Factors Influencing the Live Birth Rate of Fresh Embryo Transfer in Infertile Women with Endometrial Cavity Fluid: A Case Control Study

Caixin Yue

School of medicine, Cheeloo College of Medicine, Shandong University

Hong Lv

Center for reproductive medicine, Cheeloo College of Medicine, Shandong University

Jia-nan Lv

Center for reproductive medicine, Cheeloo College of Medicine, Shandong University

Lei Yan (✉ yanlei@sdu.edu.cn)

School of medicine, Cheeloo College of Medicine, Shandong University

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Abstract

Background: The pregnancy outcome of infertile patients with endometrial cavity fluid undergoing fresh embryo transfer is controversial. We aimed to build a logistic regression model including age, ovarian function and ovarian stimulation protocols to predict the live birth rate of fresh embryo transfer in patients with endometrial cavity fluid.

Methods: Patients underwent in vitro fertilization/intracytoplasmic sperm injection were selected from January 2014 to September 2020 in the Reproductive Hospital affiliated to Shandong University, and the presence of endometrial cavity fluid was indicated by ultrasound before fresh embryo transfer. A total of 2383 patients with endometrial cavity fluid suggested by ultrasound before fresh embryo transfer were enrolled in this study. Clinical and laboratory parameters that may influence live birth rate were analysed. Univariate, multivariate analysis and Receiver operating characteristic curve were conducted to evaluate the relationship between the predictors and live birth rate.

Results: The overall live birth rate was 1224/283 (51.38%), multivariate logistic analysis indicated that only transfer 2 embryos increased the probability of live birth (aOR: 2.274; 95%CI: 1.539- 3.361; $P<0.01$), other factors will reduce the chance of live birth, age ≥ 35 (aOR: 0.509; 95%CI: 0.410-0.632; $P<0.01$), history of endometritis (aOR: 0.236; 95%CI: 0.076-0.729; $P=0.012$), Gonadotropin start-up dose >150 IU (aOR: 0.785; 95% CI: 0.635-0.970; $P=0.025$), endometrial thickness on trigger day <8 mm (aOR: 0.286; 95%CI: 0.166-0.492; $P<0.01$), the gonadotropin releasing hormone agonist short regimen (aOR: 0.611; 95%CI: 0.467-0.799; $P<0.01$). In addition, we found that hydrosalpinx did not significantly affect the live birth rate after fresh embryo transfer in endometrial cavity fluid patients.

Conclusions: For infertile patients with endometrial cavity fluid before fresh embryo transfer, age <35 years, endometrial thickness ≥ 8 mm on trigger day and transfer two embryos might obtain better pregnancy outcomes, and history of endometritis, gonadotropin releasing hormone agonist short regime and Gonadotropin start-up dose >150 IU might be opposite.

Background

With the continuous development of assisted reproduction technology (ART) and embryo laboratory technology, embryo culture and transplantation technology is becoming more mature, and the role of uterine environment affecting endometrial receptivity (ER) in pregnancy has been paid more and more attention. Endometrial Cavity Fluid (ECF) is fluid containing blood, mucus, and endometrial secretions (1), the prevalence of ECF in IVF is 1-8% (2), and the generation of ECF is related to hydrosalpinx, polycystic ovary syndrome (PCOS), intrauterine subclinical infection and other factors (3). Some studies suggested that the deficiency of nutrients in ECF, the toxicity of cytokines, oxidative stress on embryos and the “washing-out” effect of fluid on embryos jointly affect the implantation rate of embryos (4), another study suggested that ECF reduced implantation rate due to the superphysiological level of estrogen (5). Due to the lack of typical clinical manifestations, ECF has been less studied, so the source of ECF and its effect

on pregnancy outcome remain unclear. Analysis of 3724 fresh embryo transfer patients showed that ECF reduced clinical pregnancy rate and live birth rate (6), while *He, R.H., et al* believed that ECF anteroposterior diameter<3.5mm had no significant influence on implantation rate and clinical pregnancy rate of in vitro fertilization-embryo transfer (IVF-ET) (7). Actually, in clinical practice, many patients with ECF chose to cancel ET due to concerns about the impact of ECF on pregnancy outcomes, which undoubtedly extended the waiting time for some patients, and time is extremely precious for in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) couples who are longing to have a child of their own.

