gradient in a 45% and 90% Pure Sperm-100 platform. The semen samples were examined after thawing as well as after optimization according to the WHO standard [25]. The volume of washed sperm sample used for insemination was 0.5 mL.

Intrauterine Insemination and Luteal Phase Support

Insemination was performed by one of our center's gynecologists. The prepared sperm was gently inserted within 1 cm of the fundal extend of the uterine cavity using a soft catheter. The patient then rested for 10-15 minutes in a supine position. Daily treatment with 200 mg micronized progesterone or 20

mg dydrogesterone was used for 15 days after IUI-D. Some patients with a history of recurrent spontaneous abortion received an injection of 2000 IU human chorionic gonadotropin three times (every three days).

Diagnosis of pregnancy

The serum β-hCG concentration was quantified approximately 16 days after insemination. For women who had positive serum β-hCG, ultrasound confirmation of pregnancy was carried out two weeks later. A clinical pregnancy was defined after sonographic evidence of the gestational sac was observed. Live birth was defined as a live-born delivery at least 28 weeks after IUI-D.

Statistical Analysis

The observations of any variables were not completely independent of each other when all IUI-D cycles were included. Consequently, classical statistical analyses with the assumption that samples are independent could not be used for the entire data from all cycles. In this case, generalized estimating equations (GEEs) that allow analysis of correlated observations [26] were used to evaluate the effects of these variables on IUI outcome, as in previous studies [8,14]. The outcome measure response variable was whether pregnancy existed per cycle. The outcome measure used as a response variable was whether pregnancy existed per cycle. For independent samples, two-group comparisons were performed by two-tailed Student's t-test or Mann-Whitney U tests for continuous variables (expressed as the means ± standard deviations (SDs)) or by the χ^2 test for categorical variables (expressed as frequencies and percentages). Moreover, stepwise multivariate logistic analysis was used to construct a predictive model for the pregnancy rate in independent samples. The initial analysis included the variables shown in Tables 2-5. Variables were removed stepwise when the Wald test *P*-value for a given variable was over 0.05. Only statistically significant variables were included in the final model. All analyses were performed using the software Statistical Package Social Science (SPSS) 22.0. For all statistical tests, $P < 0.05$ defined statistical significance.

Results

A total of 2,091 IUI-D cycles from 1,165 patients were included in our analysis, comprising 909 natural, 860 gonadotropin-induced, and 322 clomiphene citrate plus gonadotropin-induced cycles. Patients underwent an average of 3.50 years of infertility (SD: 2.7) and 1.8 (SD: 0.9) treatment cycles. The mean age of the women at treatment was 27.8 years (SD: 3.8). A total of 687 pregnancies occurred among 2,091 treatment cycles, with 592 live-birth deliveries, which represents a 32.9% pregnancy rate and a 28.3% live birth rate per cycle. In addition, 648 patients were pregnant, and 578 patients had live births after IUI-D among 1,165 patients; of the patients who became pregnant, 610 had become pregnant once, 37 became pregnant twice and 1 became pregnant three times during our research period. The characteristics of the included patients are summarized in Table 2.

Univariate Analysis

Continuous variable comparison between the cycles yielding positive and negative outcomes for pregnancy

The continuous variables were compared between cycles yielding positive and negative outcomes for pregnancy (Table 2). The mean age of the women was significantly lower in cycles resulting in live birth (P = 0.01). None of the other selected factors (Table 2) significantly varied between the cycles with or without clinical pregnancy (P > 0.05).

Univariate analysis of categorical factors related to patient demographic and clinical features

We further stratified the patients by demographic and clinical features and then compared the clinical pregnancy rate (CPR) and live birth rate (LBR) among the different patient stratifications (Table 3 to Table 5). The results indicated that an interval of ≥ +19 h was associated with a significantly decreased CPR and LBR relative to -19 h ~ -1 h (13.0%, 11.6% vs. 34.7%, 29.4%; P <0.01 and <0.01) and -1 h ~ +19 h intervals (13.0%, 11.6% vs. 34.1%, 29.7%; P <0.01 and <0.01). Moreover, we found that the cycles carried out in patients ≥ 35 years old yielded a lower CPR and LBR relative to those in patients who were younger (23.9%, 19.7% vs. 33.4%, 28.8%; P = 0.04 and 0.03). Patients who had only one mature follicle had a lower CPR and LBR than those who had two or more mature follicles (31.7%, 27.2% vs. 37.3%, 32.8%). There were no significant associations of CPR and LBR with the other categorical variables included in Table 3 - Table 5.

