Reproductive Biology and Endocrinology

RESEARCH

Open Access

Comparison of vaginal versus intramuscular progesterone in programmed cycles for frozen-thawed blastocyst transfer in patients with endometriosis

Ziqi Jin[1,2,3†], Guoxia Yang[1,2,3†], Tianrui Wen[1,2,3†], Benyu Miao[1,2,3†], Chen Wang[1,2,3], Qingyan Zhang[1,2,3], Fang Gu[1,2,3] and Yanwen Xu[1,2,3*]

Abstract

Background Previous studies have shown that due to the presence of endometrium progesterone resistance in patients with endometriosis, it is considered that higher levels of progesterone may be required to achieve live birth during programmed frozen-thawed embryo transfer (FET) cycles. Currently, the optimal progesterone support in FET cycles remains a contentious issue, and it mainly focused on the general infertile population, without specific attention to infertile patients with endometriosis. This study aimed to compare the pregnancy outcomes between vaginal or intramuscular progesterone administration in patients with endometriosis, and to determine whether the stage of endometriosis moderates the differences.

Methods This retrospective cohort study included patients with endometriosis who underwent their first single frozen-thawed blastocyst transfer in a programmed cycle from January 2018 to April 2024 at a university-affiliated reproductive medical center. According to the routes of luteal support, patients were divided into vaginal progesterone and intramuscular progesterone groups. Analyses were conducted using multivariate regression models and subgroup analysis. Interaction tests were employed to determine whether the revised American Society for Reproductive Medicine (r-ASRM) stages of endometriosis moderated the differences between the routes of progesterone administration and pregnancy outcomes.

Results A total of 825 programmed frozen-thawed blastocyst transfer cycles were included in the analysis, with 362 cases using vaginal progesterone and 463 cases using intramuscular progesterone. In the overall cohort, clinical pregnancy rate of the vaginal progesterone group was 49.17%, comparable to 44.06% of the intramuscular progesterone group (aOR 0.82, 95% CI 0.61–1.11). Similarly, there was no statistically significant difference in miscarriage rates between the two groups (16.85% versus 24.51%; aOR 1.57, 95% CI 0.90–2.75). In the subgroup analysis in patients classified as r-ASRM stages I-II, clinical pregnancy rate of vaginal progesterone group was significantly higher than that of intramuscular group (aOR 0.74, 95% CI 0.58–0.93, $P=0.011$). Whereas, in patients with stages III-IV, no significant

†Ziqi Jin, Guoxia Yang, Tianrui Wen and Benyu Miao are co-first authors and contributed equally to this work.

*Correspondence:
Yanwen Xu
xuyanwen@mail.sysu.edu.cn
Full list of author information is available at the end of the article

differences in pregnancy outcomes between the two groups were detected. Interaction tests between the routes of progesterone administration and r-ASRM stages were significant ($P=0.036$).

Conclusions In the first single frozen-thawed blastocyst transfer cycles for endometriosis patients with r-ASRM stages I-II, vaginal progesterone favours a higher clinical pregnancy rate compared to the intramuscular progesterone.

Keywords Endometriosis, r-ASRM, Vaginal progesterone, Intramuscular progesterone, Clinical pregnancy

Introduction

Endometriosis is a common chronic inflammatory gynecological disorder, affecting up to 25% to 50% of infertile women [1, 2]. It is characterized by the presence of endometrial glands and stroma outside the uterine cavity [3]. Symptoms of this disease include chronic pelvic pain, dysmenorrhea, and infertility, associated with a variety of negative impacts on reproduction [3–6]. An updated large-scale study which included 162,082 donor oocyte or embryo cycles (137,182 from Society for Assisted Reproductive Technology and 24,900 from the Human Fertilisation and Embryology Authority databases) showed that patients with endometriosis had a significantly lower live birth rate even after adjusting for confounding factors (aOR 0.89, 95% CI 0.81–0.97, $P=0.008$), emphasizing decreased endometrium receptivity in endometriosis [7].

It is well known that changes in the endogenous environment of endometriosis include increased local estrogen synthesis and progesterone resistance, as well as ongoing inflammation that affects both local tissues and the entire body [8]. Due to the possible endometrium progesterone resistance [9, 10], it is considered that higher levels of progesterone may be required to achieve live birth during programmed frozen-thawed embryo transfer (FET) cycles [11]. A recent study revealed that progesterone administration for endometriosis patients with lower progesterone levels (< 10.6 ng/mL) before transfer achieved similar live birth rate to the general population, indicating higher progesterone administration may benefit endometriosis patients [12]. However, Bourdon et al.'s study demonstrated that in infertile patients who achieved live birth during programmed FET cycles, there was no significant difference in serum progesterone levels on the day of transfer between those with and without endometriosis [13]. It is worth noting that serum progesterone levels do not correspond to the effective local progesterone levels in the endometrium, as they are unrelated [14, 15].

In programmed cycles, the addition of exogenous progesterone plays a decisive role in converting the endometrium to the mid-secretory phase that can accommodate embryo implantation [16]. Both vaginal and intramuscular routes are the preferred methods for progesterone administration. Due to concerns about daily intramuscular injections of progesterone, as well as issues such as local pain and inflammation at the injection site [17], patients undergoing assisted reproductive treatment (ART) generally prefer the vaginal route [18–20], which has gradually predominated in most in vitro fertilization (IVF) centers worldwide [21]. However, assessment of clinical efficacy is challenged by serum progesterone levels can not truly reflect the local effect of vaginal progesterone. In addition, the optimal progesterone support in programmed FET cycles remains a contentious issue, with studies comparing pregnancy outcomes of two routes of progesterone administration in FET cycles reporting conflicting data [22–25]. For luteal support, there is currently no universally recognized optimal route of progesterone administration. Of note, researches on the better route of progesterone administration mostly focused on the efficacy in the general infertile population, without specific attention to infertile patients with endometriosis.

Considering the high prevalence of endometriosis among women of reproductive age and the increasing application of FET, it is necessary to study the interrelationship between routes of progesterone administration and pregnancy outcomes, which may be benefitial for clinicians in decision for prescribing progesterone for infertile women with varying degrees of endometriosis. Therefore, in this study, we aimed to compare the effectiveness of vaginal progesterone and intramuscular progesterone for programmed FET cycles among patients with endometriosis, and to evaluate whether the revised American Society for Reproductive Medicine (r-ASRM) stages of endometriosis moderates the difference in pregnancy outcomes.

