Optimal Endometrial Preparation Protocols for Frozen-thawed Embryo Transfer Cycles by Maternal Age

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Research

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Abstract

Background: It is paramount to consider the appropriate preparation of the endometrium to receive the transferred embryos as the amount of frozen embryo transfer (FET) cycles is increasing worldwide. However, there remains lack of evidence about what is the most optimal protocol of endometrial preparation regarding pregnancy outcomes in different subgroup of infertile women. This retrospective cohort study was aim to explore the best endometrial preparation protocols among different maternal age groups.

Methods: A total of 16870 FET cycles were categorized into three groups based on endometrial preparation protocols: Natural cycle (NC n=3893), artificial cycles (AC, n=11459) and AC with pretreatment with GnRH-a (AC+GnRH-a, n=1518). Logistic regression was performed to investigate the independent effect of endometrial preparation protocols on IVF pregnancy outcomes. Subgroup analyses were conducted to evaluate the most optimal endometrial preparation protocols for different maternal age groups.

Results: In overall populations, after controlling for potential confounders, the incidence of live birth (NC as reference; AC: adjusted odds ratio (aOR) =0.840, 95%CI 0.774-0.912; AC+GnRHa: aOR=0.907, 95%CI 0.795-1.034) in NC was significantly higher than that of AC, while comparable to that of AC+GnRH-a. The early miscarriage rate (AC: aOR=1.413, 95%CI 1.220-1.638; AC+GnRHa: aOR=1.537, 95%CI 1.232-1.919) was significantly lower in NC compared to either AC group. In younger women, the live birth rates (AC: aOR=0.894, 95%CI 0.799-1.001; AC+GnRHa: aOR=1.111, 95%CI 0.923-1.337) were comparable between the three groups, with a slightly higher in AC+GnRH-a. Early miscarriage rate was only significantly lower in NC compared to that of AC without GnRH-a (aOR=1.452, 95%CI 1.159-1.820). While in older women, the incidence of live birth (AC: aOR=0.811, 95%CI 0.718-0.916; AC+GnRHa: aOR=0.760, 95%CI 0.626-0.923) was significantly higher, and early miscarriage (AC: aOR=1.358, 95%CI 1.114-1.655; AC+GnRHa: aOR=1.717, 95%CI 1.279-2.305) was significantly lower in NC compared to those of two AC groups.

Conclusions: NC protocol is associated with lower early miscarriage late in overall IVF population. There is a mild favor of AC+GnRH-a in younger women, while the priority of NC is remarkable in older women. Maternal age should be a considerable factor when determine endometrial preparation method for FET.

Introduction

Since the first report on successful pregnancy after frozen-thawed embryo transfer (FET) in 1983, the amount of FET has been increasing worldwide, particularly driven by the advanced vitrification technique allowing safe and efficient cryopreservation, storage and warming of embryos [1, 2]. Current evidence indicates that FET cycles not only produce non-inferior live birth rate to fresh cycles, but reduce multiple pregnancy rate by selecting single good-quality blastocyst for transfer, and minimize the risk of ovarian hyperstimulation syndrome [3–5]. Regardless, the optimal method of endometrial preparation remains a topic of ongoing debate.

The major endometrial preparation protocols can be generally classified into natural cycles (NC) and artificial cycles (AC). In NC, follicle development and ovulation are required to maintain the physiological hormonal milieu for endometrial growth and embryo implantation. Alternatively, in AC, this physiological process is overridden by the administration of exogenous estrogen and progesterone. AC with GnRH-a pretreatment is applied to minimize the risk of premature ovulation and prevent cycle cancelation [6], it was also reported to increase live birth in patients with adenomyosis [7, 8].

In clinical practice, AC is increasing adopted as NC may not be possible in patients with ovulatory disorders. Additionally, AC provides a better control of FET timing and transfer, which is convenient for both patients and physicians. However, questions remain unanswered whether AC is equivalent to NC regarding pregnancy outcomes and safety, as several recent publications have reported a higher risk of hypertensive disorders in the absence of a corpus luteum [9–11]. In this study, we aim to compare pregnancy outcomes after different endometrial preparation protocols in FET cycles, and to explore the optimal protocols in different age groups.