Multiple GEEs analysis and model building

A predictive model for the clinical pregnancy rate and live birth rate was created by GEE analysis based on all 2,091 IUI-D cycles with predictor variables with P < 0.05, as shown in Tables 3-5. The CPR was only associated with the I-O time interval. The LBR was associated with not only the I-O interval but also the patient's age and the number of mature follicles. Compared with the -19 h ~ -1 h interval, the ≥ +19 h I-O interval resulted in a significantly decreased CPR (OR 0.29; 95% CI 0.17-0.48; P< 0.01) and LBR (OR 0.32; 95% CI 0.19-0.55; P< 0.01). No significant difference was observed between the -19 h ~ -1 h interval and the -1 h ~ +19 h interval. We found a significant decrease in the LBR as age increased beyond 35 years (OR 0.61; 95% CI 0.38-0.98; P=0.04), as well as a significant increase in women with two or more mature follicles (OR 1.27; 95% CI 1.01-1.60; P=0.04). No significant difference was observed in the CPR between women who were younger or older than 35 years or for women with different numbers of mature follicles.

Discussion

In the present study, we correlated the live birth rate with a series of demographic and cycle-specific factors in 2,091 IUI-D cycles from 1,165 infertile couples. To the best of our knowledge, this was the first study to investigate the effect of I-O interval on the PR of IUI-D based on the ovation prediction by ultrasound combined with the trend of LH and E_2 regulation or HCG injection time. The overall live birth rate of 28.3% per cycle and the clinical pregnancy rate of 32.9% per cycle are comparable to previous data reported in China [27]. These results enable us to highlight some female prognostic factors for LBR.

Our data showed that the I-O interval, combined with the woman's age and number of mature follicles, can predict the LBR per cycle. The odds of having a live birth significantly decreased when the I-O interval was ≥19 h.

The HCG injection time has been mostly used as a reference time point to optimize the timing of insemination in IUI [28-31]. Different studies have investigated the effects of altering the time interval from HCG administration to insemination on IUI outcome, including IUI 3–5 minutes vs. IUI 24-32 hours after HCG injection [31], IUI simultaneously with HCG injection vs. IUI 34-36 hours after HCG injection [29], and IUI 36 hours vs. IUI 24 hours after HCG injection [28]. However, even in a stimulated cycle, spontaneous premature LH rise could occur before the dominant follicle diameter reached 18 mm[32]. Thus, IUI performed 24-36 h after HCG injection might be too late in these cycles.

Detecting spontaneous LH rise is another widely accepted way to schedule IUI-D insemination. However, this method still has some limitations. The urinary LH kit used by most studies has been reported to have a relatively high false-negative rate, which could cause high cancellations and inappropriate insemination time [19]. Only one prospective study [24] used blood samples to detect spontaneous LH rise in the IUI cycle, but the limitation of this study is its neglect of serum E_2 levels. Moreover, too much blood collection for serum LH measurement also decreases its clinical application.

The classic theory of ovulation indicates that the rise in estrogen levels during the late proliferative phase triggers the pre-ovulatory LH surge, which in turn is followed by ovulation [33]. In natural cycle IVF treatment, when the LH surge reaches a peak or a descending slope with a decreasing E_2, oocyte retrieval is scheduled the next morning or on the same day to achieve the highest oocyte retrieval rate [34].

