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Effect of Embryo Retention on in Vitro Fertilization Outcomes: A Retrospective Analysis

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Abstract

Purpose

To evaluate the incidence of embryo retention (ER) during single blastocyst embryo transfer (SET) cycles and assess its impact on reproductive outcomes in IVF.

Methods

In this retrospective cohort study, 4,593 SET cycles from 3,061 patients treated at a single IVF center between October 2021 and July 2024 were analyzed. Cycles were categorized according to the occurrence of ER during embryo transfer (ER group, n = 106; control group, n = 4,487). Both fresh and frozen transfers using autologous or donor oocytes were included. The primary outcome was live birth rate (LBR). Secondary outcomes included implantation, biochemical pregnancy, clinical pregnancy, ongoing pregnancy, and miscarriage rates. Logistic regression analyses were performed to evaluate associations between ER and reproductive outcomes.

Results

ER occurred in 2.3% of SET cycles. Implantation and early pregnancy outcomes were significantly lower in the ER group compared with controls. After adjustment, ER remained significantly associated with lower implantation (aOR 0.84, 95% CI 0.76–0.92; $p < 0.001$), biochemical pregnancy (aOR 0.49, 95% CI 0.32–0.75; $p < 0.001$), and clinical pregnancy rates (aOR 0.53, 95% CI 0.34–0.82; $p = 0.004$). No statistically significant associations were observed for ongoing pregnancy, live birth, or miscarriage.

Conclusion

ER during single blastocyst transfer appears to be associated with impaired early IVF outcomes, while its effect on live birth remains uncertain.

INTRODUCTION

Embryo transfer (ET) is the final stage of the in vitro fertilization (IVF) process and plays a crucial role in determining treatment outcomes. Despite major advances in laboratory technologies and the clinical management of assisted reproductive technologies (ART), the embryo transfer (ET) procedure has remained largely unchanged since its first description by Edwards and colleagues in 1980 (1). While IVF success depends on several factors, including endometrial receptivity, embryo quality, and the clinician's technique increasing evidence suggests that embryo-related factors may have a greater impact on reproductive outcomes than currently available approaches for assessing endometrial receptivity (2).

The optimization of the embryo transfer (ET) procedure therefore remains essential for improving clinical outcomes in IVF cycles. Several procedural aspects have been shown to influence implantation and pregnancy rates, including the use of ultrasound-guided ET (3), removal of cervical mucus prior to transfer (4), the use of soft rather than rigid catheters (5, 6), optimal embryo deposition approximately 1–2 cm from the uterine fundus (7), and minimizing the transfer volume and the presence of air bubbles to reduce uterine contractions (8). Furthermore, systematic reviews and meta-analyses have consistently demonstrated that post-transfer bed rest does not improve implantation, clinical pregnancy, or live birth rates, whereas immediate mobilization after ET does not compromise IVF outcomes (9). Despite these improvements in technique, embryo transfer may still be associated with technical complications, even when performed by experienced operators (10). Among these complications, ER within the transfer catheter represents a relevant concern given its potential to adversely affect ART outcomes. ER is a relatively uncommon event that requires immediate retransfer of the embryo (11).

The mechanisms underlying ER are not fully understood but may involve factors such as contamination of the catheter with cervical mucus or blood, the viscosity of the culture medium, uterine anatomical characteristics, or operator-related technical aspects (6). Moreover, prolonged exposure of retained embryos to suboptimal conditions, together with increased uterine contractions during the retransfer procedure, may adversely affect implantation rates (12). Previous studies investigating the impact of ER have reported inconsistent results. While some studies have suggested that ER and subsequent repeated transfer are associated with reduced implantation and pregnancy rates (13–14), others have found no significant differences in clinical outcomes (10, 15). Given the critical role of embryo transfer quality in determining IVF success, further investigation is warranted to clarify the relationship between ER and reproductive outcomes. Therefore, the aim of this study was to evaluate the incidence of ER in both fresh and frozen single blastocyst transfer cycles and to assess its potential impact on IVF outcomes.

