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Effect of prior cesarean delivery on the outcomes of intracytoplasmic sperm injection

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Objective: This study was conducted to investigate the impact of previous delivery mode on pregnancy outcomes in patients with secondary infertility after frozen-thawed embryo transfer.
Methods: This prospective observational study included 140 patients experiencing secondary infertility. Of these, 70 patients had a previous cesarean delivery (CD), while the remaining 70 patients had a previous normal vaginal delivery (NVD). The primary outcome was the implantation rate. The secondary outcomes included rates of clinical pregnancy, chemical pregnancy, miscarriage, and ectopic pregnancy.
Results: The comparison of all fertility outcomes between the two groups revealed no statistically significant differences. The implantation rate was 40.4% in the CD group and 41.7% in the NVD group (p=0.842). The clinical pregnancy rate was 50% in the CD group and 49.3% in the NVD group (p=0.932), while the chemical pregnancy rate was 14.6% in the CD group and 19% in the NVD group (p=0.591). The miscarriage rates in the CD and NVD groups were 20% and 17.6%, respectively (p=0.803). One case of tubal ectopic pregnancy occurred in the NVD group (1.4%).
Conclusion: The mode of prior delivery did not significantly impact pregnancy outcomes following frozen-thawed embryo transfer.

Keywords: Cesarean section; Embryo implantation; Frozen embryo transfer; Intracytoplasmic sperm injection; Normal vaginal delivery

Introduction

In the coming years, during which both unmet need and overuse are anticipated to coexist, the usage of cesarean sections (CS) is expected to continue rising globally [1]. Despite being a frequently necessary surgical procedure, CS may lead to increased risk of several obstetric complications in subsequent pregnancies. These complications include infection, hemorrhage, malplacentation, cesarean scar pregnancy, morbidly adherent placenta, and uterine rupture [2]. CS has been suggested to potentially increase a woman's susceptibility to subfertility or even infertility [3,4]. This detrimental effect on fertility could be attributed to infection, the formation of adhesions, disruption of the placental bed [5], or the hostile uterine environment created by the presence of the CS niche [6].

In assisted reproductive treatment (ART) cycles, embryos are directly transferred into the uterine cavity, eliminating the need for the tubal component. Consequently, the implantation process is considered the most critical factor in predicting pregnancy outcomes [7]. The current understanding of the impact of prior CS on intracytoplasmic sperm injection (ICSI) outcomes is limited, with inconsistencies observed in the clinical pregnancy rate (CPR), miscarriage rate (MR), and live birth rate (LBR). Several studies, including those by Diao et al. [8] in 2021, Patounakis et al. [9] in 2016, and Zhang et al. [10] in 2016, have found no detrimental effect of previous CS on ICSI outcomes. However, other researches have indicated significant reductions in the LBR and CPR in patients with prior CS, including the studies of Riemma et al. [11] in 2021, Zhao et al. [7] in 2021, Wang et al. [12] in 2020, Vissers et al. [6] in 2020, and van den Tweel et al. [13] in 2019. Given these conflicting results, a clear need exists for more robust studies. Therefore, this study was undertaken to investigate

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the influence of prior delivery mode on ICSI outcomes. Notably, the majority of the aforementioned studies were retrospective, making the prospective nature of our study its most distinctive feature.

Methods

1. Study design and participants

The sample size was determined using PASS 2020 Power Analysis and Sample Size Software (PASS 2020; NCSS; available at ncss.com/software/pass). To evaluate the effect of a previous CS on ICSI outcomes in women using frozen-thawed embryos, a minimum total sample size of 140 eligible female patients experiencing secondary infertility and undergoing *in vitro* fertilization (IVF) was computed. This sample size, divided into two groups of 70, was deemed necessary to assess the proportional difference in the implantation rate between groups, considering a 95% confidence level and 80% power and using the chi-square test [14]. A total of 140 patients experiencing secondary infertility were selected from a private IVF/ICSI center in Alexandria, Egypt between January 2022 and March 2023. Patients who fulfilled the inclusion criteria were recruited on the day of blastocyst freezing. For comparison between groups, 70 patients had a history of one previous CS (termed the cesarean delivery [CD] group), while the remaining 70 had a history of normal vaginal delivery (NVD). Before couples were enrolled in our study, they underwent a standard examination protocol, which included semen analysis, ovarian reserve testing, transvaginal ultrasonography for uterine assessment, and antral follicle count.

The inclusion criteria were as follows: women 20 to 35 years old with a body mass index of 18 to 30 kg/m^2, who were indicated for the freeze-all technique due to their high risk of developing ovarian hyperstimulation syndrome, the presence of treatable tubal or uterine anomalies discovered during controlled ovarian stimulation (COS), or elevated serum progesterone levels. The exclusion criteria included women with severe forms of endometriosis, congenital uterine anomalies, a scarred uterus resulting from previous myomectomy, moderate to severe intrauterine adhesions, fibroid uteri, or poor-quality embryos. Additionally, women with untreated hydrosalpinx and those who had undergone all fresh transfers were excluded. The study did not include patients undergoing preimplantation genetic testing.