Therefore, the purpose of this study is to improve the live birth rate of ECF patients, that is, by studying the related predictors of the live birth rate of fresh embryo transfer in ECF patients, it is expected to be helpful in the selection of ECF embryo transfer strategies, to guide the reproductive prognosis of ECF patients, and to maximize the utilization of patients' embryos, which can not only relieve psychological stress but also achieve a higher live birth rate.

Methods

Study Population

In this study, patients underwent IVF/ICSI were selected from January 2014 to September 2020 in the Reproductive Hospital affiliated to Shandong University, and the presence of ECF was indicated by ultrasound before fresh ET (Figure 1). The electronic medical records related to ART were gathered and analyzed. The inclusion and exclusion criteria for this study are as follows, inclusion criteria: age $\leq$ 40 years, ultrasound suggested endometrial line separation or ECF, eggs were collected from January 2014 to September 2020, the first cycle and fresh ET were carried out during this period, controlled ovarian stimulation (COS) protocols for patients with gonadotropin releasing hormone agonist (GnRH-a) prolong/long/short protocol and GnRH antagonist (GnRH-A) regimen. Exclusion criteria: untreated uterine malformation (septate uterus), untreated submucosa and multiple uterine leiomyomas, untreated adenomyosis, chromosomal abnormalities (number and structure of chromosomal, excluding polymorphism), performing preimplantation genetic testing (PGT) and lacking for the relevant examination or data. Patients using donated oocytes and sperm also contained in this research. Finally, a total of 2383 patients were enrolled, including 1224 live births and 1159 non-live births.

Controlled ovarian stimulation and oocyte retrieval

All patients received standard COS, oocytes retrieval, IVF/ICSI, ET and luteal support procedures. In terms of the use of COS protocols, in the live birth group: GnRH-a prolong regimen 35 (2.9%), GnRH-a long regimen 963 (78.7%), GnRH-a short regimen 121 (9.8%), GnRH-A regimen 105 (8.6%); in the non-live birth group: GnRH-a prolong regimen 48 (4.1%), GnRH-a long regimen 762 (65.7%), GnRH-a short regimen 230 (19.9%), GnRH-A regimen 119 (10.3%). In simple terms, the GnRH-a prolong regimen is a full dose (3.75mg) of long-acting GnRH α (leuprorelin acetate, Livzon) injected at 2 to 5 days of the menstrual cycle, then Gonadotropin (Gn) starts in 28-40 days or started with Gn in 28-40 days after 1-2 full dose (or half)

injections of long-acting GnRHa. The GnRH-a long regimen generally starts in mid-luteal phase, 5-7 days after ovulation or oral cycle contraceptives with 3-7 days of dose remaining, and Gn starts 13-21 days after short-acting GnRHa (leuporelin acetate, Takeda, 1.88mg st or 0.05-0.1mg qd) is injected. The GnRH-a short regimen is basically Gn initiation 1-2 days after short-acting GnRHa (0.1mg qd) is injected at 2-4 days of the menstrual cycle. In the GnRH-A regimen, Gn is initiated directly between 2 and 4 days of the menstrual cycle, but GnRH-A (Cetrorelix, Merck Serono) is added later when the largest follicles exceed 12mm. Gn dosage was determined individually according to the patient's age, body mass index (BMI), height, weight, ovarian reserve function-Antral follicle count (AFC), Anti-Mullerian Hormone (AMH), basal follicle stimulating hormone (FSH), and other factors. In the process of ovulation stimulation, the dosage and type were adjusted according to the patient's serum hormone level and the size and number of follicles monitored by ultrasound. Generally speaking, when two or three dominant follicles up to 20mm in diameter, Human chorionic gonadotropin (HCG, 4000-10000IU) was administered according to the patient's body weight, ovarian reserve, estrogen level and number of follicles (pay attention to the number of small follicles), but in the a GnRH-A regimen, generally trigger when three dominant follicle ≥ 17 mm or two dominant follicle ≥ 18 mm in diameter. Oocyte retrieval was performed under ultrasound monitoring 34-36 hours after the trigger.