According to this theory, we modified our IUI procedure. Urinary LH was tested for follicles ≥ 14 mm until it was positive or for follicles ≥ 18 mm, and then serum LH and E_2 quantification was performed. When LH was ≥ 35 IU/L, we retested LH and E_2. Based on the regulation trend of LH and E_2 or HCG injection time, insemination was scheduled. This method could minimize the frequency of blood sampling and detect premature spontaneous LH rise as early as possible. Sequential transvaginal ultrasound checks were carried out to monitor ovulation for up to three days, which allowed us to obtain a relatively more exact I-O interval. Using this protocol, we can obtain a better outcome, indicating that this method could be used in scheduling exact and better insemination times in the IUI-D cycle.

As spermatozoa have a longer survival period in the uterus than the ovulated oocyte [35], it is reasonable that the probability of conception increases when spermatozoa are available in the reproductive tract before ovulation occurs; in other words, spermatozoa wait for oocyte [36]. Interestingly, our data indicate that there is an equal probability of conception when the interval length between insemination and ovulation is ≤19 h, regardless of which one occurred first, suggesting that such an interval is long enough for both spermatozoa and oocyte to maintain their activity and complete fertilization. After 19 hours, the activity of the spermatozoa or the oocyte may sharply decrease, and the LBR would drop significantly.

Patient age was another predictor for the CPR and LBR in our study. The CPR and LBR were significantly decreased for women ≥35 years old, similar to the results of previous studies [37,38]. This could be attributed to decreasing ovarian reserves with increasing age [39]. Furthermore, women presenting with two more mature follicles on the ovulation day were more likely to achieve pregnancy than women with only one mature follicle.

The main limitations of our study include our relatively small sample size and possible sample selection bias from the retrospective analysis. Studies based on a larger sample size and prospective design should be carried out in the future to confirm these results. In conclusion, our results have shown for the first time that the time interval between insemination and ovulation highly correlates with the live birth rate in IUI-D cycles. We also identified the I-O interval as a new prognostic factor for the outcome of IUI-D and highlighted that within 19 hours of ovulation is the proper time window for fertilization in the IUI-D procedure.

Declarations

Ethics approval and consent to participate

This study was performed in accordance with guidelines outlined in the Declaration of Helsinki. All methods and information collection protocols were approved by the Ethics Committee for the Clinical Application of Human Assisted Reproductive Technology of Northwest Women's and Children's Hospital.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

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

Author contributions:

XM: Data analysis, Data collection and manuscript writing. HW: Project development. Pei-jun Liu: Project development. Juan-zi Shi: manuscript writing and editing

Funding:

No funding support.

ACKNOWLEDGMENTS

We thank all the clinicians, scientists and embryologists in the Northwest Women and Children's Hospital for their assistance with data collection, and all the patients for their contribution to this study.