Materials and methods

Patients

This retrospective cohort study was conducted from January 2018 to April 2024. Anonymous data were extracted from the Clinical Reproductive Medicine Management System/Electronic Medical Record Cohort Database of the Center for Reproductive Medicine at the First Affiliated Hospital of Sun Yat-sen University. The study was approved by the hospital's ethics committee and institutional review board (approval no. 2024419). Informed

consent was waived due to the retrospective nature of the study. All treatments were performed in accordance with relevant guidelines and regulations.

Patients meeting the following criteria were included in this study: (i) infertile patients diagnosed with endometriosis and staged surgically via laparoscopy, (ii) the first single frozen-thawed blastocyst transfer cycle, (iii) programmed cycles where exogenous hormone replacement was used for endometrial preparation. Exclusion criteria were as follows: (i) presence of uterine pathology (including adenomyosis, intrauterine adhesions, uterine malformation and submucous myoma), (ii) a thinner endometrial thickness (< 7 mm on the day of progesterone administration), (iii) patients using other luteal support protocols (including human chorionic gonadotropin (hCG) injections, oral alone, vaginal and intramuscular progesterone co-administration), (iv) patients with endocrine and autoimmune diseases. A flowchart of the patient-selection process is shown in Fig. 1.

Diagnosis of endometriosis

This study ultimately only analyzed endometriosis patients who underwent laparoscopic surgery and staging prior to ART. Women who were suspected of having endometriosis based on transvaginal ultrasound or medical history but did not undergo laparoscopic examination were not included in this study.

The staging was based on the revised classification by the ASRM, which categorizes endometriosis into four stages (I-IV) [26]. Surgical reports for each patient were obtained from the patients' clinics.

Laboratory protocols

The procedures for IVF stimulation, oocyte retrieval, intracytoplasmic sperm injection (ICSI), blastocyst culture, use of vitrification techniques, and embryo transfer have been described previously [27, 28]. Embryo assessment was performed on the morning of the 5th day after oocyte retrieval, with each blastocyst graded based on the Gardner scoring system [29]. Good-quality embryos were defined as those meeting specific criteria: the blastocoel fully expanded the embryo (Grade 3), a loosely compacted inner cell mass with several cells (Grade B), and a trophectoderm with few cells forming a loose epithelium (Grade B). Embryos with a score below the 3-BB grade were defined as poor quality. For preimplantation genetic testing (PGT) cycles, a trophectoderm biopsy was performed on the 5th or the 6th day following oocyte retrieval, with blastocysts being vitrified after the biopsy.

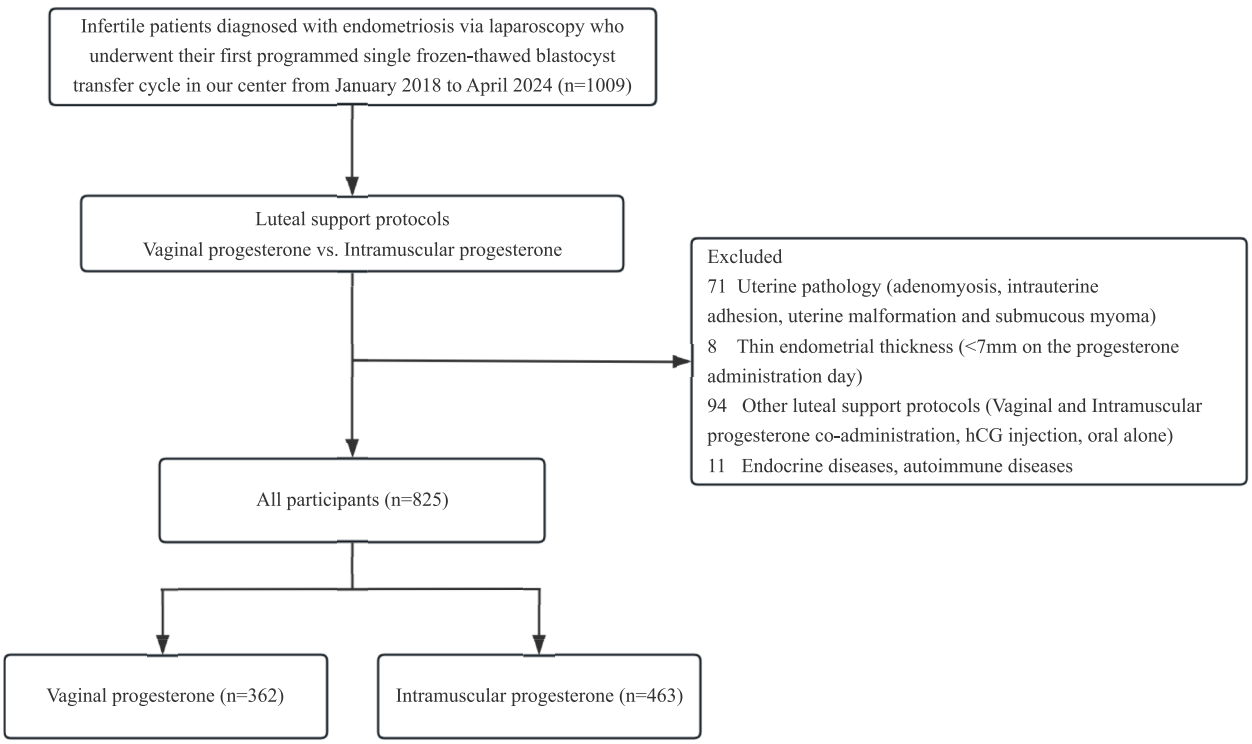

Fig. 1 Flowchart of patients. Note: hCG = human chorionic gonadotropin

Endometrial preparation and luteal support

Based on patients' characteristics as well as physicians' preferences and judgment, hormone replacement treatment (HRT) cycles with or without gonadotropin-releasing hormone agonist (GnRHa) pretreatment were used as endometrial preparation protocols. Patients were administered estradiol (E_2) valerate (2 mg twice daily) (Progynova; Schering AG, Berlin, Germany) until the endometrial thickness reached at least 7 mm after 12–14 days. At this point, intramuscular progesterone (60 mg daily) or vaginal progesterone (Crinone, Fleet Laboratories Limited, Watford, Hertfordshire, UK) was given. Blastocyst transfer was performed on the 6th day after progesterone administration. Embryos were transferred under ultrasound guidance using Cook catheters (Curved Embryo Transfer Catheter; Cook Medical, Bloomington, USA). For GnRHa-HRT cycles, 3.75 mg GnRHa pretreatment was provided 28 days before the start of endometrial preparation. The subsequent endometrial preparation protocol and embryo transfer timing were the same as for the HRT protocol. If pregnancy was confirmed, luteal support continued until 10–12 weeks of gestation.