Materials And Methods

Study Design and Population

This was a retrospective cohort study of women underwent IVF/ICSI treatment in our fertility center from January 2015 to June 2019. Excluding criteria included cycles with no viable embryos available for transfer or underwent Pre-implantation genetic diagnosis, and cycles in which transferred embryos came from different ovarian stimulation cycles, or mixed with cleavage and blastocyst stage embryos. The eligible cycles were classified into three groups according to the endometrial preparation protocols: natural cycle (NC), artificial cycle (AC) without GnRH-a, and AC with GnRH-a pretreatment (AC + GnRH-a).

Cryopreservation and thawing

Standard regimens for controlled ovarian stimulation (COH) were applied by clinicians in our center based on the individual ovarian reserve and response, including long agonist protocols, antagonist protocols, and clomiphene-based mild stimulation protocols. Ovulation was triggered with either human chorionic gonadotropin (hCG, Lizhu; Zhuhai, China) 6 500 – 10 000 IU or a single subcutaneous bolus of triptorelin (Diphereline; Ipsen, France) 0.2 mg, oocyte retrieval was performed 34–36 hours after. Insemination of mature oocytes was performed by conventional IVF or ICSI according to the sperm parameters. Details on embryo culture, vitrification, thawing and transfer procedures have been described in our previous studies [12, 13]. Embryos on Day 3 were graded according to the morphology criteria [14]. Good and fair embryos were cryopreserved or undergoing blastocyst culture. The Gardner grading system was used to evaluate the blastocyst quality [15]. Only blastocysts better than grade 3CC were selected for vitrification. The laboratory procedures were performed by well-trained embryologists, each with over

5 years of laboratory experience. There were no substantial changes of laboratory practices over the course of study.

Endometrial preparation protocols

The patients were allocated to different endometrial preparation protocols based on the experience of the clinician and patients' characteristic. NC was chosen if the patient had regular menses or refused to take medication, AC with or without GnRH-a was selected in patients with irregular menses, or who lived at considerable distance and did not wish to be frequently monitored.

NC: Surveillance of the cycle was done from day 8–10 of the cycle with vaginal ultrasound until the leading follicle was ≥ 18 mm or the urine LH surge was observed. Ovulation may occur spontaneously or triggered by 10 000 IU hCG (Choriomon, IBSA, Lugano, Switzerland). Oral administration of 20 mg progesterone twice daily was prescribed for 3/5 days before FET of cleavage/blastocyst stage embryo.

AC: endometrial preparation was started from day 2–3 of menstrual cycle with daily administration of 4–8 mg oral estradiol valerate (Progynova; Bayer, Germany) for 15 days. Intramuscular administration of 60 mg progesterone daily (ZheJiang XianJu Pharmaceuticals, China) was prescribed for 4/6 days before FET of cleavage/blastocyst stage embryo.

AC + GnRH-a: The injection of leuproreline acetate (Diphereline; Ipsen, France) 3.75 mg i.m., was administered during the mid-luteal phase of the menstrual cycle, twenty-nine days later the hormone replacing protocol as in AC was started.

In all three protocols, serum estradiol (E2), progesterone (P) levels, and endometrium thickness were measured on hCG day in NC or on progesterone day in AC. One-two blastocysts or 1–3 cleavage stage embryos were transferred under ultrasound guidance. From the day of embryo transfer. Luteal support was prescribed with 20 mg progestin tablets (Duphaston; Abbott, Netherlands) orally twice daily and 90 mg progestin gel (Crinone; Merck, Germany) vaginally daily until a serum beta hCG assay was performed 11 (if blastocyst was transferred) or 13 days (if cleavage embryo was transferred) after FET. Luteal support was continued to 12 weeks of gestation if pregnancy was resulted.

Definition of clinical outcomes

Clinical pregnancy was defined as the observation of at least one gestational sac on transvaginal ultrasound at 6–7 weeks of gestation. Ongoing pregnancy was defied as a pregnancy proceeding beyond 12 weeks of gestation. Early miscarriage was defined as the pregnancy loss before 12 weeks of gestation. Live birth was defined as an infant born alive after 28 weeks of gestation.