References

1. Vander Borght M, Wyns C. Fertility and infertility: Definition and epidemiology. Clin Biochem. 2018 Dec;62:2-10.
2. Craig LB, Peck JD, Janitz AE. The prevalence of infertility in American Indian/Alaska Natives and other racial/ethnic groups: National Survey of Family Growth. Paediatr Perinat Epidemiol. 2019 Mar;33(2):119-125.
3. Practice Committee of the American Society for Reproductive Medicine. Electronic address aao. Management of nonobstructive azoospermia: a committee opinion. Fertil Steril. 2018 Dec;110(7):1239-1245.
4. Kandavel V, Cheong Y. Does intra-uterine insemination have a place in modern ART practice? Best Pract Res Clin Obstet Gynaecol. 2018 Nov;53:3-10.
5. Chen L, Zhu L, Cai C, et al. Clinical and neonatal outcomes of intrauterine insemination with frozen donor sperm. Syst Biol Reprod Med. 2018 Aug;64(4):240-245.
6. De Geyter C, Calhaz-Jorge C, Kupka MS, et al. ART in Europe, 2014: results generated from European registries by ESHRE: The European IVF-monitoring Consortium (EIM) for the European Society of Human Reproduction and Embryology (ESHRE). Hum Reprod. 2018 Sep 1;33(9):1586-1601.
7. Mokdad C, Clavier B, Perdrix A, et al. [Prognosis factors in donor semen insemination: a 10-years follow-up study of 188 patients]. Gynecol Obstet Fertil. 2013 Feb;41(2):96-104.
8. Thijssen A, Creemers A, Van der Elst W, et al. Predictive value of different covariates influencing pregnancy rate following intrauterine insemination with homologous semen: a prospective cohort study. Reprod Biomed Online. 2017 May;34(5):463-472.
9. Atasever M, Kalem MN, Hatirnaz S, et al. Factors affecting clinical pregnancy rates after IUI for the treatment of unexplained infertility and mild male subfertility. J Turk Ger Gynecol Assoc. 2016;17(3):134-8.
10. Michau A, El Hachem H, Galey J, et al. Predictive factors for pregnancy after controlled ovarian stimulation and intrauterine insemination: A retrospective analysis of 4146 cycles. J Gynecol Obstet Hum Reprod. 2019 Dec;48(10):811-815.
11. Huyghe S, Verest A, Thijssen A, et al. Influence of BMI and smoking on IUI outcome with partner and donor sperm. Facts Views Vis Obgyn. 2017 Jun;9(2):93-100.
12. Isa AM, Abu-Rafea B, Alasiri SA, et al. Age, body mass index, and number of previous trials: are they prognosticators of intra-uterine-insemination for infertility treatment? Int J Fertil Steril. 2014 Oct;8(3):255-60.
13. Cochet T, Gatimel N, Moreau J, et al. Effect of unilateral tubal abnormalities on the results of intrauterine inseminations. Reprod Biomed Online. 2017 Sep;35(3):314-317.

14. Zarek SM, Hill MJ, Richter KS, et al. Single-donor and double-donor sperm intrauterine insemination cycles: does double intrauterine insemination increase clinical pregnancy rates? Fertil Steril. 2014 Sep;102(3):739-43.
15. Luco SM, Agbo C, Behr B, et al. The evaluation of pre and post processing semen analysis parameters at the time of intrauterine insemination in couples diagnosed with male factor infertility and pregnancy rates based on stimulation agent. A retrospective cohort study. Eur J Obstet Gynecol Reprod Biol. 2014 Aug;179:159-62.
16. Kohn TP, Kohn JR, Ramasamy R. Effect of Sperm Morphology on Pregnancy Success via Intrauterine Insemination: A Systematic Review and Meta-Analysis. J Urol. 2018 Mar;199(3):812-822.
17. Rodriguez-Purata J, Latre L, Ballester M, et al. Clinical success of IUI cycles with donor sperm is not affected by total inseminated volume: a RCT. Hum Reprod Open. 2018;2018(2):hoy002.
18. Kosmas IP, Tatsioni A, Fatemi HM, et al. Human chorionic gonadotropin administration vs. luteinizing monitoring for intrauterine insemination timing, after administration of clomiphene citrate: a meta-analysis. Fertil Steril. 2007 Mar;87(3):607-12.
19. El Hachem H, Antaki R, Sylvestre C, et al. Timing therapeutic donor inseminations in natural cycles: human chorionic gonadotrophin administration versus urinary LH monitoring. Reprod Biomed Online. 2017 Aug;35(2):174-179.
20. Robb PA, Robins JC, Thomas MA. Timing of hCG administration does not affect pregnancy rates in couples undergoing intrauterine insemination using clomiphene citrate. J Natl Med Assoc. 2004 Nov;96(11):1431-3.
21. Claman P, Wilkie V, Collins D. Timing intrauterine insemination either 33 or 39 hours after administration of human chorionic gonadotropin yields the same pregnancy rates as after superovulation therapy. Fertil Steril. 2004 Jul;82(1):13-6.
22. Lee J, Hwang S, Lee J, et al. Effect of insemination timing on pregnancy outcome in association with female age, sperm motility, sperm morphology and sperm concentration in intrauterine insemination. J Obstet Gynaecol Res. 2018 Jun;44(6):1100-1106.
23. Cohlen B, Bijkerk A, Van der Poel S, et al. IUI: review and systematic assessment of the evidence that supports global recommendations. Hum Reprod Update. 2018 May 1;24(3):300-319.
24. Blockeel C, Knez J, Polyzos NP, et al. Should an intrauterine insemination with donor semen be performed 1 or 2 days after the spontaneous LH rise? A prospective RCT. Hum Reprod. 2014 Apr;29(4):697-703.
25. Lu JC, Huang YF, Lu NQ. [WHO Laboratory Manual for the Examination and Processing of Human Semen: its applicability to andrology laboratories in China]. Zhonghua Nan Ke Xue. 2010 Oct;16(10):867-71.
26. Hanley JA, Negassa A, Edwardes MD, et al. Statistical analysis of correlated data using generalized estimating equations: an orientation. Am J Epidemiol. 2003 Feb 15;157(4):364-75.
27. Zhou Z, Chen L, Wu H, et al. Assisted reproductive technology in Beijing, 2013-2015. Reprod Biomed Online. 2018 Nov;37(5):521-532.