Definition of clinical outcomes

We adopted the consensus reached by the ASRM in 2017 to define clinical outcomes [30]. Clinical pregnancy was defined as the presence of one or more gestational sacs observed via ultrasound examination. Miscarriage referred to spontaneous pregnancy loss before 22 weeks of intrauterine gestation. Ectopic pregnancy was diagnosed of extrauterine pregnancy. Biochemical pregnancy was identified only by detecting beta hCG in either serum or urine tests. Ongoing pregnancy was indicated as pregnancy lasting after 12 weeks. Live birth was defined as the delivery of at least one live baby after 22 weeks of gestation. Preterm birth referred to delivery occurring between 22 and 37 completed weeks of gestation. Low birth weight was characterized as a birth weight below 2500 g, while macrosomia was defined as a birth weight exceeding 4000 g.

Statistical analysis

For continuous variables, the data were presented as mean ± standard deviation (for normally distribution) or median (interquartile range) (for skewed distribution). Group comparisons were conducted using Student's *t*-test or Mann–Whitney U nonparametric test, depending on the normality of the variable distribution. For categorical variables, the data were presented as frequencies or percentages, and comparisons were conducted using chi-square tests or Fisher's exact tests.

Univariate analysis was used to assess the impact of various variables on clinical pregnancy rates. We used multivariate logistic regression models to calculate the crude odds ratios (OR) and adjusted odds ratios (aOR) with 95% confidence intervals (CI), and analyzed the relationship between routes of progesterone administration and pregnancy outcomes. Both the crude model and multivariable adjustment models were utilized. Covariates were included in final models as potential confounding factors if they altered the estimated effect of routes of progesterone administration on pregnancy outcomes by more than 10%, were significantly associated with pregnancy outcomes based on recently published studies and clinical experience. In adjusted model I, adjusted variables included female age, body mass index (BMI), PGT utilization, blastocyst developmental stage (Day 5 versus Day 6), blastocyst grade, and endometrial thickness on the day of progesterone administration. In adjusted model II, adjusted variables included all covariates in model I plus infertility duration, infertility type, gravidity, parity, endometrial preparation protocols, basal serum luteinizing hormone (LH) levels, and endometriosis r-ASRM stages. In adjusted model III, adjusted variables included all covariates in model II plus basal follicle-stimulating hormone (FSH) levels and serum E_2 levels on the day of embryo transfer. Subgroup analyses were conducted by female age, BMI, endometrial thickness on the day of progesterone administration, blastocyst grade, blastocyst developmental stage, endometrium preparation protocols, PGT utilization and endometriosis r-ASRM stages using stratified logistic regression models with adjustment for confounders. The interactions among subgroups were assessed using the likelihood ratio test. The results are presented as forest plots. To prevent reduced statistical power and bias from directly excluding missing data, we employed multiple imputation, using five replications and the Markov chain Monte Carlo (MCMC) method in the R MI procedure [31]. This approach was used to address missing data on anti-Müllerian hormone (AMH) and serum hormone levels. We performed confounder adjustments on the imputed data in the multiple regression analysis and compared these results with those derived from the full data cohort.

All statistical analyses were conducted using the R statistical software, version 3.4.3 (The R Foundation, Vienna, Austria). A two-sided P-value < 0.05 was considered to indicate statistical significance.

Results

Characteristics of the study cohort

In this study, a total of 1,009 patients with endometriosis, who were diagnosed and staged via laparoscopy, and underwent their first programmed single

FET cycles between January 2018 and April 2024 were included. Among them, 184 cases were excluded due to uterine pathology, thinner endometrium (endometrial thickness of < 7 mm on the day of progesterone administration), using other luteal support protocols and with endocrine diseases and autoimmune diseases. Details were shown in Fig. 1. Finally, 825 FET cycles were included, and 382 pregnancies were achieved, leading to a clinical pregnancy rate of 46.30%. In all programmed FET cycles, 43.88% (362/825) of the patients received vaginal progesterone administration, while the remaining patients (463/825) received intramuscular progesterone administration.

Table 1 Baseline characteristics of patients using vaginal progesterone versus intramuscular progesterone for luteal support

Characteristics	All participants (*N* = 825)	Vaginal progesterone (*N* = 362)	Intramuscular progesterone (*N* = 463)	*P*-value
Woman's age at oocyte retrieval (y)	32.69 ± 3.71	32.40 ± 3.60	32.92 ± 3.78	0.055
Man's age at oocyte retrieval (y)	34.65 ± 4.85	34.32 ± 4.68	34.90 ± 4.97	0.091
BMI (kg/m2)	20.65 ± 2.57	20.51 ± 2.56	20.76 ± 2.58	0.168
Infertility duration(y)	3.00 (1.50–4.00)	2.00 (1.00–4.00)	3.00 (2.00–5.00)	< 0.001
Infertility type (%)				0.050
Primary	482 (58.42%)	216 (59.67%)	266 (57.45%)	
Secondary	299 (36.24%)	120 (33.15%)	179 (38.66%)	
Other[a]	44 (5.33%)	26 (7.18%)	18 (3.89%)	
Gravidity, median (Range)	0.64 (0.00–5.00)	0.62 (0.00–4.00)	0.65 (0.00–5.00)	0.647
Parity, median (Range)	0.14 (0.00–2.00)	0.15 (0.00–2.00)	0.13 (0.00–2.00)	0.634
No. of miscarriages, median (Range)	0.40 (0.00–4.00)	0.39 (0.00–4.00)	0.41 (0.00–4.00)	0.746
Basal serum FSH (mIU/ml)	5.99 ± 1.94	6.15 ± 2.20	5.86 ± 1.70	0.114
Basal serum LH (mIU/ml)	3.50 ± 1.57	3.65 ± 1.64	3.37 ± 1.51	0.012
Basal serum E_2 (pg/ml)	33.00 (25.00–45.00)	32.00 (25.00–45.00)	33.00 (24.00–45.00)	0.386
AMH (ng/ml)	3.11 (1.84–4.88)	3.11 (1.90–4.68)	3.11 (1.72–5.26)	0.065
No. of retrieved oocytes	15.38 ± 7.60	15.40 ± 7.70	15.37 ± 7.53	0.957
PGT utilization, n (%)	153 (18.55%)	65 (17.96%)	88 (19.01%)	0.700
Endometriosis r-ASRM stages[b], n (%)				0.643
Stages I-II	347 (42.06%)	149 (41.16%)	198 (42.76%)	
Stages III-IV	478 (57.94%)	213 (58.84%)	265 (57.24%)	
Endometrial thickness on the day of P administration (mm)	9.72 ± 1.50	9.73 ± 1.57	9.71 ± 1.45	0.823
Serum LH at transfer (IU/L)	1.41 (0.24–4.52)	0.43 (0.21–4.68)	1.65 (0.31–4.51)	0.970
Serum E_2 at transfer (pg/mL)	125.00 (88.00–174.25)	118.00 (88.00–164.00)	130.00 (88.00–178.50)	0.002
Serum P at transfer (ng/ml)	8.80 (3.60–12.80)	4.20 (2.40–7.50)	11.50 (8.60–14.50)	< 0.001
Endometrium preparation protocols, n (%)				0.774
HRT	401 (48.61%)	178 (49.17%)	223 (48.16%)	
GnRHα-HRT	424 (51.39%)	184 (50.83%)	240 (51.84%)	
Morphology score, n (%)				0.226
< 4BC	80 (9.70%)	30 (8.29%)	50 (10.80%)	
≥ 4BC	745 (90.30%)	332 (91.71%)	413 (89.20%)	
Blastocyst developmental stage, n (%)				0.692
Day 5	544 (66.02%)	241 (66.76%)	303 (65.44%)	
Day 6	280 (33.98%)	120 (33.24%)	160 (34.56%)	