MATERIALS AND METHODS

Study Design and Participants

This retrospective cohort study was conducted at IVI Rome (Italy) between October 2021 and July 2024. A total of 3,061 patients undergoing single blastocyst embryo transfer (SET) were included. Overall, 4,593 SET cycles were analyzed, including 4,487 transfers without ER and 106 transfers in which ER occurred during the embryo transfer procedure. Both fresh and frozen SET cycles using autologous or donor oocytes were included. Preimplantation genetic testing (PGT) was performed in women older than 35 years. Cycles were eligible for inclusion only if endometrial thickness was ≥7 mm at the time of embryo transfer. Cycles with incomplete clinical data due to loss to follow-up were excluded. The study protocol was approved by the Institutional Ethics Committee (approval number 2501-ROM-010-LM), and all procedures were conducted in accordance with relevant guidelines and regulations. Given the retrospective design of the study, the requirement for informed consent was waived.

Laboratory IVF procedures

Oocyte insemination was performed using intracytoplasmic sperm injection (ICSI). Fertilization was assessed approximately 16–18 hours after injection through the identification of two pronuclei (2PN), and the fertilization rate was subsequently calculated. Embryos were cultured under controlled laboratory conditions until the blastocyst stage (day 5–6). Embryo development was monitored using a time-lapse incubation system, allowing continuous assessment of developmental kinetics without removing embryos from the incubator. Blastocysts were graded according to the Gardner scoring system (Gardner 1999) based on the degree of blastocoel expansion as well as the morphology of theinner cell mass (ICM) and trophectoderm (TE) (16). Embryo selection for transfer was based on a combination of blastocyst morphology, morphokinetic parameters obtained from time-lapse monitoring, and preimplantation genetic testing (PGT) results when available (Meseguer et al., 2011; Meseguer et al., 2012) (16,17).

Recent evidence suggests that paternal factors may significantly influence embryo development and morphokinetic parameters. Meseguer et al. demonstrated that time-lapse incubation combined with morphokinetic selection algorithms improves embryo classification and is associated with higher implantation and pregnancy rates compared with conventional embryo assessment (Meseguer et al., 2014) (18). Similarly, in a retrospective analysis including 2,726 blastocysts, Pellegrini et al. (2024) reported that sperm quality can influence the timing of early cleavage events and blastocyst development, emphasizing the relevance of paternal factors in embryo developmental dynamics and embryo selection strategies (19).

Embryo Transfer Procedures

Embryo transfers were performed under transabdominal ultrasound guidance with a moderately full bladder to optimize uterine visualization. All procedures were carried out by experienced clinicians according to a standardized protocol. A two-catheter system was used, consisting of an outer rigid catheter and an inner soft catheter. After placement of a speculum and gentle cleansing of the cervix, the outer catheter was advanced to the level of the internal cervical os. The embryo was then loaded into the inner catheter in approximately 20 µL of culture medium, with small air bubbles included to facilitate ultrasound visualization. Under continuous ultrasound guidance, the embryo was deposited approximately 1.0-1.5 cm from the uterine fundus. Following the transfer, the catheter was returned to the embryology laboratory, flushed with culture medium, and examined microscopically to confirm the absence of retained embryos. In cases of ER, the embryo was immediately reloaded and retransferred.

Endometrial Preparation and luteal support

The type of endometrial preparation was determined according to the physician’s clinical judgment and the individual characteristics of each patient and included either a natural cycle (NC) or hormone replacement therapy (HRT). For patients undergoing HRT, endometrial preparation was initiated 2–3 days after the onset of menstruation with oral estradiol valerate (6 mg/day; Progynova 2 mg, Schering). After

10–14 days of treatment, endometrial thickness was assessed by transvaginal sonography (TVS). Patients with an endometrial thickness >7 mm, quiescent ovaries, serum estradiol levels >100 pg/mL, and serum progesterone levels <1 ng/mL were scheduled for single embryo transfer (SET).

Luteal support in HRT cycles was initiated five days before SET with vaginal micronized progesterone (Progeffik, 200 mg; Effik Laboratories) administered at a dose of 400 mg every 12 hours. In women with serum progesterone levels <9.75 ng/mL on the day of embryo transfer, an additional subcutaneous progesterone injection (Pleyris, 25 mg; IBSA) was administered. Hormonal support was continued until 12 weeks of gestation.

In patients undergoing a natural cycle, follicular development was monitored by transvaginal ultrasound. Ovulation was triggered with human chorionic gonadotropin (hCG) (Ovitrelle 250 µg; Merck Serono) when endometrial thickness exceeded 7 mm and the leading follicle measured 16–20 mm. Embryo transfer was scheduled seven days after hCG administration. Luteal support was initiated five days before SET with vaginal micronized progesterone (Progeffik; Effik Laboratories) at a dose of 200 mg every 12 hours and was continued until 12 weeks of gestation.