Embryo transfer and luteal support

Insemination followed the conventional scheme or intracytoplasmic sperm injection or half of both are conducted 4-6 hours after follicular aspiration, depending on the sperm quality. Embryos were graded according to morphological criteria such as the number and size of blastomere and the percentage of fragmentation (8). On day 3, two high-quality embryos were selected for transplantation and the remaining embryos were further cultured to blastocysts for cryopreservation. If Day 3 had not portable embryos, we would continue to develop to the blastocyst for single blastocyst transplant and remaining blastocyst cryopreservation. Luteal support began on the day of oocyte retrieval with oral dydrogesterone (Duphaston, Abbott) 20 mg twice daily and vaginal progesterone soft capsule (Utrogestan, Besins) 200mg daily or vaginal progesterone gel (Crinone, Merck Serono) 90 mg daily and oral dydrogesterone (Duphaston, Abbott) 10 mg twice daily. Blood HCG was tested 2 weeks after ET, if HCG was positive, vaginal progesterone is continued until clinical pregnancy, and oral progesterone is used until about 10 weeks of gestation, when the placenta is functional and exogenous progesterone supplementation is not allowed.

Pregnancy outcome measures

Biochemical pregnancy is defined as serum HCG greater than 10mIU/ml at two weeks after embryo transfer. Biochemical pregnancy abortion is defined as blood HCG (+), but the pregnancy sac is not discovered and menstruated naturally. Clinical pregnancy is defined as the presence of pregnancy sac and fetal heart in the uterine cavity observed under ultrasound. Ectopic pregnancy is defined as blood HCG (+), but the pregnancy sac, fetal bud and fetal heart are not detected in the uterine cavity, and abnormal low echo area is detected outside the uterine cavity with pregnancy sac and fetal bud. Abortion

is defined as termination of pregnancy under 28 weeks of gestation, fetal weight less than 1000g, which occurred before 12 weeks as early miscarriage, occurred in 12 weeks, and latter as the late-term abortion. Live birth is defined as a fetus with vital signs delivered after 28 weeks of gestation, which occurred before 37 weeks of gestation for preterm birth, occurred in 37 weeks and latter for term birth. Endometritis was diagnosed as CD38/CD138 positive by endometrial biopsy. The sperm source in this study is the sperm source of the embryo underwent fresh ET. All patients in the study were followed regularly until the end of pregnancy.

Statistical Analysis

Data analysis was performed using SPSS 26.0 (IBM Corp, Armonk, NY, USA), continuous variables were expressed by mean $\pm$ standard deviation and calculated by independent sample *T-test*, while categorical variables were expressed by frequency and percentage and analyzed by *Chi-square* or *Fisher exact test*. Univariate regression analysis was performed for variables with $P < 0.1$, and the results were represented by P value and OR (95CI%). $P < 0.05$ was considered statistically significant. Multivariate regression analysis was carried out on significant variables to determine the relevant predictors. Receiver operating characteristic (ROC) curve was used to analyze the accuracy of the prediction model and Graphpad Prism (v.8.0; GraphPadSoftware, USA) drew the forest plot for a visual description.

Results

A total of 2383 patients with ECF suggested by ultrasound before fresh embryo transplantation were included in this study, including 1224 (51.36%) live births and 1159 (48.64%) non-live births. In the live birth group, 177 (7.43%) had preterm birth and 1,047 (43.93%) had term birth. In the non-live birth group, there were 794 cases of non-pregnancy (33.32%), 182 cases of biochemical pregnancy abortion (7.64%), 167 cases of early abortion (7.01%), 15 cases of late abortion (0.63%), and 1 case (0.04%) of induced labor due to "fetal death" at 37+6 weeks of gestation. Ectopic pregnancy occurred in 23 cases (0.97%) and intrauterine and extrauterine pregnancies at the same time occurred in 2 cases (0.08%).

The characteristics of patients were presented in Table 1. The two groups were comparable in AMH, basic FSH, Luteinizing hormone (LH), prolactin (PRL), scope of ECF, hysteroscopic surgical history, but there were statistically significant differences between the two groups in age, infertility type, basic estrogen (E_2), history of cesarean section, history of intrauterine adhesion and history of endometritis. The proportion of age < 35 in the live birth group was larger than that in the non-live birth group. In the live birth group, 621 (26.2%) had primary infertility and 603 (49.3%) had secondary infertility meanwhile in the non-live birth group, 472 (40.7%) had primary infertility and 687 (59.3%) had secondary infertility. The basal E_2 of the non-live birth group was higher than that of the live birth group and the proportion of the history of cesarean section, intrauterine adhesion and endometritis was also higher in the non-live birth group. There are differences in the two groups in BMI, unprocessed hydrosalpinx, history of endometrial polyps, but no statistical significance.