Data are expressed as mean ± SD or percentage of outcome

BMI body mass index, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, E_2 estradiol, *P* progesterone, *AMH* antimüllerian hormone, *PGT* preimplantation genetic testing, *HRT* hormone replacement treatment, *GnRHa* gonadotropin-releasing hormone agonist

Other[a]: Patients who did not meet the diagnostic criteria for infertility, mainly included patients treated with PGT

Endometriosis r-ASRM stages[b]: The revised American Society of Reproductive Medicine distinguishes four stages of endometriosis, where stages I and II are fairly mild types, and stages III and IV are advanced disease

Comparison of baseline characteristics between the two groups

The baseline characteristics were shown in Table 1. Among these patients, the vaginal progesterone group had a shorter infertility duration (2.00 (1.00–4.00) versus 3.00 (2.00–5.00), $P<0.001$), higher basal serum LH levels (3.65 ± 1.64 versus 3.37 ± 1.51, $P=0.012$), and lower E_2 (118.00 (88.00–164.00) versus 130.00 (88.00–178.50), $P=0.002$) and progesterone levels (4.20 (2.40–7.50) versus 11.50 (8.60–14.50), $P<0.001$) on the day of transfer. However, the two groups were comparable with regards to female age, BMI, gravidity, parity, basal serum FSH and E_2, endometriosis r-ASRM stages, blastocyst grade, blastocyst developmental stage, endometrial thickness on the day of progesterone administration, and endometrium preparation protocols (all $P>0.050$). In addition, there were no significant differences in clinical pregnancy rates, miscarriage rates, biochemical pregnancy rates, ectopic pregnancy rates, ongoing pregnancy rates, live birth rates and neonatal perinatal outcomes between the two groups (all $P>0.050$) (Table 2).

Supplemental Table 1 shows the results of stratification into four groups based on serum progesterone levels on the day of transfer. The results indicate that there were no significant differences in pregnancy outcomes among the different progesterone level groups, both in the whole cohort and when stratified according to the routes of progesterone administration.

Association of different routes of progesterone administration and pregnancy outcomes

A univariate regression analysis was conducted to assess the impact of each variable on clinical pregnancy rate (Supplemental Table 2). Overall, the infertility duration, basal serum FSH levels, and blastocyst developmental stage were negatively correlated with clinical pregnancy rates, while PGT utilization and blastocyst grade were positively correlated with clinical pregnancy rates. Endometriosis r-ASRM stages and the two routes of progesterone administration had no significant impact on clinical pregnancy rates.

Multivariate logistic regression models were used to evaluate the relationship between the two routes of

Table 2 Reproductive outcomes of two different routes of progesterone administration

Variables	All participants($N=825$)	Vaginal progesterone ($N=362$)	Intramuscular progesterone ($N=463$)	*P*-value
Clinical pregnancy rate, n (%)	382 (46.30%)	178 (49.17%)	204 (44.06%)	0.144
Miscarriage rate, n (%)	80 (20.94%)	30 (16.85%)	50 (24.51%)	0.067
Biochemical pregnancy rate, n (%)	84 (10.18%)	43 (11.88%)	41 (8.86%)	0.154
Ectopic pregnancy rate, n (%)	11 (2.87%)	7 (3.91%)	4 (1.96%)	0.254
Ongoing pregnancy rate, n (%)	294 (35.64%)	142 (39.23%)	152 (32.83%)	0.057
Live birth rate, n (%)	245 (29.70%)	117 (32.32%)	128 (27.65%)	0.145
Pregnancy complications, n (%)				0.370
GH	20 (8.16%)	8 (6.84%)	12 (9.38%)	
GDM	25 (10.20%)	14 (11.97%)	11 (8.59%)	
PROM	8 (3.27%)	3 (2.56%)	5 (3.91%)	
Placenta previa	8 (3.27%)	6 (5.13%)	2 (1.56%)	
Placental abruption	18 (7.35%)	6 (5.13%)	12 (9.38%)	
Type of birth, n (%)				0.508
Preterm	24 (9.80%)	13 (11.11%)	11 (8.59%)	
Term	221 (90.20%)	104 (88.89%)	117 (91.41%)	
Method of delivery, n (%)				0.845
Cesarean section	169 (68.98%)	80 (68.38%)	89 (69.53%)	
Normal vaginal delivery	76 (31.02%)	37 (31.62%)	39 (30.47%)	
Gender, n (%)				0.074
Female	132 (53.88%)	70 (59.83%)	62 (48.44%)	
Male	113 (46.12%)	47 (40.17%)	66 (51.56%)	
Birth weight (g)	3259.92 ± 482.41	3249.91 ± 507.85	3269.06 ± 459.74	0.757
LBW, n (%)	13 (5.31%)	6 (5.13%)	7 (5.47%)	0.905
Macrosomia, n (%)	11 (4.49%)	6 (5.13%)	5 (3.91%)	0.645

GH gestational hypertension, *GDM* gestational diabetes mellitus, *PROM* Premature rupture of membranes, *LBW* low birth weight

Table 3 Relationship between different routes of progesterone administration and clinical pregnancy rates and miscarriage rates in different models

Outcome	Crude model[a]		Adjusted model I[b]		Adjusted model II[c]		Adjusted model III[d]	
	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
Clinical pregnancy rate								
Vaginal progesterone	Reference		Reference		Reference		Reference	
Intramuscular progesterone	0.81 (0.62, 1.07)	0.144	0.81 (0.61, 1.09)	0.165	0.83 (0.61, 1.11)	0.210	0.82 (0.61, 1.11)	0.197
Miscarriage rate								
Vaginal progesterone	Reference		Reference		Reference		Reference	
Intramuscular progesterone	1.60 (0.97, 2.66)	0.068	1.48 (0.88, 2.49)	0.143	1.57 (0.91, 2.70)	0.102	1.57 (0.90, 2.75)	0.112