Outcomes and statistical analysis

The primary outcome of the study was the live birth rate (LBR), defined as the delivery of a live infant at ≥24 weeks of gestation. Secondary outcomes included implantation rate (IR), defined as the number of gestational sacs per embryo transferred; biochemical pregnancy rate (BPR), defined as a serum β-hCG level >20 IU/L measured 12 days after embryo transfer; clinical pregnancy rate (CPR), defined as ultrasound confirmation of a gestational sac with fetal heartbeat at 6–7 weeks of gestation; ongoing pregnancy rate (OPR), defined as a viable intrauterine pregnancy beyond 12 weeks of gestation; and miscarriage rate (MR), defined as the loss of a clinical pregnancy before 24 weeks of gestation. All clinical outcomes were calculated per embryo transfer. Live birth rate was calculated among cycles with available delivery outcome data. Categorical variables were compared between study groups using the chi-square test, while continuous variables were analyzed using independent-samples t-tests or non-parametric alternatives when the assumption of normality was not satisfied. All analyses were performed at the single embryo transfer level. Associations between ER and reproductive outcomes were evaluated using logistic regression models. Reproductive outcomes were analyzed as binary variables, although they are presented descriptively as rates. Univariate logistic regression models were first used to estimate crude associations. Multivariate logistic regression analyses were subsequently performed to adjust for clinically relevant covariates selected for each outcome-specific model. These included patient, cycle, embryological, and procedural variables, such as age of the transferred oocyte, female BMI, embryo quality, day of embryo transfer, endometrial thickness, cycle type, embryologist category, and insemination-related variables. Results are reported as odds ratios (ORs) with corresponding 95% confidence intervals (CIs). A two-sided p-value <0.05 was considered statistically significant. Multicollinearity was assessed prior to model inclusion, and model fit was evaluated. All statistical analyses were performed using R software (version 4.5.1). Missing data were handled using a

complete-case approach for regression analyses. Descriptive statistics were based on available data; therefore, totals may differ across variables due to missing values.

RESULTS

A total of 4,593 single blastocyst embryo transfer cycles were analyzed, including 106 cycles complicated by ER and 4,487 cycles without ER (control group). Baseline demographic, clinical, and laboratory characteristics were comparable between the two groups, with no statistically significant differences observed (all $p > 0.05$; Table 1). Additional stimulation, insemination, and laboratory parameters are reported in Supplementary Table S1 and similarly showed no statistically significant differences between groups. Group differences in reproductive outcomes were reported in Table 2, whereas logistic regression models were used to estimate crude and adjusted associations (Table 3). The implantation rate was significantly higher in the control group compared with the ER group (55.4% vs. 39.2%, $p = 0.001$). In univariate logistic regression analysis, ER was significantly associated with a lower likelihood of implantation (OR 0.85, 95% CI 0.77–0.94, $p = 0.001$; Table 3).

Similarly, both biochemical and clinical pregnancy rates were significantly lower in the ER group compared with controls (58.1% vs. 42.5%, $p = 0.002$; OR 0.53, 95% CI 0.36–0.79, $p = 0.001$, and 52.0% vs. 38.7%, $p = 0.010$; OR 0.58, 95% CI 0.39–0.87, $p = 0.008$, respectively).

The ongoing pregnancy rate was lower in the ER group compared with the control group (32.1% vs. 39.9%). Univariate comparisons did not show a statistically significant difference ($p = 0.12$), whereas univariate logistic regression suggested a borderline association (OR 0.66, 95% CI 0.44–0.99, $p = 0.047$), which was attenuated after adjustment (aOR 0.65, 95% CI 0.41–1.01, $p = 0.05$).
Live birth rates were lower in cycles with ER (28.0% vs. 36.8%), although this difference did not reach statistical significance (OR 0.67, 95% CI 0.43–1.04, $p = 0.07$). Miscarriage rates were comparable between groups (10.4% vs. 13.3%; OR 0.71, 95% CI 0.38–1.34, $p = 0.29$). Figure 1 shows a consistent downward trend across reproductive outcomes in cycles complicated by ER, with the greatest differences observed in early outcomes and attenuation at later stages.