Controlled Ovarian Stimulation and IVF/ICSI Characteristics

Controlled ovulation stimulation characteristics and factors that may affect live birth are shown in Table 2. In the live birth group, 35 (2.9%) of the GnRH-a prolong regimen, 963 (78.7%) of the GnRH-a long regimen, 105 (8.6%) of the GnRH antagonist regimen, 121 (9.8%) of the GnRH-a short regimen while in the non-live birth group, 48 (4.1%) of the GnRH-a prolong regimen, 762 (65.7%) of the GnRH-a long regimen, 119 (10.3%) of the GnRH antagonist regimen, 230 (19.9%) of the GnRH-a short regimen, there was statistical difference between the two groups in the application of the COS protocols, among which there was a great difference in the application of the GnRH-a long program and the GnRH-a short program. And in the choice of way of insemination, the proportion of ICSI in the live birth group (25.7%) was higher than that in the non-live birth group (22.5%). We also found that when Gn start-up dose $\leq 150\text{IU}$, Gn total amount $\leq 2000\text{IU}$, endometrial thickness on the trigger day $\geq 8\text{mm}$, AFC >7 , transferring cleavage stage embryo and 2 embryos, higher live birth rate could be obtained. There were no significant differences between the two groups in Gn duration, E_2 levels on the trigger day, number of oocyte retrieval and sperm source of transplanted embryos. The selection of relevant predictive factors and cut-off values of the two groups was based on previously published articles (9) and the diagnosis and treatment standards of our center. As for the selection of cut-off BMI value, we referred to the BMI range recommended by Chinese experts for Chinese people (18.5-23.9 is normal, <18.5 is too low, and ≥ 24 is overweight or obesity). Cut-off value of Gn start-up dose was selected according to the average value of the two groups. Endometrial thickness on trigger day was considered to meet the transplantation requirements when endometrial thickness $>8\text{mm}$ according to the diagnosis and treatment standards of our center, although sometimes 7mm is considered to have no significant impact, and if the endometrial thickness is too thick, we suggest patients to have an examination first to exclude endometrial lesions, so we choose 0.8cm-1.4cm as the reference.

Logistic Regression and Predictions

We conducted univariate regression analysis on the factors that might affect the live birth rate, and the results were shown in Table 3. Variables with $P < 0.05$ were included in the multivariate analysis, such as age, BMI, infertility type, history of caesarean section, history of intrauterine adhesion, history of endometritis, COS protocols, Gn start-up dose, total amount of Gn, endometrial thickness on trigger day, AFC, embryo transfer period, number of ET, the results are shown in Table 4. Only transfer two embryos increased the probability of live birth (aOR: 2.274; 95% CI: 1.539-3.361; $P < 0.01$), other factors will reduce the chance of live birth, age ≥ 35 (aOR: 0.509; 95% CI: 0.410-0.632; $P < 0.01$), history of endometritis (aOR: 0.236; 95% CI: 0.076-0.729; $P = 0.012$), Gn start-up dose $> 150\text{IU}$ (aOR: 0.785; 95% CI: 0.635-0.970; $P = 0.025$), endometrial thickness on trigger day $< 8\text{mm}$ (aOR: 0.286; 95% CI: 0.166-0.492; $P < 0.01$). However, in the COS protocols, compared with the GnRH-a long regimen, the GnRH-a prolong regimen and GnRH antagonist regimen did not significantly affect the live birth rate of fresh ET in patients with ET, while the GnRH-a short regimen reduced the live birth rate (aOR: 0.611; 95% CI: 0.467-0.799; $P < 0.01$). We selected factors such as age, history of endometritis, COS protocols, Gn start-up dose, endometrial thickness on trigger day and number of embryos transfer into the logistic regression model. The P value of Hosmer-Lemshaw test

was 0.924, indicating that the model fit was good. The area under the ROC (Receiver Operating Characteristic) curve of the prediction model for live birth rate of ECF (Figure 2) is 0.662 (95%CI: 0.640-0.684). The results of multivariate analysis are shown in the forest diagram (Figure 3).