BMI body mass index, *PGT* preimplantation genetic testing, *r-ASRM* The revised American Society of Reproductive Medicine, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, E_2 estradiol, *P* progesterone, *OR* odds ratio, *CI* confidence interval

[a] No adjustments for other covariates

[b] Adjusted for woman's age at oocyte retrieval, BMI, PGT utilization, blastocyst developmental stage, morphology score, endometrial thickness on the day of P administration

[c] Adjusted for all covariables in model I plus infertility duration, infertility type, gravidity, parity, endometrial preparation, basal serum LH, endometriosis r-ASRM stages

[d] Adjusted for all covariables in model II plus basal serum FSH, serum E_2 at transfer

progesterone administration and pregnancy outcomes (Table 3). Clinical pregnancy rates were 49.17% (178/362) in vaginal progesterone group versus 44.06% (204/463) in intramuscular progesterone group, respectively. Miscarriage rate in the vaginal progesterone group was 16.85% (30/178), compared to 24.51% (50/204) in the intramuscular progesterone group. In models unadjusted, partially adjusted, or the fully adjusted, we all found that the differences were not statistically significant in clinical pregnancy rates (aOR 0.82, 95% CI 0.61–1.11, $P=0.197$) and miscarriage rates (aOR 1.57, 95% CI 0.90–2.75, $P=0.112$) (fully adjusted model) between the two groups.

Subgroup analyses

To assess whether the relationship between the two routes of progesterone administration with pregnancy outcomes is stable in different subgroups, we conducted subgroup analyses and interaction tests. The results of the subgroup analysis based on the r-ASRM stages of endometriosis showed that in patients with stages I-II, clinical pregnancy rate was 43.43% in the intramuscular progesterone subgroup, significantly lower than 53.69% in the vaginal progesterone subgroup (aOR 0.74, 95% CI 0.58–0.93, $P=0.011$). However, in patients with stages III-IV, there was no significant difference in clinical pregnancy rates between the two groups (aOR 1.02, 95% CI 0.84–1.23, $P=0.855$). Additionally, the interaction testing of two routes of progesterone administration and r-ASRM stages was highly significant (*P* for interaction = 0.036) (Fig. 2). As miscarriage rate was considered, there was no statistically significant difference between the two groups in patients with stages I-II (aOR 1.48, 95% CI 0.99–2.23, $P=0.065$) and stages III-IV (aOR 1.11, 95% CI 0.78–1.60, $P=0.559$) (Supplemental Fig. 1).

We compared the subgroups divided by the r-ASRM stages of endometriosis (Supplemental Table 3). The results showed that subgroup with r-ASRM stages III-IV had a shorter infertility duration (2.00 (1.00–4.00) versus 3.00 (2.00–5.00), $P<0.001$), lower ovarian reserve indicated by lower AMH level (2.93 (1.65–4.46) versus 3.56 (2.25–5.26), $P<0.001$), higher basal FSH level (6.16 ± 2.04 versus 5.75 ± 1.77, $P=0.003$), and lower number of oocytes collected (13.91 ± 7.03 versus 17.41 ± 7.90, $P<0.001$). In addition, significant higher proportion of patients in this subgroup received GnRHa downregulation (62.55% versus 36.02%, $P<0.001$), resulted in lower LH (0.47 (0.22–3.36) versus 2.96 (0.41–6.36), $P=0.001$) and E_2 (117.00 (82.75–165.00) versus 131.00 (99.75–188.25), $P<0.001$) levels at the embryo transfer day.

We further presented the pregnancy outcomes based on whether GnRHa pretreatment was used, grouped by different r-ASRM stages of endometriosis patients, and further sub-grouped by the two methods of progesterone administration (Supplementary Table 4). The results showed that among these 347 mild patients of endometriosis with stage I-II (222 cases of stage I and 125 cases of stage II), HRT group had a higher clinical pregnancy rate (52.25% vs 40.00%, $P=0.028$) and live birth rate (34.23% vs 24.00%, $P=0.047$) compared