28. Rahman SM, Karmakar D, Malhotra N, et al. Timing of intrauterine insemination: an attempt to unravel the enigma. Arch Gynecol Obstet. 2011 Oct;284(4):1023-7.
29. Aydin Y, Hassa H, Oge T, et al. A randomized study of simultaneous hCG administration with intrauterine insemination in stimulated cycles. Eur J Obstet Gynecol Reprod Biol. 2013 Oct;170(2):444-8.
30. Vlahos NF, Coker L, Lawler C, et al. Women with ovulatory dysfunction undergoing ovarian stimulation with clomiphene citrate for intrauterine insemination may benefit from administration of human chorionic gonadotropin. Fertil Steril. 2005 May;83(5):1510-6.
31. Mostafa S. Mostafa, Ahmed M. El Huseiny, Soliman BS. Effect of postponing hCG injection after intrauterine insemination on pregnancy rate. Middle East Fertility Society Journal. 2014;19(3):4.
32. Antaki R, Dean NL, Lapensee L, et al. An algorithm combining ultrasound monitoring and urinary luteinizing hormone testing: a novel approach for intrauterine insemination timing. J Obstet Gynaecol Can. 2011 Dec;33(12):1248-52.
33. Moghissi KS. Prediction and detection of ovulation. Fertil Steril. 1980 Aug;34(2):89-98.
34. Bodri D, Kawachiya S, Kondo M, et al. Oocyte retrieval timing based on spontaneous luteinizing hormone surge during natural cycle in vitro fertilization treatment. Fertil Steril. 2014 Apr;101(4):1001-7 e2.
35. Kucuk T. Intrauterine insemination: is the timing correct? J Assist Reprod Genet. 2008 Aug;25(8):427-30.
36. Wilcox AJ, Weinberg CR, Baird DD. Timing of sexual intercourse in relation to ovulation. Effects on the probability of conception, survival of the pregnancy, and sex of the baby. N Engl J Med. 1995 Dec 7;333(23):1517-21.
37. Vargas-Tominaga L, Alarcon F, Vargas A, et al. Associated factors to pregnancy in intrauterine insemination. JBRA Assist Reprod. 2020 Jan 30;24(1):66-69.
38. Sicchieri F, Silva AB, Silva A, et al. Prognostic factors in intrauterine insemination cycles. JBRA Assist Reprod. 2018 Mar 1;22(1):2-7.
39. Thijssen A, Creemers A, Van der Elst W, et al. Predictive factors influencing pregnancy rates after intrauterine insemination with frozen donor semen: a prospective cohort study. Reprod Biomed Online. 2017 Jun;34(6):590-597.