Statistical analysis

The demographic characteristics and clinical outcomes were described as mean ± SD for continuous variables and as frequency with proportion for categorical variables. The differences between groups were tested using the ANOVA test for continuous variables and the Pearson's chi-square test for categorical variables. Multivariable logistic regression was performed to investigate the effect of endometrial preparation protocol on pregnancy outcome after controlling for potential confounders, endometrial preparation method group was included as a categorical variable, and NC was selected as the reference. The results were reported as adjusted odds ratios (aORs) with 95% CIs. Subgroup analysis stratified by maternal age (<35 year or ≥ 35 year) was performed to explore the most optimal endometrial preparation protocol for different age groups. All statistical analyses were performed by using the two-sided 5% level of significance in the statistical package Stata, Version 19 (StataCorp, College Station, TX, USA).

Results

Totally there were 16870 FET cycles included in the study: 3893 NC cycles, 11459 AC cycles without GnRH-a and 1518 AC cycles with GnRH-a pretreatment. The demographic data were shown in Table I. It is of note that the maternal age in the Group of AC cycles without GnRH-a was about 2 years younger than the other two groups. When stratified by maternal age, there were 9341 cycles with younger age (<35 years), and 7529 cycles with advanced age (≥ 35 years).

Table I
Maternal and treatment characteristics between different endometrial preparation protocols in overall population.

	NC	AC	AC+GnRH-a	*P*
cycles (n)	3893	11459	1518	
maternal age (y)	35.15±4.57	33.51±4.77	35.22±4.97	0.000
cycle rank (n)	1.83±1.10	1.58±0.95	1.85±1.19	0.000
BMI (kg/m^2)	21.28±2.81	21.61±3.07	21.77±3.28	0.000
Nulliparity (%)	1595(40.97)	5105(44.55)	656(43.21)	0.000
Duration of infertility (y)	3.67±3.02	3.57±2.80	4.12±3.31	0.000
Indication of treatment, n (%)				0.000
Pelvic	1379(35.42)	3896(34.00)	495(32.61)	
Ovulatory disorder	38(0.98)	1085(9.47)	100(6.59)	
Endometriosis	331(8.50)	709(6.19)	234(15.42)	
Immunology	60(1.54)	148(1.29)	13(0.86)	
Male	623(16.00)	1596(13.93)	181(11.92)	
Combined	568(14.59)	1640(14.31)	215(14.16)	
Idiopathic	894(22.96)	2385(20.81)	280(18.45)	
Fertilization method, n (%)				0.000
IVF	2640(67.81)	8139(71.03)	1063(70.03)	
ICSI	1235(31.72)	3211(28.02)	444(29.25)	
IVF+ICSI	18(0.46)	109(0.95)	11(0.72)	
Embryo stage, n (%)				0.000
D3	1484(38.12)	3537(30.87)	731(48.16)	
D5	2409(61.88)	7922(69.13)	787(51.84)	
Number of embryos transferred (n)	1.70±0.63	1.73±0.63	1.91±0.65	0.000
Number of top embryos (n)	1.32±0.88	1.36±0.88	1.52±0.94	0.000
Endometrium thickness (mm)	9.90±1.74	9.42±1.54	9.86±1.74	0.000
E2 (pg/ml)	401.27±360.76	440.87±491.23	428.81±527.90	0.000
P (ng/ml)	0.56±0.36	0.29±0.21	0.27±0.21	0.000

NC, natural cycle; AC, artificial cycle; GnRH-a, gonadotropin releasing hormone agonist; BMI, body mass index; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; E2, estrogen; P, progesterone