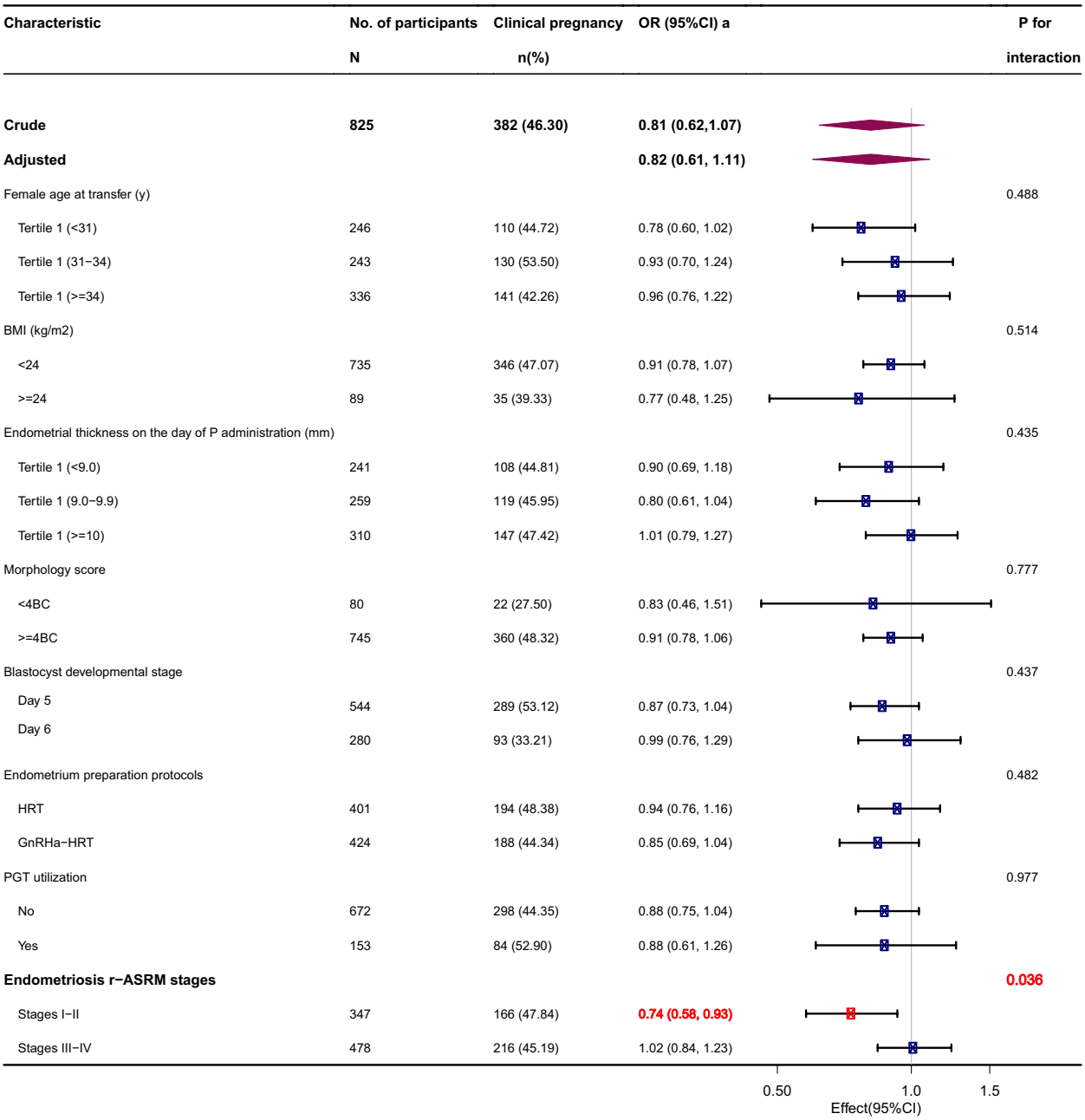

Fig. 2 Effect of different routes of progesterone administration on clinical pregnancy rates in each subgroup. Note: BMI = body mass index, P = progesterone, HRT = hormone replacement treatment, GnRHa = gonadotropin-releasing hormone agonist, PGT = preimplantation genetic testing, r-ASRM = The revised American Society of Reproductive Medicine, OR = odds ratio, CI = confidence interval. [a]Adjusted for woman's age at oocyte retrieval, BMI, PGT utilization, blastocyst developmental stage, morphology score, endometrial thickness on the day of P administration, endometrial preparation protocols

with the GnRHa pretreatment group, and this trend was consistent across both subgroups of progesterone administration methods. However, in severe patients with stage III-IV, the clinical pregnancy rate of the HRT subgroup with vaginal progesterone was significantly lower than that of the GnRHa pretreatment subgroup (37.21% vs 51.97%, $P = 0.034$), while there was no significant difference in the intramuscular progesterone subgroups and the entire population.

Sensitivity analyses

After addressing the missing data with multiple imputation, the results of the multivariate logistic regression analyses using multiple adjustment strategies were

consistent with those of participants who had complete data (Supplemental Table 5).

Discussion

Main findings

In this study, we provided evidence to support that vaginal progesterone administration favours higher clinical pregnancy rate in endometriosis patients with r-ASRM stages I-II, as compared with intramuscular progesterone administration.

Comparison with other studies

There are many studies comparing different routes of progesterone administration in programmed FET cycles, and the results are conflicting. Most studies showed that intramuscular and vaginal progesterone have similar pregnancy outcomes [32]. However, a recent randomized trial demonstrasted that patients using vaginal progesterone experienced reduced live birth rate and increased miscarriage rate compared to those using intramuscular progesterone [33]. In contrast, other studies highlighted the advantages of vaginal progesterone [34]. Many of these studies focused on the general infertile population, with endometriosis in the exclusion criteria.

Endometriosis is dominated by a disruption of progesterone signaling pathways, resulting in progesterone resistance with a weakened response of the endometrium to progesterone and reduced success rate of fertility treatments [3, 35]. It was reported that endometriosis patients with serum progesterone levels higher than 118 nmol/L (37.1 ng/mL) doubled the live birth rates of those with lower serum progesterone levels in HRT-FET cycles, suggesting that endometriosis patients may need intensive progesterone adminstration to overcome progesterone resistance [11]. However, a recent study found that endometriosis patients had similar live birth rates compared to the control group [13]. Furthermore, there were no significant differences in serum progesterone levels on the day of FET between pregnant women in both groups, suggesting that endometriosis patients may not require higher progesterone levels to achieve successful live births.

To the best of our knowledge, no study has been published investigating the differences in pregnancy outcomes between different routes of progesterone administration specifically in programmed cycles of endometriosis patients, a typical population with progesterone resistance. Progesterone administrated through vaginal induces a strong local progestogenic effect on the uterus and endometrium, which can be locally absorbed by cervical cells, and rapidly transported to the endometrial cells, thereby achieving a high progestogen concentration in the uterine cavity [36, 37]. Evidence is needed to answer the question whether vaginal progesterone can help to overcome progesterone resistance in endometriosis, considering its strong local effect. In addition, the impact of the severity of endometriosis remains to be clarified.

Implications

In the present study, all patients were classified by the laporoscopy surgery. Our results showed that clinical pregnancy rate of the vaginal progesterone group was significantly higher than those in the intramuscular group in patients with stage I-II. It means that strong local effect of progesterone may benefit endometrium environment of patients with mild endometriosis. Our findings highlight the importance of individualized management of endometriosis according to the r-ASRM stages. Nevertheless, we did not observe the same advantage for vaginal progesterone over intramuscular administration in patients with endometriosis classified as stages III-IV. The main reason may be due to this subgroup received significant more GnRHa downregulation which may improve endometrium environment [38]. Endometriosis is characterized by excessive local E_2 biosynthesis and aberrant E_2 action within the endometrium, particularly evident in stages III-IV [3]. GnRHa downregulation with extremely low E_2 level for a long time may benefit this pathological changes [39], evidenced by lower LH and E_2 levels at the embryo transfer day in our study.

Strengths and limitations

The strengths of this study are as follows: (i) This is the first study to investigate the impact of different progesterone administration routes on pregnancy outcomes in patients with endometriosis. (ii) It was conducted exclusively among endometriosis patients only diagnosed by laparoscopy, which helps to define the different endometriosis severity, thus lessen the heterogeneity of the study cohort. Additionally, we performed subgroup analyses to determine if the association could be influenced by the r-ASRM stages of endometriosis. (iii) We collected more variables and developed multiple regression models adjusted for confounding factors to quantify the association between different progesterone administration routes and pregnancy outcomes.

However, there are also some limitations in this study. (i) The study lacks reasons for selecting specific progesterone administration routes, such as patient comfort,

convenience, and medication cost, which may introduce bias. (ii) As a retrospective study, it is not possible to control all the bias. However, multivariate regression analysis models were used to adjust for confounding factors between the two groups. Similarly, subgroup analyses and sensitivity analyses also supported the robustness of the findings. (iii) Patients were divided into two groups based on clinical practice rather than randomization. Future well-designed prospective randomized studies are still needed to explore the optimal luteal support protocol for endometriosis patients. (iv) Last but not the least, a bigger sample size may increase the statistical power of our study.