To further evaluate the association between ER and reproductive outcomes, multivariate logistic regression models were adjusted for outcome-specific clinically relevant covariates.
After adjustment, ER remained significantly associated with lower implantation (aOR 0.84, 95% CI 0.76–0.92, $p < 0.001$), biochemical pregnancy (aOR 0.49, 95% CI 0.32–0.75, $p < 0.001$), and clinical pregnancy rates (aOR 0.53, 95% CI 0.34–0.82, $p = 0.004$).

In contrast, no statistically significant associations were observed for ongoing pregnancy (aOR 0.65, 95% CI 0.41–1.01, $p = 0.05$), live birth (aOR 0.70, 95% CI 0.44–1.13, $p = 0.14$), or miscarriage (aOR 0.62, 95% CI 0.31–1.24, $p = 0.17$) (Table 3).

DISCUSSION

In this large retrospective cohort study including 4,593 single blastocyst transfer cycles, ER during embryo transfer was associated with significantly lower implantation and early pregnancy rates. However, no statistically significant association was observed between ER and live birth after adjustment for relevant confounders. These findings suggest that ER may be associated with impaired early implantation dynamics, although its impact on the ultimate probability of achieving a live birth remains uncertain.

The observed discrepancy between early reproductive outcomes and live birth rates should be interpreted cautiously. In particular, findings for ongoing pregnancy were not consistent across statistical approaches, with non-significant crude comparisons and only borderline associations in regression models. This lack of robustness suggests that these results should be interpreted with caution. ER is unlikely to represent an isolated causal event; rather, it may reflect underlying procedural complexity. Factors such as cervical mucus or blood contamination, uterine anatomical characteristics, or technically difficult embryo transfers may both increase the likelihood of ER and independently impair implantation. Therefore, ER may act as a proxy for difficult transfer conditions rather than being directly responsible for the observed reduction in early outcomes.

From a biological perspective, the difference between early and late reproductive outcomes may indicate that ER primarily affects the initial stages of implantation. Mechanical manipulation during retransfer, transient uterine contractions, or brief exposure of the embryo to suboptimal conditions could interfere with early embryo–endometrium interaction. However, embryos that successfully implant following retransfer appear to retain their developmental competence. The similar miscarriage rates observed between groups support the hypothesis that, once implantation is established, prior ER does not substantially affect subsequent pregnancy progression.

The lack of a statistically significant difference in live birth rates should not be interpreted as evidence of equivalence between groups. The relatively small number of ER events in our cohort may have limited the statistical power to detect moderate differences in live birth, as suggested by the width of the confidence intervals. Therefore, a clinically meaningful effect of ER on live birth cannot be definitively excluded.

The clinical relevance of ER remains debated in the literature. Some studies have suggested that difficult embryo transfers or repeated catheter insertions are associated with reduced implantation and pregnancy rates, potentially due to mechanical endometrial trauma, increased uterine contractility, or prolonged embryo exposure outside optimal culture conditions. Conversely, other studies have reported no significant differences in live birth outcomes when immediate retransfer is performed under standardized conditions. Our findings are consistent with this latter body of evidence, suggesting that although ER is associated with impaired early outcomes, its impact on final treatment success remains uncertain when promptly managed.

This study has several strengths. The large sample size and the restriction to single blastocyst transfers reduce confounding related to embryo number and developmental stage. In addition, the use of

multivariable models allowed adjustment for several clinically relevant covariates.

Nevertheless, important limitations should be acknowledged. First, the retrospective design precludes causal inference. Second, residual confounding cannot be excluded, particularly for procedural variables such as transfer difficulty, uterine contractility, or operator technique, which were not directly captured. Third, although the overall cohort was large, the number of ER cases was relatively limited, reducing the ability to detect small or moderate differences in live birth. Furthermore, the analysis was conducted at the embryo transfer level, and potential clustering of multiple cycles within the same patient may have influenced the estimates. Finally, the single-center design may limit the generalizability of these findings to other clinical settings.

From a clinical perspective, ER should be considered a procedural event requiring attention, but not necessarily a determinant of treatment failure. While optimizing embryo transfer technique remains essential to minimize ER occurrence, clinicians may cautiously reassure patients that immediate retransfer does not appear to markedly compromise the likelihood of achieving a live birth, although some degree of uncertainty remains.