Discussion

Nowadays, with the development of assisted reproductive technology and the popularity of transvaginal sonography (TVS), more and more people choose to use vaginal ultrasound to monitor follicular development and endometrial thickness, so that the detection rate of ECF without typical clinical symptoms increases. The detection rate of ECF under TVS is about 4-14%. The generation of ECF is not only related to hydrosalpinx, PCOS, intrauterine subclinical infection, but also may be related to cervical stenosis caused by various reasons (10). However, the related factors affecting the live birth rate of ECF patients after embryo transfer are still unclear. Therefore, the purpose of this study is to identify the related risk factors and provide guidance for the reproductive prognosis of ECF patients.

This study analyzed the factors influencing the live birth rate of patients with ECF before fresh ET, in this single-center case-control study, we found that age, COS protocols, history of endometritis, Gn start-up dose, endometrial thickness on trigger day and number of ET were independent risk factors for live birth rate. When age was used as a predictor, we found that older patients (≥ 35 years) had significantly lower live birth rates than younger patients, which was confirmed by multivariate analysis. This is consistent with other literature reporting that age is an independent risk factor and a strong predictor of clinical pregnancy rate, live birth rate, miscarriage rate, and other pregnancy outcomes (11, 12).

Syndecan-1 (CD138) is a proteoglycan secreted specifically on the surface of plasma cells, many studies have confirmed the role of CD138 in the diagnosis of endometritis and believe that CD138 is a reliable indicator for the diagnosis of chronic endometritis (CE) (13, 14), CE can negatively affect the pregnancy outcome significantly (15), the secretion of IL-6 (interleukin-6), IL-1 β and TNF- α (Tumor necrosis factor- α) in CE is increased (16), and the increased cytokines may reduce ER and damage the implantation window, thus affecting the pregnancy outcome (17). *Fan, X. et al.* study believed that CD138 was a reliable indicator for predicting the outcome of embryo transfer pregnancy (18), which was consistent with our conclusion.

AFC and AMH are the most sensitive indexes to predict ovarian function, and are also the main basis for clinicians to choose COS program (19), in addition, the clinician will also consider a patient's height and weight in determining Gn starting dose. AMH was comparable between the two groups, and although AFC was different, adjusted AFC had no significant effect on live birth rate. Even so, we found that different ovarian stimulation regimens still had an impact on live birth rate. The effect of GnRH-a prolong regimens and GnRH-a on live birth rate was similar to that of the GnRH-a long regimens, but the live birth rate of the GnRH-a short regimens was low, which may be due to its poor ER. However, the effect of COS protocols on pregnancy outcome is controversial. It has been shown by *Song, J., et al.*, that live birth rate of fresh embryo can be significantly improved by GnRH-a prolong protocol compared to the GnRH-

a long protocol (20), while *Tavmergen, E., et al* suggested that GnRH-a short regimens reduce clinical pregnancy rate and live birth rate (21). In the selection of Gn start-up dose, we found that initial dose >150IU was an independent factor influencing the live birth rate of ECF patients even after adjusting for relevant factors. Two retrospective studies of 150IU and 225IU start-up dose showed that there was no statistical difference in clinical pregnancy rate and number of frozen embryos between the two groups. But a small amount of start-up was recommended for economic reasons (22, 23). A multicenter Randomized Controlled Trial (RCT) also supports women with regular menstruation should be initiated with a standard dose of 150IU (24). In addition, we also found that in ECF patients with untreated hydrosalpinx, hydrosalpinx did not significantly affect the live birth rate after fresh embryo transfer in ECF patients, which is inconsistent with previous studies showing that hydrosalpinx reduces pregnancy rate (25). The effect of COS protocols, Gn start-up dose and untreated hydrosalpinx on pregnancy outcome needs further analysis and verification.

Now, people are paying more attention to endometrial thickness. A retrospective cohort study showed that patients with endometrial thickness <8mm on HCG days had lower clinical pregnancy rates and live birth rates (26), another analysis of 10787 fresh ET patients showed that endometrial thickness (cut-off value of 8mm) on HCG day was an independent factor in predicting clinical pregnancy rate and live birth rate (27), which coincide with our findings. However, the mechanism of how endometrium affects pregnancy outcome remains unclear. One hypothesis is that it causes E₂ receptor abnormalities as with clomiphene citrate (CC) (28), another hypothesis is that it has to do with high oxygen tension, an environment of high oxygen or oxygen metabolites is not conducive to embryo growth (29). The functional layer of endometrium has capillary network and the basal layer has spiral arteries, in patients with thin endometrium, the functional layer is thin or even absent, and the transplanted embryo will be closer to the basal layer with high oxygen tension, thus affecting the pregnancy outcome (28), further studies are needed to prove the mechanism.