Conclusion

In conclusion, we found that vaginal progesterone led to a significantly higher clinical pregnancy rate compared to intramuscular progesterone in patients with mild endometriosis (r-ASRM stages I-II) undergoing FET, suggesting that vaginal progesterone is an attractive option to improve the outcomes of ART for patients with mild endometriosis.

Abbreviations

r-ASRM	The revised American Society for Reproductive Medicine
FET	Frozen-thawed embryo transfer
ART	Assisted reproductive treatment
IVF	In vitro fertilization
RCT	Randomized controlled trial
hCG	Human chorionic gonadotropin
PGT	Preimplantation genetic testing
HRT	Hormone replacement treatment
GnRHa	Gonadotropin-releasing hormone agonist
E_2	Estradiol
aOR	Adjusted odds ratios
CI	Confidence intervals
BMI	Body mass index
LH	Luteinizing hormone
FSH	Follicle-stimulating hormone
MCMC	Markov chain Monte Carlo
AMH	Anti-Müllerian hormone

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12958-025-01354-7.

Supplementary Material 1: Supplemental Figure 1. Effect of different routes of progesterone administration on miscarriage rates in each subgroup. Note: BMI = body mass index, P = progesterone, HRT = hormone replacement treatment, GnRHa = gonadotropin-releasing hormone agonist, PGT = preimplantation genetic testing, r-ASRM = The revised American Society of Reproductive Medicine, OR = odds ratio, CI = confidence interval. [a] Adjusted for woman's age at oocyte retrieval, BMI, PGT utilization, day of embryo development at transfer, morphology score, endometrial thickness on the day of P administration, endometrial preparation protocols. § The model failed because of the small sample size.

Supplementary Material 2: Supplemental Table 1. Comparison of pregnancy outcomes in different routes of progesterone administration according to the serum P level on the transfer day.

Supplementary Material 3: Supplemental Table 2. Univariate analysis for clinical pregnancy rates.

Supplementary Material 4: Supplemental Table 3. Baseline characteristics and reproductive outcomes of patients with endometriosis divided by the r-ASRM stages[a].

Supplementary Material 5: Supplemental Table 4. Reproductive outcomes of patients with endometriosis divided by the r-ASRM stages with and without GnRHa pretreatment, with sub-stratification by progesterone administration methods.

Supplementary Material 6: Supplemental Table 5. Relationship between different routes of progesterone administration and clinical pregnancy rates and miscarriage rates in different models — by using data after multiple imputation.

Acknowledgements

We thank all patients that participated in the retrospective cohort.

Authors' contributions

Yanwen Xu and Benyu Miao were responsible for the study design and revision of the manuscript; Chen Wang, Qingyan Zhang and Fang Gu handled patient recruitment. Tianrui Wen contributed to data collection and statistical work. Ziqi Jin and Guoxia Yang interpreted of data and wrote the manuscript. All authors read and approved the final manuscript.

Funding

This study was supported by grants from the National Key Research and Development Program of China (2023YFC2705503), Natural Science Foundation of Guangdong Province (2023A1515012109), National Natural Science Foundation of China (82071716).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee and institutional review board of the first affiliated Hospital of Sun Yat-sen University (approval no. 2024419). Informed consent was waived due to the retrospective nature of the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Reproductive Medicine Center, The First Affiliated Hospital of Sun Yat-Sen University, No. 1 of Zhongshan 2nd Road, Guangzhou, Guangdong 510080, China. [2]Key Laboratory for Reproductive Medicine of Guangdong Province, Guangzhou, Guangdong, China. [3]Guangdong Provincial Clinical Research Center for Obstetrical and Gynecological Diseases, Guangzhou, Guangdong, China.

Received: 29 November 2024 Accepted: 30 January 2025
Published online: 06 February 2025

References

1. Vercellini P, Vigano P, Bandini V, Buggio L, Berlanda N, Somigliana E. Association of endometriosis and adenomyosis with pregnancy and infertility. Fertil Steril. 2023;119(5):727–40.
2. Salmeri N, Vigano P, Cavoretto P, Marci R, Candiani M. The kisspeptin system in and beyond reproduction: exploring intricate pathways and