CONCLUSION

ER during single blastocyst transfer appears to be an infrequent procedural event associated with poorer early IVF outcomes, while its impact on live birth remains uncertain. When promptly recognized and managed with immediate retransfer, ER does not appear to substantially compromise the probability of achieving a live birth, although a moderate effect cannot be excluded. These findings support the importance of careful embryo transfer technique while providing cautious reassurance to clinicians and patients. Further prospective, multicentre studies incorporating detailed procedural variables and appropriate statistical approaches are needed to better define the clinical significance of ER.

Declarations

Consent to participate

All patients provided informed consent for the use of anonymized clinical data for research purposes according to institutional policies.

Consent for publication

Not applicable.

Ethical approval

This study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Institutional Ethics Committee (approval number: 2501-ROM-010-LM).

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No funding was received for conducting this study.

Author Contribution

LM and LP contributed equally to this work. MC performed the final revision of the manuscript.CZ, LL, and AP reviewed and approved the final version of the manuscript.

Data Availability

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

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Tables

TABLE 1 - Baseline characteristics of the study population.

Variable	Control group (n=4487)	ER group (n=106)	p-value
Female age (years, mean ± SD)	41.29 ± 5.30	41.72 ± 4.98	0.335
Female BMI (kg/m², mean ± SD)	22.51 ± 3.72	23.22 ± 4.42	0.076
Male age (years, mean ± SD)	41.57 ± 7.63	42.10 ± 7.57	0.473
Male BMI (kg/m², mean ± SD)	25.34 ± 3.39	25.20 ± 3.25	0.710
PGT-A cycles, n (%)	1702 (37.9%)	44 (41.5%)	0.516
Endometrial thickness (mm, mean ± SD)	8.63 ± 2.56	8.63 ± 1.43	0.451
Oocyte origin, n (%)			
Donor oocytes	3024 (67.4%)	71 (67.0%)	1.000
Autologous oocytes	1463 (32.6%)	35 (33.0%)	
Embryo quality, n (%)			
Grade A	1456 (34.0%)	33 (33.0%)	0.076
Grade B	2396 (55.9%)	49 (49.0%)	
Grade C	432 (10.1%)	18 (18.0%)	
Grade D	2 (0.05%)	0 (0.0%)	
Cycle type, n (%)			
Stimulated cycle	68 (1.5%)	1 (0.9%)	0.888
Natural cycle	561 (12.5%)	13 (12.3%)	
HRT cycle	3857 (86.0%)	92 (86.8%)	
Ovarian stimulation			
Duration of stimulation (days, mean ± SD)	10.87 ± 9.79	10.89 ± 4.31	0.988
Day of embryo transfer, n (%)			
Day 5	3810 (84.9%)	89 (84.0%)	0.894
Day 6	677 (15.1%)	17 (16.0%)	

Blastocysts were graded morphologically using the Gardner & Schoolcraft system (expansion 1–6 and ICM/TE quality A–C). Blastocysts with patterns 4AA, 4AB or 5AA were categorized as High Quality (A), while patterns 3AB or 4BB were Intermediate (B), and those with any C component (e.g., 4BC, 5BC) were considered Low Quality (C). Data may not sum to totals due to missing values. Embryo quality data were missing for 201 cycles in the control group and 6 cycles in the ER group. One cycle had missing data for cycle type.

TABLE 2 – IVF and clinical outcomes of ER.

Outcome	Control group (n/N, %)	ER group (n/N, %)	p-value
Implantation rate	2486/4487 (55.4%)	42/106 (39.2%)	0.001
Biochemical pregnancy	2608/4487 (58.1%)	45/106 (42.5%)	0.002
Clinical pregnancy	2329/4487 (52.0%)	41/106 (38.7%)	0.010
Ongoing pregnancy	1791/4487 (39.9%)	34/106 (32.1%)	0.126
Miscarriage	597/4487 (13.3%)	11/106 (10.4%)	0.463
Live birth	1453/3948 (36.8%)	28/100 (28.0%)	0.089

TABLE 3 - Univariate and multivariate analysis of ER.