P <0.05 was considered as statistically significant

The IVF/ICSI pregnancy outcomes per FET cycle for the three groups in overall population are showed in Figure I. The results of logistic regression are shown in Table II. After controlling for potential confounders, the incidences of clinical pregnancy (NC as reference; AC: adjusted odds ratio (aOR) = 0.923, 95%CI 0.852-1.000; AC + GnRH-a: aOR = 1.008, 95%CI 0.855–1.110) were comparable between the three groups. The incidences of live birth (AC: aOR = 0.840, 95%CI 0.774–0.912; AC + GnRH-a: aOR = 0.907, 95%CI 0.795–1.034) and ongoing pregnancy (AC: aOR = 0.837, 95%CI 0.771–0.901; AC + GnRH-a: aOR = 0.891, 95%CI 0.782–1.016) in NC were significantly higher than that of AC, while comparable to that of AC + GnRH-a. The early miscarriage rate (AC: aOR = 1.413, 95%CI 1.220–1.638; AC + GnRH-a: aOR = 1.537, 95%CI 1.232–1.919) was significantly lower in NC compared to either AC group.

Table II
Crude and adjusted ORs of different endometrial preparation protocols in overall population.

	NC	AC	P_1	AC+GnRH-a	P_2
Clinical pregnancy rate					
Crude OR(95%CI)	reference	1.157(1.075,1.244)	0.000	1.009(0.896,1.136)	0.884
Adjusted OR(95%CI)	reference	0.923(0.852,1.000)	0.054	1.008(0.855,1.110)	0.905
Ongoing pregnancy rate					
Crude OR(95%CI)	reference	1.068(0.992,1.149)	0.082	0.906(0.802,1.022)	0.108
Adjusted OR(95%CI)	reference	0.837(0.771,0.901)	0.000	0.891(0.782,1.016)	0.085
Live birth rate					
Crude OR(95%CI)	reference	1.057(0.981,1.138)	0.145	0.911(0.806,1.029)	0.133
Adjusted OR(95%CI)	reference	0.840(0.774,0.912)	0.000	0.907(0.795,1.034)	0.144
Early miscarriage rate					
Crude OR(95%CI)	reference	1.198(1.042,1.376)	0.011	1.451(1.173,1.796)	0.001
Adjusted OR(95%CI)	reference	1.413(1.220,1.638)	0.000	1.537(1.232,1.919)	0.000

NC, natural cycle; AC, artificial cycle; GnRH-a, gonadotropin releasing hormone agonist

P_1: AC vs. NC, P_2: AC+GnRH-a vs. NC

P <0.05 was considered as statistically significant

The pregnancy outcomes stratified by maternal age are shown in Figure II. Logistic regression indicated that in younger women (Table III), the live birth (AC: aOR = 0.894, 95%CI 0.799–1.001; AC + GnRH-a: aOR = 1.111, 95%CI 0.923–1.337) and clinical pregnancy rates (AC: aOR = 0.976, 95%CI 0.870–1.095; AC + GnRH-a: aOR = 1.197, 95%CI 0.988–1.450) were comparable between the three groups, although slightly higher in AC + GnRH-a. Ongoing pregnancy rate (AC: aOR = 0.889, 95%CI 0.794–0.995; AC + GnRH-a: aOR = 1.074, 95%CI 0.891–1.204) was only significantly higher, and early miscarriage rate (AC: aOR = 1.452, 95%CI 1.159–1.820; AC + GnRH-a: aOR = 1.317, 95%CI 0.929–1.868) was only significantly lower in NC compared to that of AC. While in older women (Table IV), except clinical pregnancy rates (AC: aOR = 0.901, 95%CI 0.804–1.010; AC + GnRH-a: aOR = 0.903, 95%CI 0.756–1.077) were comparable across groups, the incidences of live birth (AC: aOR = 0.811, 95%CI 0.718–0.916; AC + GnRHa: aOR = 0.760, 95%CI 0.626–0.923) and ongoing pregnancy (AC: aOR = 0.881, 95%CI 0.719–0.915; AC + GnRH-a: aOR = 0.765, 95%CI 0.632–0.926) were significantly higher, and early miscarriage (AC: aOR = 1.358, 95%CI 1.114–1.655; AC + GnRH-a: aOR = 1.717, 95%CI 1.279–2.305) was significantly lower in NC compared to those of two AC groups.

Table III
Crude and adjusted ORs of different endometrial preparation protocols in younger women.