Tables

Table 1 IUI cycle classification based on the time interval from insemination to ovulation in IUI-D course

	Group1	Group2	Group3	Croup4
Day1: 8:00 a.m.	UC	UC	UC	UC
Day1: 3:00 p.m.	UC	UC	UC and Ovu.	UC
Day1: 4:00 p.m.	Insem.	Insem.	Insem.	–
Day2: 8:00 a.m.	–	–	–	UC and Ovu.
Day2: 9:00 a.m.				Insem.
Day2: 11:00 a.m.	UC and not Ovu.	UC and Ovu.	–	–
Interval	≥+19h	-1~ +19 h	-8 ~ -1 h	-19 ~ -1 h
Interval	Insemination preceded ovulation by more than 19 hours	Insemination preceded ovulation by fewer than 19 hours	Ovulation preceded insemination by 1hour to 8 hours	Ovulation preceded insemination by 1 hour to 19 hours

Abbreviation: UC: ultrasound check; **Ovu:** ovulation; **Insem:** insemination; **I-O interval:** the interval between insemination and ovulation, **-** means ovulation happens before insemination, **+** means contrary situation.

Therefore, the interval from insemination to ovulation can be classified into three types: **≥+19h** (ovulation is ≥ 19h after insemination), **-1~+19 h** (ovulation is <19h after insemination), **-19~ -1 h** (ovulation is <19h before insemination) (Table 1).

Table 2 Continuous variables comparison between cycles yielding positive and negative outcomes for pregnancy

Variables [a]	Total	Pregnancy	No pregnancy	*P*-value	Live birth	No live birth	P-value
No. of cycles	2091	687	1404		592	1499	
No. of patients	1165	648	517		578	587	
Age at treatment (years)	27.8 ± 3.8	27.6 ± 3.4	27.9 ± 3.8	0.06	27.5 ± 3.6	28.0±3.8	**0.01**
Cycle number	1.8 ± 0.9	1.8 ± 0.9	1.8 ± 0.9	0.16	1.8 ± 0.9	1.8±0.9	0.40
Duration of infertility (years)	3.5 ± 2.7	3.5 ± 2.4	3.5 ± 2.8	0.51	2.4 ± 0.1	2.7±0.1	0.25
Diameter of leading follicle (mm)	19.0 ± 1.6	19.0 ± 1.6	18.9 ± 1.6	0.14	19.0 ± 1.5	19.0±1.6	0.69
Duration of COS (days) [b]	6.0 ± 3.1	6.2 ± 3.1	6.0 ± 3.1	0.36	6.1 ± 3.1	6.0±3.1	0.69
Thickness of endometrium (mm) [c]	11.0 ± 2.1	11.0 ± 2.1	10.9 ± 2.1	0.23	11.0 ± 2.0	10.9±2.1	0.26

[a] Two-tailed Student's t tests were carried out for each variable to obtain the *P*-values. Statistically significant results ($P < 0.05$) are marked in bold.

[b] This measure is only analyzed for the patients receiving controlled ovarian stimulation (COS) treatment (n = 1182).

[c] Thickness of endometrium was evaluated by ultrasound check on the day of insemination.

Abbreviation: No.: number; **COS**: controlled ovarian stimulation;

Table 3 Univariate analysis on categorical factors related to patients demographic features

Parameter	Total	CPR (%)	*P*-value	LBR (%)	*P*-value
Cycle number			0.61		0.77
<3	1658	32.6 (540/1658)		28.5 (472/1658)	
>=3	433	34.0 (147/433)		27.7 (120/433)	
Age of patient(years)			**0.04**		**0.03**
<35	1974	33.4 (659/1974)		28.8 (569/1974)	
>=35	117	23.9 (28/117)		19.7 (23/117)	
Pregnancy History			0.58		0.35
Primary infertility	1816	33.1 (601/1816)		28.7 (521/1816)	
Secondary infertility	275	31.3 (86/275)		25.8 (71/275)	
Patency of fallopian tube			0.71		0.69
Two tube	1955	32.7 (640/1955)		28.2 (551/1955)	
one tube	136	34.6 (47/136)		30.1 (41/136)	

Abbreviation: CPR: clinical pregnancy rate per cycle; **LBR**: live birth rate per cycle

Table 4 Univariate analysis on categorical factors related to ovarian stimulation