The study got approved from the ethical committee of Faculty of Medicine, Alexandria University on January 20, 2022 with a serial number (0201611). Before participating in the study, each patient was counseled and provided written informed consent.

2. Ovarian stimulation and ICSI

All patients underwent COS using a fixed antagonist protocol. The initial dose of gonadotropin was adjusted according to anti-Müllerian hormone (AMH) level: a dose of 225 IU was administered to average responders with AMH levels ranging from 1 to 3 ng/mL, a dose of 300 IU was given to poor responders with AMH levels below 1 ng/mL, and a dose of 150 IU was given to high responders with AMH levels above 3 ng/mL. On the 5th day of stimulation, the gonadotropin doses were further modified based on the patient's response, and 0.25 mg of subcutaneous Cetrotide (Merck Darmstadt), was added daily. When at least three follicles of 17 mm or larger were observed, an ovulation trigger was administered, followed by oocyte retrieval after 35 to 36 hours. At our center, ICSI was routinely performed for all cases. This was followed by grading of the blastocysts according to the Gardner scoring system [15] and the subsequent freezing of all blastocysts.

3. Endometrial preparation for frozen-thawed embryo transfer cycle

All participating women were administered oral estradiol valerate (8 mg/day) starting on the 2nd day of the menstrual cycle. On day 10 of treatment, the thickness of the endometrium was evaluated using vaginal ultrasonography. Once the endometrial thickness reached or exceeded 7 mm, all participants were given additional treatment. This included 400 mg of progesterone in vaginal suppositories twice daily and 100 mg of progesterone administered intramuscularly each day, in conjunction with the ongoing estrogen treatment. On the 6th day of progesterone treatment, a frozen-thawed embryo was transferred. The administration of both estrogen and progesterone continued until week 9 or 10 of gestation.

4. Outcomes

The primary outcome under consideration was the implantation rate, defined as the ratio of the total number of gestational sacs observed via transvaginal ultrasound to the total number of transferred embryos per group. The secondary outcomes included rates of clinical pregnancy, chemical pregnancy, miscarriage, and ectopic pregnancy. Clinical pregnancy was determined by the ultrasonographic visualization of a viable embryo within the uterine cavity 4 weeks after embryo transfer (ET). The CPR was calculated by dividing the number of clinical pregnancies by the number of ET procedures. Chemical pregnancy was defined as a positive pregnancy test result 11 days after ET, followed by abnormally increasing or subsequently decreasing human chorionic gonadotropin levels, coupled with the absence of a visualized gestational sac on transvaginal ultrasound. The chemical pregnancy rate was calculated by dividing the total number of chemical pregnancies by the total number of positive pregnancy tests following ET. The MR was computed by dividing the total number of pregnancies that failed to progress after the visual-

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Table 1. Demographic characteristics of the examined groups

Parameter	CD (n = 70)	NVD (n = 70)	*p*-value
Age (yr)	30.80 ± 3.17	29.52 ± 3.84	0.063[a]
BMI (kg/m^2)[c]	25.64 ± 3.67	25.96 ± 3.41	0.704[a]
AFC	22.0 (13.0–35.0)	24.0 (11.0–45.0)	0.546[a]
Basal AMH level	3.91 ± 3.83	4.07 ± 3.24	0.171[a]
Cause of infertility[b]			0.074[c]
Ovarian	17 (24.3)	12 (17.1)	
Tubal	4 (5.7)	3 (4.3)	
Male sex	28 (40.0)	23 (32.9)	
Combined (male and female)	13 (18.6)	10 (14.3)	
Unexplained	8 (11.4)	22 (31.4)	

Values are presented as mean±standard deviation, median (interquartile range), or number (%).
CD, cesarean delivery; NVD, normal vaginal delivery; BMI, body mass index; AFC, antral follicle count; AMH, anti-Müllerian hormone.
[a]Mann-Whitney *U* test; [b]Chi-square test; [c]Monte-Carlo test.