We also found that the number of embryos transfer is also one of the influencing factors. Two embryos transferred have a higher chance of live birth. Although transfer of two embryos significantly increase the live birth rate, it also increase the incidence of multiple pregnancies (30), multiple pregnancies can increase the incidence of adverse pregnancy complications and even death for both mother and fetus (31). Comprehensive consideration should be taken in clinical work to maximize the benefit of patients.

Conclusions

In conclusion, by analyzing the factors influencing the live birth rate of ECF patients during fresh embryo transfer, age <35 years, endometrial thickness ≥ 8 mm on trigger day and transfer two embryos might obtain better pregnancy outcomes, and history of endometritis, gonadotropin releasing hormone agonist short regime and Gonadotropin start-up dose >150IU might be opposite. In addition, we found that hydrosalpinx did not significantly affect the live birth rate after fresh embryo transfer in endometrial cavity fluid patients. This study has certain guiding significance for clinical practice, which can avoid excessive treatment such as aspiration and prevent some patients waste unnecessary waiting time by

cancelling ET due to the fear of affecting the pregnancy outcome. Of course, this study is a single-center retrospective study, and it occurs selection bias inevitably, especially in the selection of COS protocols, patients' tendency to choose COS regimens due to social and psychological factors may also be related confounding factors, which cannot be avoided in this study. We only analyzed the related factors affecting the live birth rate of ECF patients, and did not conduct a subgroup analysis on the amount of ECF, which is also a problem we will solve later. The relevant conclusions of this study still need to be verified by prospective, multi-center clinical trials.

List Of Abbreviations

ART: assisted reproduction technology; ER: endometrial receptivity; ECF: Endometrial Cavity Fluid; PCOS: polycystic ovary syndrome; IVF-ET: in vitro fertilization-embryo transfer; IVF/ICSI: in vitro fertilization/intracytoplasmic sperm injection; COS: controlled ovarian stimulation; GnRH-a: gonadotropin releasing hormone agonist; GnRH-A: GnRH antagonist; PGT: preimplantation genetic testing; Gn: Gonadotropin; BMI: body mass index; AFC: Antral follicle count; AMH: Anti-Mullerian Hormone; FSH: basal follicle stimulating hormone; HCG: Human chorionic gonadotropin; LH: Luteinizing hormone; PRL: prolactin; E2: estrogen; TVS: transvaginal sonography; CE: chronic endometritis; TNF :Tumor necrosis factor; IL: interleukin; RCT: Randomized Controlled Trial; CC: clomiphene citrate

Declarations

Ethics approval

The research has been approved by the Ethics Committee of the Reproductive Hospital affiliated to Shandong University.

Consent for publication

Not applicable

Availability of data and materials

The datasets used in this study are available from the corresponding author on reasonable request.

Competing interests

All authors declare that they have no conflict of interest, whether of a financial or other nature.

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Authors' contributions

Lei Yan contributed to the design and guidance of the study. Data collection were by Caixin Yue, Jia-nan Lv and Hong Lv, statistical analysis was by Caixin Yue, and Caixin Yue drafted the manuscript. All authors meet the final approval of the version to be published.