potential links between endometriosis and polycystic ovary syndrome. Rev Endocr Metab Disord. 2024;25(2):239–57.
3. Vercellini P, Vigano P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. Nat Rev Endocrinol. 2014;10(5):261–75.
4. Coccia ME, Rizzello F, Mariani G, Bulletti C, Palagiano A, Scarselli G. Impact of endometriosis on in vitro fertilization and embryo transfer cycles in young women: a stage-dependent interference. Acta Obstet Gynecol Scand. 2011;90(11):1232–8.
5. Kuivasaari P, Hippelainen M, Anttila M, Heinonen S. Effect of endometriosis on IVF/ICSI outcome: stage III/IV endometriosis worsens cumulative pregnancy and live-born rates. Hum Reprod. 2005;20(11):3130–5.
6. Barnhart K, Dunsmoor-Su R, Coutifaris C. Effect of endometriosis on in vitro fertilization. Fertil Steril. 2002;77(6):1148–55.
7. Paffoni A, Casalechi M, De Ziegler D, Cicinelli E, Somigliana E, Vigano P, Vitagliano A. Live birth after oocyte donation in vitro fertilization cycles in women with endometriosis: a systematic review and meta-analysis. JAMA Netw Open. 2024;7(1):e2354249.
8. Zondervan KT, Becker CM, Missmer SA. Endometriosis. N Engl J Med. 2020;382(13):1244–56.
9. Marquardt RM, Kim TH, Shin JH, Jeong JW. Progesterone and estrogen signaling in the endometrium: what goes wrong in endometriosis? Int J Mol Sci. 2019;20(15):3822.
10. Lessey BA, Young SL. Homeostasis imbalance in the endometrium of women with implantation defects: the role of estrogen and progesterone. Semin Reprod Med. 2014;32(5):365–75.
11. Alsbjerg B, Kesmodel US, Humaidan P. Endometriosis patients benefit from high serum progesterone in hormone replacement therapy-frozen embryo transfer cycles: a cohort study. Reprod Biomed Online. 2023;46(1):92–8.
12. Guedj NS, Coroleu B, Alvarez M, García S, Polyzos NP. Role of serum progesterone levels and subcutaneous progesterone supplementation in endometriosis patients undergoing artificial cycle frozen embryo transfer. Hum Reprod. 2024;39:i78–9.
13. Bourdon M, Sorel M, Maignien C, Guibourdenche J, Patrat C, Marcellin L, et al. Progesterone levels do not differ between patients with or without endometriosis/adenomyosis both in those who conceive after hormone replacement therapy-frozen embryo transfer cycles and those who do not. Hum Reprod. 2024;39(8):1692–700.
14. Clemenza S, Sorbi F, Noci I, Capezzuoli T, Turrini I, Carriero C, et al. From pathogenesis to clinical practice: emerging medical treatments for endometriosis. Best Pract Res Clin Obstet Gynaecol. 2018;51:92–101.
15. Barra F, Scala C, Ferrero S. Current understanding on pharmacokinetics, clinical efficacy and safety of progestins for treating pain associated to endometriosis. Expert Opin Drug Metab Toxicol. 2018;14(4):399–415.
16. Groenewoud ER, Cohlen BJ, Macklon NS. Programming the endometrium for deferred transfer of cryopreserved embryos: hormone replacement versus modified natural cycles. Fertil Steril. 2018;109(5):768–74.
17. Tavaniotou A, Smitz J, Bourgain C, Devroey P. Comparison between different routes of progesterone administration as luteal phase support in infertility treatments. Hum Reprod Update. 2000;6(2):139–48.
18. Huisman D, Raymakers X, Hoomans EH. Understanding the burden of ovarian stimulation: fertility expert and patient perceptions. Reprod Biomed Online. 2009;19(Suppl 2):5–10.
19. Yanushpolsky E, Hurwitz S, Greenberg L, Racowsky C, Hornstein M. Crinone vaginal gel is equally effective and better tolerated than intramuscular progesterone for luteal phase support in in vitro fertilization-embryo transfer cycles: a prospective randomized study. Fertil Steril. 2010;94(7):2596–9.
20. Beltsos AN, Sanchez MD, Doody KJ, Bush MR, Domar AD, Collins MG. Patients' administration preferences: progesterone vaginal insert (Endometrin(R)) compared to intramuscular progesterone for Luteal phase support. Reprod Health. 2014;11:78.
21. Vaisbuch E, de Ziegler D, Leong M, Weissman A, Shoham Z. Luteal-phase support in assisted reproduction treatment: real-life practices reported worldwide by an updated website-based survey. Reprod Biomed Online. 2014;28(3):330–5.
22 Shapiro DB, Pappadakis JA, Ellsworth NM, Hait HI, Nagy ZP. Progesterone replacement with vaginal gel versus i.m. injection: cycle and pregnancy outcomes in IVF patients receiving vitrified blastocysts. Hum Reprod. 2014;29(8):1706–11.
23. Kaser DJ, Ginsburg ES, Missmer SA, Correia KF, Racowsky C. Intramuscular progesterone versus 8% Crinone vaginal gel for luteal phase support for day 3 cryopreserved embryo transfer. Fertil Steril. 2012;98(6):1464–9.
24. Berger BM, Phillips JA. Pregnancy outcomes in oocyte donation recipients: vaginal gel versus intramuscular injection progesterone replacement. J Assist Reprod Genet. 2012;29(3):237–42.
25. Haddad G, Saguan DA, Maxwell R, Thomas MA. Intramuscular route of progesterone administration increases pregnancy rates during non-downregulated frozen embryo transfer cycles. J Assist Reprod Genet. 2007;24(10):467–70.
26. Revised American Society for Reproductive Medicine classification of endometriosis: 1996. Fertil Steril. 1997;67(5):817–21. https://doi.org/10.1016/s0015-0282(97)81391-x.
27. Hu X, Liu Y, Zhang X, Lee P, Wen Y, Ding C, et al. Oocyte degeneration after ICSI is not an indicator of live birth in young women. Front Endocrinol (Lausanne). 2021;12:705733.
28. Xiong Y, Chen Q, Chen C, Tan J, Wang Z, Gu F, Xu Y. Impact of oral antibiotic treatment for chronic endometritis on pregnancy outcomes in the following frozen-thawed embryo transfer cycles of infertile women: a cohort study of 640 embryo transfer cycles. Fertil Steril. 2021;116(2):413–21.
29. Gardner DK, Surrey E, Minjarez D, Leitz A, Stevens J, Schoolcraft WB. Single blastocyst transfer: a prospective randomized trial. Fertil Steril. 2004;81(3):551–5.
30. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, et al. The international glossary on infertility and fertility care, 2017. Fertil Steril. 2017;108(3):393–406.
31. Farrar D, Fairley L, Santorelli G, Tuffnell D, Sheldon TA, Wright J, et al. Association between hyperglycaemia and adverse perinatal outcomes in south Asian and white British women: analysis of data from the Born in Bradford cohort. Lancet Diabetes Endocrinol. 2015;3(10):795–804.
32. Abdelhakim AM, Abd-ElGawad M, Hussein RS, Abbas AM. Vaginal versus intramuscular progesterone for luteal phase support in assisted reproductive techniques: a systematic review and meta-analysis of randomized controlled trials. Gynecol Endocrinol. 2020;36(5):389–97.
33. Devine K, Richter KS, Jahandideh S, Widra EA, McKeeby JL. Intramuscular progesterone optimizes live birth from programmed frozen embryo transfer: a randomized clinical trial. Fertil Steril. 2021;116(3):633–43.
34. Jiang L, Luo ZY, Hao GM, Gao BL. Effects of intramuscular and vaginal progesterone supplementation on frozen-thawed embryo transfer. Sci Rep. 2019;9(1):15264.
35. Lin SC, Li WN, Lin SC, Hou HT, Tsai YC, Lin TC, et al. Targeting YAP1 ameliorates progesterone resistance in endometriosis. Hum Reprod. 2023;38(6):1124–34.
36. Ho CH, Chen SU, Peng FS, Chang CY, Yang YS. Luteal support for IVF/ICSI cycles with Crinone 8% (90 mg) twice daily results in higher pregnancy rates than with intramuscular progesterone. J Chin Med Assoc. 2008;71(8):386–91.
37. Chi HB, Liu NN, Li R, Tao LY, Chen LX, Qiao J. Comparison of vaginal gel and intramuscular progesterone for in vitro fertilization and embryo transfer with gonadotropin-releasing hormone antagonist protocol. Chin Med J (Engl). 2018;131(13):1557–61.
38. Vannuccini S, Clemenza S, Rossi M, Petraglia F. Hormonal treatments for endometriosis: the endocrine background. Rev Endocr Metab Disord. 2022;23(3):333–55.
39. Cao X, Chang HY, Xu JY, Zheng Y, Xiang YG, Xiao B, et al. The effectiveness of different down-regulating protocols on in vitro fertilization-embryo transfer in endometriosis: a meta-analysis. Reprod Biol Endocrinol. 2020;18(1):16.

Publisher's Note