Outcome	OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Implantation rate	0.85 (0.77–0.94)	**0.001**	0.84 (0.76–0.92)	**<0.001**
Biochemical pregnancy	0.53 (0.36–0.79)	**0.001**	0.49 (0.32–0.75)	**<0.001**
Clinical pregnancy	0.58 (0.39–0.87)	**0.008**	0.53 (0.34–0.82)	**0.004**
Ongoing pregnancy	0.66 (0.44–0.99)	**0.047**	0.65 (0.41–1.01)	0.055
Live birth	0.67 (0.43–1.04)	0.073	0.70 (0.44–1.13)	0.141
Miscarriage	0.71 (0.38–1.34)	0.293	0.62 (0.31–1.24)	0.176

Figures

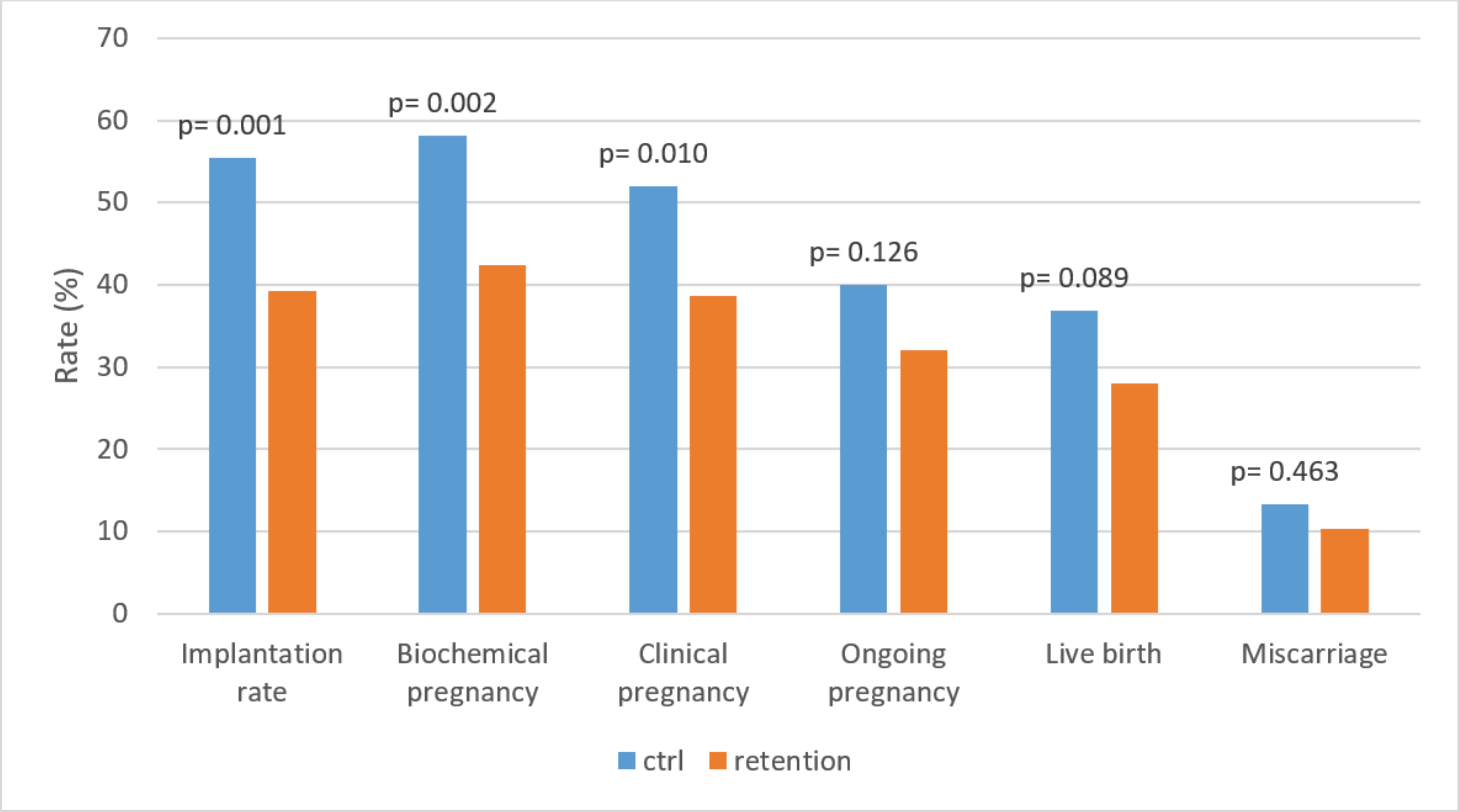


Figure 1

Comparison of reproductive outcomes in cycles with and without ER.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTableS1.docx