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Tables

Table 1 Demographic and baseline characteristics of two group

Characteristic		Live birth (n=1224)	Non-Live birth (n=1159)	P value
Age (years)	<35, n(%)	1033(84.4%)	794(68.51%)	<.001
	≥35, n(%)	191(15.6%)	365(31.49%)	
BMI (kg/m2)	<18.5, n(%)	89(7.3%)	59(5.1%)	<.054
	18.5–23.99, n(%)	799(65.3%)	753(64.9%)	
	≥24, n(%)	336(27.4%)	347(30.0%)	
Type of infertility	Primary infertility, n(%)	621(26.2%)	472(40.7%)	<.001
	Secondary infertility, n(%)	603(49.3%)	687(59.3%)	
AMH(ng/ml)		3.77±2.81	3.88±21.2	.852
Basal hormone level	FSH(IU/L)	6.96±1.86	7.09±2.25	.108
	LH(IU/L)	5.61±2.79	5.56±4.52	.737
	E2(pg/ml)	45.73±74.29	52.77±93.98	.043
Prolactin, (ng/ml)		16.89±8.71	17.35±17.39	.411
Endometrial cavity fluid scope	local, n(%)	90(7.4%)	90(7.8%)	.703
	Whole layer, n(%)	1134(92.6%)	1069(92.2%)	
Cesarean section history	No, n(%)	1191(97.3%)	1100(94.9%)	.002
	Yes, n(%)	33(2.7%)	59(5.1%)	
Untreated hydrosalpinx	No, n(%)	1176(96.1%)	1096(94.6%)	.080
	Yes, n(%)	48(3.9%)	63(5.4%)	
Intrauterine adhesion history	No, n(%)	1176(96.1%)	1089(94.0%)	.017
	Yes, n(%)	48(3.9%)	70(6.0%)	
Endometrial polyp history	No, n(%)	924(75.5%)	911(78.6%)	.071
	Yes, n(%)	300(24.5%)	248(21.4%)	
Endometritis history	No, n(%)	1220(99.7%)	1141(98.4%)	.002
	Yes, n(%)	4(0.3%)	18(1.6%)	
hysteroscopic surgery history	No, n(%)	893(72.9%)	859(74.1%)	.522
	Yes, n(%)	331(27.1%)	300(25.9%)	

BMI: Body mass index AMH: Anti-Mullerian Hormone FSH: Follicle stimulating hormone

LH: luteinizing hormone E2: estradiol

Table 2 Controlled ovarian stimulation and IVF/ICSI cycles characteristics

Characteristic		Live birth (n=1224)	Non-Live birth (n=1159)	P value
Ovarian stimulation protocol	GnRH-a long regime, n(%)	963(78.7%)	762(65.7%)	<.001
	GnRH-a prolong regime, n(%)	35(2.9%)	48(4.1%)	
	GnRH-A regimen, n(%)	105(8.6%)	119(10.3%)	
	GnRH-a short regime, n(%)	121(9.8%)	230(19.9%)	
Fertilization method	IVF, n(%)	898(73.4%)	894(77.1%)	.028
	ICSI, n(%)	314(25.7%)	261(22.5%)	
	IVF/ICSI, n(%)	12(0.9%)	4(0.4%)	
Gn duration(days)	≤11, n(%)	795(64.9%)	781(67.4%)	.209
	>11 , n(%)	429(35.1%)	378(32.6%)	
Gn start-up dose(IU)	≤150, n(%)	870(71.1%)	673(58.1%)	<.001
	>150, n(%)	354(28.9%)	486(41.9%)	
Total Gn dose(IU)	≤2,000, n(%)	742(60.6%)	617(53.2%)	<.001
	>2,000, n(%)	482(39.4%)	542(46.8%)	
Endometrial thickness on trigger day(mm)	<8, n(%)	20(1.6%)	77(6.6%)	<.001
	8-14, n(%)	1120(91.5%)	1025(88.4%)	
	>14, n(%)	84(6.9%)	57(5.0%)	
Estradiol on trigger day(pg/ml)	<2,000, n(%)	283(23.1%)	296(25.5%)	.346
	2000-3000, n(%)	379(30.9%)	338(29.2%)	
	>3000, n(%)	562(46.0%)	525(45.3%)	
AFC(no.)	≤7, n(%)	117(9.6%)	184(15.9%)	<.001
	>7, n(%)	1107(90.4%)	975(84.1%)	
Oocyte retrieval(no.)	≤15, n(%)	1098(89.7%)	1022(88.2%)	.235
	>15, n(%)	126(10.3%)	137(11.8%)	
Stage of embryo transfer	Cleavage, n(%)	1009(82.4%)	844(72.8%)	<.001
	Blastocyst, n(%)	215(17.6%)	315(27.2%)	

Number of embryo transfer	1, n(%)	252(20.6%)	400(34.5%)	<.001
	2, n(%)	972(79.4%)	759(65.5%)	
Sperm origin	husband, n(%)	1134(92.6%)	1092(94.2%)	.122
	donor, n(%)	90(7.4%)	67(5.8%)	

GnRH-a: gonadotropin releasing hormone agonist GnRH-A: GnRH antagonist

ICSI: intracytoplasmic sperm injection AFC: antral follicle count IVF: in vitro fertilization