Parameter	Total	CPR (%)	*P*-value	LBR (%)	*P*-value
Method of Ovu. induction			0.08		0.21
Natural cycle	909	30.7 (279/909)		26.6 (242/909)	
Gn.	860	35.6 (306/860)		30.3 (261/860)	
CC plus Gn.	322	31.7 (102/322)		27.6 (89/322)	
Start time of Ovu. induction			1.00		0.72
<D10 of menstruation	878	34.5 (303/878)		29.3 (257/878)	
>=D10 of menstruation	304	34.5 (105/304)		30.6 (93/304)	
Number of dominant follicles			**0.03**		**0.03**
1	1667	31.7 (529/1667)		27.2 (453/1667)	
≥2	424	37.3 (158/424)		32.8 (139/424)	
Progynova Used for endometrium growth			0.34		0.19
No drug	1452	33.5 (487/1452)		29.2 (424/1452)	
Use drugs	639	31.3 (200/639)		26.3 (168/639)	
Type of endometrium			0.32		0.44
Type A or A-B	351	29.6 (104/351)		25.6 (90/351)	
Type B or B-C	1523	33.8 (514/1523)		28.7 (437/1526)	
Type C	217	31.8 (69/217)		30.0 (65/217)	
Time of HCG injection			0.92		0.90
No HCG	783	32.7 (256/783)		28.5 (223/783)	
≤24 hours before insem	968	32.6 (316/968)		27.9 (270/968)	
≥26 hours before insem	340	33.8		29.1 (99/340)	

	(115/340)

Abbreviation: **Insem**: insemination; **Ovu**.: ovulation**; Gn**: Gonadotropin; **CC**: clomiphene citrate; **HCG**: human chorionic gonadotropin; **CPR**: clinical pregnancy rate per cycle; **LBR**: live birth rate per cycle

Table 5 Univariate analysis on categorical factors related to insemination

Parameter	Total	CPR (%)	*P*-value	LBR (%)	*P-value*
I-O interval (h)			**< 0.01**		**< 0.01**
-19~-1	714	34.7 (248/714)		29.4 (210/714)	
-1~+19 h	1231	34.1 (420/1231)		29.7 (365/1231)	
≥19	146	13.0 (19/146)		11.6 (17/146)	
Drug for corpus luteum support			0.20		0.90
Progesterone only	2005	32.6 (653/2005)		28.3 (567/2005)	
Progesterone with HCG	86	39.5 (34/86)		29.1 (25/86)	
Sperm concentration (million/mL)			0.21		
≥10, 20	284	29.2 (83/284)		24.3 (69/284)	0.16
≥20, 30	1421	32.8 (466/1421)		28.4 (403/1421)	
≥30	386	35.8 (138/386)		31.1 (120/386)	

Abbreviation: h: hours; **CPR**: clinical pregnancy rate per cycle; **LBR**: live birth rate; **I-O**: insemination-ovulation

Table 6 IUI-D predictive model for clinical pregnancy rate per cycle

	OR	95%CI low	95%CI upp	P-value
(Intercept)	0.52	0.44	0.62	<0.01
Age of patient(years)				
>=35	0.63	0.39	1.02	0.06
I-O interval (h)				
-1~+19	0.97	0.80	1.18	0.77
>=19	0.29	0.17	0.48	**<0.01**
Number of dominant follicles				
≥2	1.24	0.99	1.56	0.06

Abbreviation: OR: odds ratio; **C.I.:** confidential interval; **I-O**: insemination-ovulation

Table 7 IUI-D predictive model for live birth rate per cycle

	OR	95%CI low	95%CI upp	P-value
(Intercept)	0.41	0.34	0.49	<0.01
Age of patient(years)				
>=35	0.61	0.38	0.98	0.04
I-O interval (h)				
-1~+19	1.01	0.83	1.24	0.93
>=19	0.32	0.19	0.55	**<0.01**
Number of dominant follicles				
≥2	1.27	1.01	1.60	0.04

Abbreviation: OR: odds ratio; **C.I.:** confidential interval; **I-O**: insemination-ovulation