	NC (n=1783)	AC (n=6870)	P_1	AC+GnRH-a (n=688)	P_2
Clinical pregnancy rate					
Crude OR(95%CI)	reference	1.019(0.981,1.213)	0.109	1.205(1.005,1.446)	0.044
Adjusted OR(95%CI)	reference	0.976(0.870,1.095)	0.682	1.197(0.988,1.450)	0.067
Ongoing pregnancy rate					
Crude OR(95%CI)	reference	0.982(0.885,1.090)	0.737	1.087(0.911,1.296)	0.356
Adjusted OR(95%CI)	reference	0.889(0.794,0.995)	0.041	1.074(0.891,1.204)	0.455
Live birth rate					
Crude OR(95%CI)	reference	0.973(0.877,1.080)	0.61	1.100(0.922,1.312)	0.29
Adjusted OR(95%CI)	reference	0.894(0.799,1.001)	0.051	1.111(0.923,1.337)	0.267
Early miscarriage rate					
Crude OR(95%CI)	reference	1.410(1.135,1.751)	0.002	1.300(0.924,1.830)	0.132
Adjusted OR(95%CI)	reference	1.452(1.159,1.820)	0.001	1.317(0.929,1.868)	0.122

NC, natural cycle; AC, artificial cycle; GnRH-a, gonadotropin releasing hormone agonist

P_1: AC vs. NC, P_2: AC+GnRH-a vs. NC

P <0.05 was considered as statistically significant

Table IV
Crude and adjusted ORs of different endometrial preparation protocols in older women.

	NC (n=2110)	AC (n=4589)	P_1	AC+GnRH-a (n=830)	P_2
Clinical pregnancy rate					
Crude OR(95%CI)	reference	1.003(0.904,1.113)	0.957	0.879(0.747,1.036)	0.124
Adjusted OR(95%CI)	reference	0.901(0.804,1.010)	0.073	0.903(0.756,1.077)	0.257
Ongoing pregnancy rate					
Crude OR(95%CI)	reference	0.904(0.809,1.009)	0.072	0.754(0.631,0.901)	0.002
Adjusted OR(95%CI)	reference	0.811(0.719,0.915)	0.001	0.765(0.632,0.926)	0.006
Live birth rate					
Crude OR(95%CI)	reference	0.896(0.801,1.002)	0.054	0.750(0.626,0.898)	0.002
Adjusted OR(95%CI)	reference	0.811(0.718,0.916)	0.001	0.760(0.626,0.923)	0.006
Early miscarriage rate					
Crude OR(95%CI)	reference	1.317(1.091,1.590)	0.004	1.685(1.271,1.235)	0.000
Adjusted OR(95%CI)	reference	1.358(1.114,1.655)	0.002	1.717(1.279,2.305)	0.000

NC, natural cycle; AC, artificial cycle; GnRH-a, gonadotropin releasing hormone agonist

P_1: AC vs. NC, P_2: AC+GnRH-a vs. NC

P <0.05 was considered as statistically significant

Discussion

It is paramount to consider the appropriate preparation of the endometrium to receive the transferred embryos as the amount of FET cycles is increasing worldwide. Our study demonstrated that NC is superior to AC regarding pregnancy outcomes in general IVF population. To the best of our knowledge, our study is the first to evaluate the optimal endometrial preparation protocols in different age groups and concludes that there is a mild favor of AC + GnRH-a in younger women, but a remarkable priority of NC in older women.

There is no doubt that the two most important factors for a successful pregnancy are the availability of a good quality, euploid embryo, a receptive endometrium and synchrony of these factors. In FET, endometrium receptivity is achieved by dedicated endometrial preparation protocols, which can largely be

divided into natural and artificial cycles. In NC, usually solely menstrual cycle monitoring is performed without any pharmacological intervention prior to ovulation, which may be spontaneous (true NC) or triggered by HCG (modified NC). However, the timing of ovulation in NC may pose scheduling difficulties, and premature ovulation may occur and increase cancellation rates. In AC, exogenous hormone is administered to prepare the endometrium for embryo implantation, with exogenous to prime the endometrium, while progesterone to complete endometrial maturation. AC with GnRH-a pretreatment offers the most control over the timing and minimize the risk of premature ovulation, but the cycle is much more prolonged and expensive.