Table 3 Univariate regression analysis of predictors influencing live birth rate

BMI: Body mass index Gn: gonadotropin GnRH-a: gonadotropin releasing hormone agonist

GnRH-A: GnRH antagonis ICSI: intracytoplasmic sperm injection IVF: in vitro fertilization

Table 4 Multivariate regression analysis of predictors influencing live birth rate

predictors		OR(95% CI)	P value
Age (years)	<35	1	<.001
	≥35	0.042(0.330-0.490)	
BMI (kg/m2)	18.5–23.99	1	.045
	<18.5	1.422(1.008-2.005)	
	≥24	0.913(0.762-1.093)	
Type of infertility	Primary infertility	1	<.001
	Secondary infertility	0.667(0.567-0.785)	
Basel estradiol (pg/ml)		0.999(0.998-1.00)	.051
Cesarean section history	No	1	.003
	Yes	0.517(0.335-0.797)	
Untreated hydrosalpinx	No	1	.081
	Yes	0.710(0.483-1.043)	
Intrauterine adhesion history	No	1	.018
	Yes	0.635(0.436-0.925)	
Endometrial polyp history	No	1	.071
	Yes	1.193(0.985-1.444)	
Endometritis history	No	1	.005
	Yes	0.208(0.070-0.616)	
Ovarian stimulation protocol	GnRH-a long regime	1	.016
	GnRH-a prolong regime	0.577(0.369-0.901)	
	GnRH-A regimen	0.698(0.528-0.923)	
	GnRH-a short regime	0.416(0.328-0.529)	
Fertilization method	IVF	1	.061
	ICSI	1.198(0.992-1.446)	
	IVF/ICSI	2.987(0.960-9.295)	
Gn start-up dose(IU)	≤150	1	<.001
	>150	0.563(0.475-0.668)	
Total Gn dose(IU)	≤2,000	1	<.001

	>2,000	0.739(0.628-0.870)	
Endometrial thickness	8-14	1	
on trigger day(mm)	<8	0.238(0.144-0.392)	<.001
	>14	1.349(0.953-1.908)	.091
Antral follicle count(no.)	≤7	1	<.001
	>7	1.786(1.394-2.286)	
Stage of embryo transfer	Cleavage	1	<.001
	Blastocyst	0.571(0.469-0.695)	
Number of embryo transfer	1	1	<.001
	2	2.033(1.691-2.443)	

predictors		OR(95% CI)	P value
Age (years)	<35	1	<.001
	≥35	0.509(0.410-0.632)	
BMI (kg/m2)	18.5–23.99	1	.177
	<18.5	1.281(0.894-1.835)	
	≥24	1.149(0.944-1.399)	
Type of infertility	Primary infertility	1	.606
	Secondary infertility	0.954(0.796-1.142)	
Cesarean section history	No	1	.882
	Yes	1.037(0.643-1.671)	
Intrauterine adhesion history	No	1	.454
	Yes	1.177(0.768-1.804)	
Endometritis history	No	1	.012
	Yes	0.236(0.076-0.729)	
Ovarian stimulation protocol	GnRH-a long regime	1	.285
	GnRH-a prolong regime	0.771(0.479-1.242)	
	GnRH-A regimen	0.829(0.617-1.114)	
	GnRH-a short regime	0.611(0.467-0.799)	
Gn start-up dose(IU)	≤150	1	.025
	>150	0.785(0.635-0.970)	
Total Gn dose(IU)	≤2,000	1	.418
	>2,000	0.418(0.923-0.760)	
Endometrial thickness on trigger day(mm)	8-14	1	<.001
	<8	0.286(0.166-0.492)	
	>14	1.260(0.879-1.805)	
Antral follicle count(no.)	≤7	1	.132
	>7	1.234(0.938-1.623)	
Stage of embryo transfer	Cleavage	1	.216
	Blastocyst	1.302(0.857-1.979)	

Number of embryo transfer	1	1	<.001
	2	2.274(1.539-3.361)	

BMI: Body mass index GnRH-A: GnRH antagonis Gn: gonadotropin

GnRH-a: gonadotropin releasing hormone agonist

Figures

Figure 1

Ultrasonic manifestation of endometrial cavity fluid

Figure 2

Figure 2

Receiver operating characteristic (ROC) curve

FIGURE 3

Figure 3

Forest plot to show the results of multivariate analysis