Figures

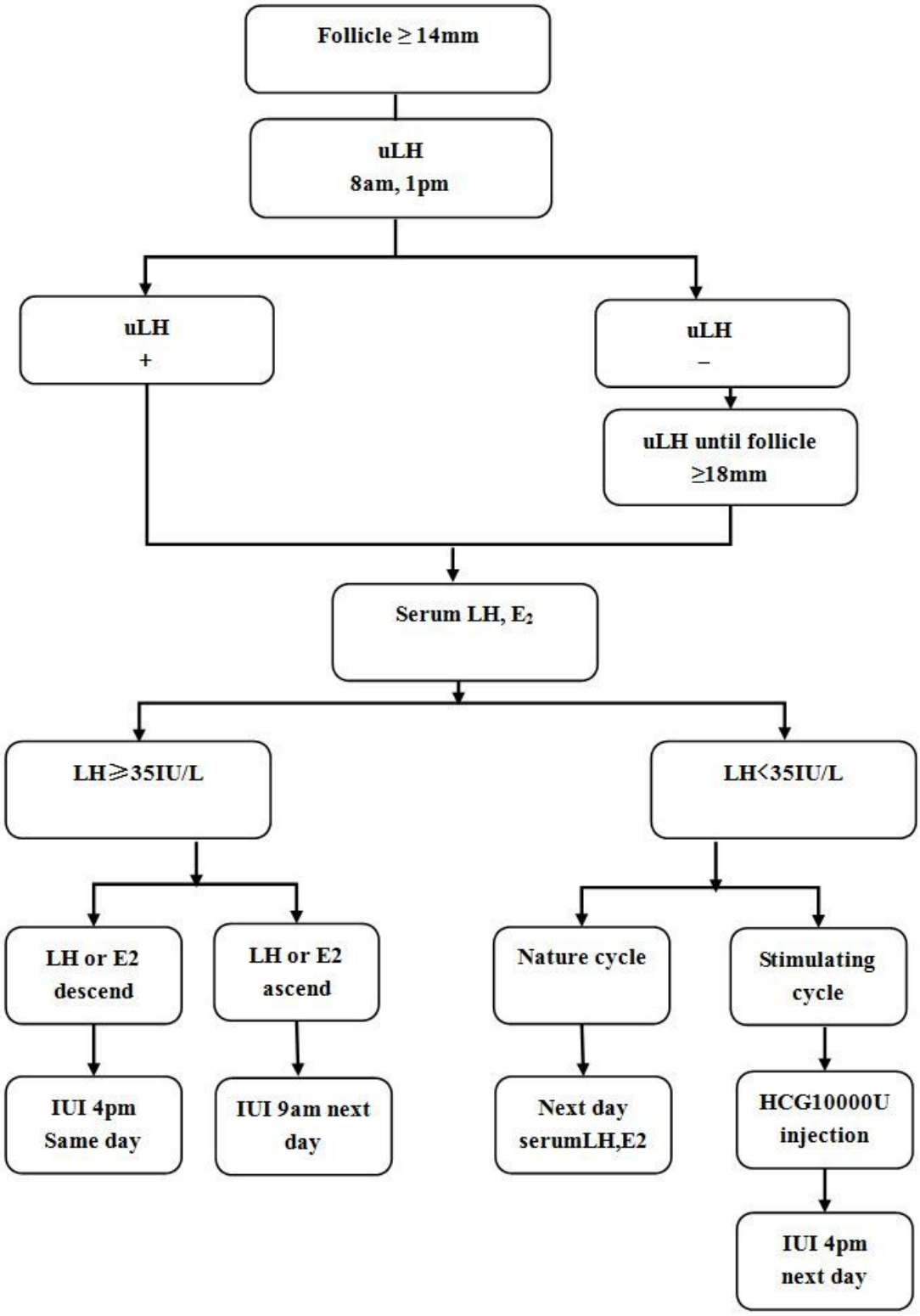

Figure 1

Algorithm for IUI timing combining dominant follicle diameter and luteinizing and estrogen hormone testing