Several studies have compared NC and AC cycles in endometrial preparation, unfortunately the results have been conflicting. Two large scale meta-analyses comparing different cycle regimens of FET failed to show a superiority of one approach over the others in terms of reproductive outcomes, however, the majority of studies included were often of a low or very low quality of evidence as most were retrospective nature with limited sample size, and some fail to report important clinical outcomes, or had a poor reporting of study methods [6, 16]. A retrospective cohort study of 1265 cycles revealed that the implantation rate was significantly higher in NC, while there were no significant differences between the groups in the clinical pregnancy, ongoing pregnancy, live birth, and miscarriages rate [17]. El-Toukhy T and coworkers conducted a prospective randomized trial of 234 patients and found that using GnRH-a prior to exogenous steroid supplementation for endometrial preparation achieved significantly higher clinical pregnancy (24% vs 11.3%, OR 2.5, 95%CI 1.2–5.5) and live birth rates (20% vs 8.5%, OR 2.9, 95%CI 1.2-8). Our study suggests that in overall population, the incidence of live birth was higher in NC compared with AC without GnRH-a, but comparable to that of AC with GnRH-a. While the early miscarriage rate in NC was significantly lower than AC, either with or without GnRH-a pretreatment.

The decreased live birth and increased early miscarriage rate in AC might due to absence of the corpus luteum. Indeed, it has been reported that pregnancies achieved in the absence of a corpus luteum (CL) are at higher risk of hypertensive disorder, preeclampsia, and cesarean section delivery [9, 11, 18]. Relaxin is ~ 6 kDa peptide hormone secreted by CL and plays a key role in the transformation of the maternal circulation during early pregnancy especially before the “corpus luteal-placental shift” [19]. Absence of CL results in undetectable level of relaxin, as well as decreased levels of certain angiogenic and immunoregulatory factors, leading to insufficient cardiovascular adaptation and adverse pregnancy outcomes [20, 21]. The mechanism of GnRH-a pretreatment in improving pregnancy outcomes in AC is unclear. It is proposed that long GnRH-a treatment suppress untimely rises of progesterone levels during hormonal supplementation, which may advance the endometrium and hamper pregnancy outcomes. Besides, animal study suggested that GnRH agonist up-regulated the uterine expression levels of key receptivity markers including Hoxa10, Hoxa11, Lif and integrin b3 mRNA and protein, as well as increased the abundance of pinopodes in adenomyosis, therefore restoring endometrial receptivity [22].

Another intriguing finding of our study is that same endometrial protocol using in different age groups results in different reproductive outcomes. In younger women, there seems to be a mild favor of AC + GnRH-a protocol as the early miscarriage rate of NC was only significantly lower than that of AC, but

comparable to that of AC + GnRH-a. While the incidence of clinical pregnancy, ongoing pregnancy and live birth were both slightly higher in AC + GnRH-a than those in NC, although no reached statistical significance. Comparative proteomic analysis indicated that GnRH-a was associated with upregulation of cytoskeleton regulation and downregulation of energy metabolism on human endometrium. Younger patients present more vigorous metabolism than the older. It is possible that the downregulation of energy-metabolism proteins under GnRH-a treatment exerts a positive effect on the endometrium receptivity of younger women, but a negative effect on that of the older. In older women, the priority of NC to both AC groups was remarkable, as the ongoing pregnancy and live birth rate of NC is much higher, and early miscarriage rate was much lower in NC compared to AC, either with or without GnRH-a pretreatment. A recent study by Liu J and coworkers suggested that in women aged 38 years or over, the endometrial preparation protocols did not affect FET outcomes. However, the study was limited by the small sample size as only 457 cycles with advanced age included [23]. Advanced maternal age is an independent risk factor of thrombotic events [24]. Moreover, exogenous hormone increases the risk of vascular thrombosis [25]. Premature estradiol elevation lead to apoptosis of trophoblast and is associated with uteroplacental insufficiency, hence further worsen the pregnancy outcomes in older women using AC protocols [26].

Strengths and limitations

This study has several strengths. First, the large sample size of 16870 cycles enhances the statistical power. Second, the clinical and laboratory practices did not substantially change over the course of study, which should minimize the possible confounders associated with pregnancy outcome. Third, we adjusted for a number of potential confounders that might otherwise have biased the findings. Last but not least, this study has good representativeness as we avoid strict inclusion and exclusion criteria. This study is mainly limited by its retrospective nature. Besides, we could not control all the confounders. Finally, as all these data come from a single fertility center, multicenter study is warranted to verify the findings.

Conclusion

In conclusion, our study reveals that NC protocol was associated with lower early miscarriage late in overall population. There is a mild favor of GnRH-a cycles in younger women, while the priority of NC protocol is remarkable in the older. Maternal age should be a considerable factor when determine endometrial preparation method for FET.

Abbreviations

FET: Frozen-thawedembryo transfer; NC: natural cycle; AC: artificial cycle; COH: controlled ovarian stimulation; aOR: adjusted odds ratio; CL: corpus luteum

Declarations

Ethical Approval and Consent to participate

This study was approved by the Institutional Review Board of the Shenzhen Zhongshan Urology Hospital. The requirement of informed consent was waived due to the retrospective nature of the study.

Consent for publication

Not applicable.

Availability of supporting data

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

Competing interests

The authors declare that they have no competing interests

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Authors’ contributions

YZ and MLM supervised the entire study, including the design, procedures and revisions to the article. ZQZ analyzed the data and drafted the manuscript. SX and XJS took part in acquisition and analysis of data. HZZ and SRX took part in critical discussion and revision of the article. All authors read and approved the final manuscript.

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Figures

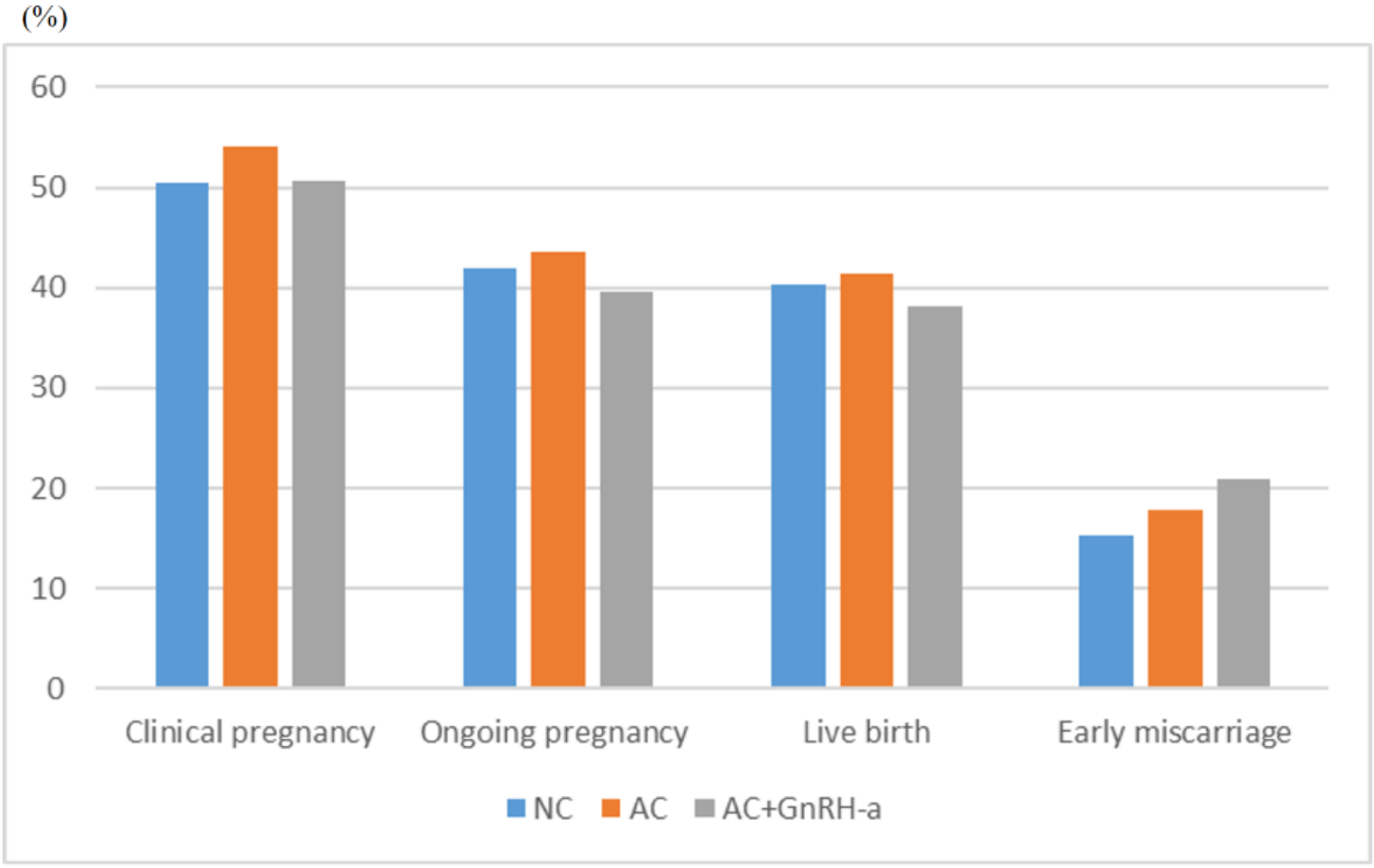

Figure 1

Pregnancy outcomes of different endometrial preparation protocols in overall population.

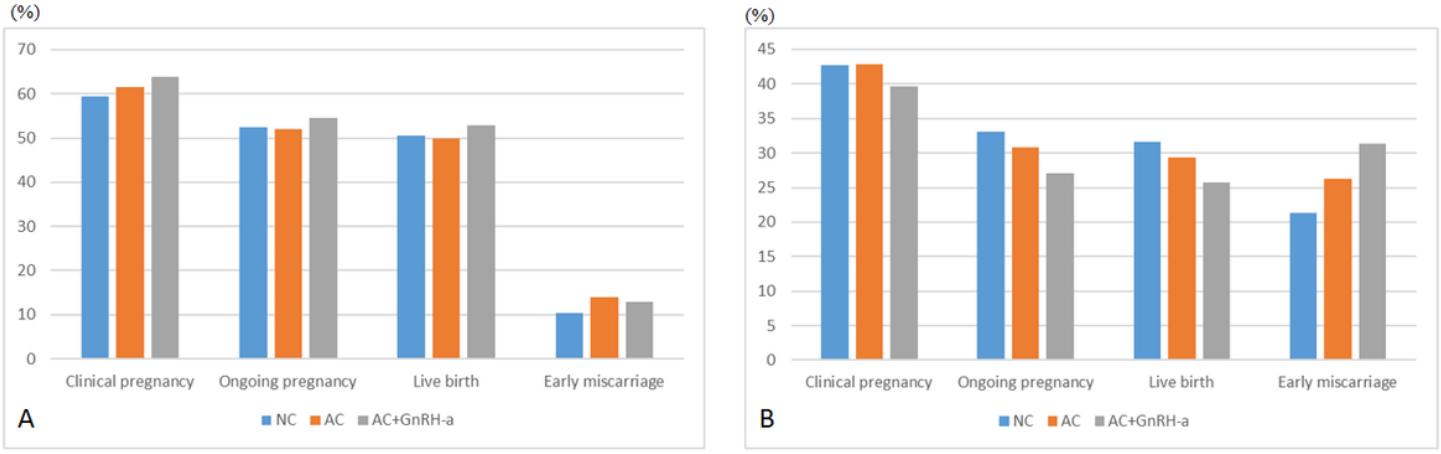

Figure 2

Pregnancy outcomes of different endometrial preparation protocols in younger (A) and older (B) women.