Table 2. Comparison of ICSI data between groups

Parameter	CD (n = 70)	NVD (n = 70)	*p*-value
No. of retrieved oocytes	14.0 (10.0–19.0)	15.0 (9.0–18.0)	0.950[a]
No. of metaphase II oocytes	12.0 (9.0–16.0)	13.0 (8.0–16.0)	0.912[a]
Fertilization rate (%)	89.18 (80.0–100.0)	90.83 (83.3–95.65)	0.594[a]
No. of embryos according to quality			0.106[b]
Excellent	22 (31.4)	31 (44.3)	
Good	22 (31.4)	12 (17.1)	
Average	26 (37.1)	27 (38.6)	
No. of transferred embryos			0.866[b]
Single FET	36 (51.5)	37 (52.9)	
Double FET	34 (48.6)	33 (47.1)	

Values are presented as median (interquartile range) or number (%).
ICSI, intracytoplasmic sperm injection; CD, cesarean delivery; NVD, normal vaginal delivery; FET, frozen-thawed embryo transfer.
[a]Mann-Whitney *U* test; [b]Chi-square test.

Table 3. Comparison of pregnancy outcomes between groups

Parameter	CD (n = 70)	NVD (n = 69[a])	*p*-value
Implantation rate	42/104 (40.4)	43/103 (41.7)	0.842[b]
Clinical pregnancy rate	35/70 (50)	34/69 (49.3)	0.932[b]
Chemical pregnancy rate	6/41 (14.6)	8/42 (19)	0.591[b]
Miscarriage rate	7/35 (20)	6/34 (17.6)	0.803[b]

Values are presented as number/total number (%).
CD, cesarean delivery; NVD, normal vaginal delivery.
[a]One patient withdrew before pregnancy results were obtained; [b]Chi-square test.

ization of an intrauterine gestational sac by the total number of clinically recognized intrauterine pregnancies. Finally, the ectopic pregnancy rate was calculated by dividing the number of ectopic pregnancies by the total number of ET procedures.

5. Statistical analysis

The data were entered into a computer and analyzed using SPSS version 20.0 (IBM Corp.) [16,17]. Qualitative data were characterized using numbers and percentages. The Kolmogorov-Smirnov test was employed to confirm the normality of the distribution. Quantitative data were described using range (minimum and maximum), mean±standard deviation, and median (interquartile range). The significance of the results obtained was determined at the 5% level.

The tests employed included. (1) The chi-square test was used to compare categorical variables across different groups. (2) The Monte-Carlo test and Fisher exact test were utilized to apply a correction to the chi-square result when over 20% of the cells had an expected count of less than 5. (3) The Mann-Whitney test was used for quantitative variables that were not normally distributed, to compare between the two studied groups.

Results

No significant differences in baseline characteristics were present between the two groups, as shown in Table 1. In terms of the ICSI outcome parameters, no statistically significant differences were observed between groups in the number of oocytes retrieved, the number of mature (metaphase II) oocytes, or the fertilization rate, as shown in Table 2. The embryos were evaluated and graded based on the criteria established by Gardner, which consider blastocoel expansion as well as the quality of the inner cell mass and the trophectoderm. Subsequently, blastocysts were assigned a grade ranging from excellent to poor, reflecting their quality from highest to lowest. This grading system was designed to facilitate the input of scores into numerical databases and to aid in statistical analysis. The grading scale is as follows [18]: excellent (≥3AA); good (3,4,5,6 AB, 3,4,5,6 BA, and 1,2 AA); average (3–6BB, 3–6AC, 3–6CA, 1–2AB, and 1–2BA); and poor (1–6BC, 1–6CB, 1–6CC, and 1–2BB). When comparing the quality of transferred embryos between the two groups under study, no statistically significant difference was found (Table 2). The maximum number of embryos transferred in any given procedure was two.

Regarding the implantation rate (Table 3), the CD group had a rate of 40.4%, while the NVD group had a value of 41.7%. This difference was not statistically significant (p=0.842). The CPR was 50% in the CD group and 49.3% in the NVD group, which was also not a statistically significant distinction (p=0.932). In the CD group, six cases were diagnosed as chemical pregnancies, making up 14.6% of the group, while in the NVD group, eight cases were diagnosed as such, ac-

Table 4. Relationship between the presence of a niche and pregnancy outcomes within the cesarean delivery group

Parameter	Subgroup (a) (no niche identified) (n=56)	Subgroup (b) (niche identified) (n=14)	*p*-value
Not pregnant	24 (42.9)	5 (35.7)	0.627[a]
Pregnant	32 (57.1)	9 (64.3)	
Chemical pregnancy rate	4 (12.5)	2 (22.2)	0.597[b]
Clinical pregnancy rate	28 (87.5)	7 (77.8)	0.597[b]

Values are presented as number (%).
[a]Chi-square test; [b]Fisher exact test.

counting for 19%. Again, no significant difference was present between the groups (p=0.591). Regarding the MR, 20% of the CD patients experienced a miscarriage, compared to 17.6% in the NVD group. This difference was not statistically significant (p=0.803). One case of tubal ectopic pregnancy arose in the NVD group (1.4%), but no significant difference was present between groups. In the NVD group, one case was withdrawn before the pregnancy results were obtained. The CD group was also divided into two subgroups (Table 4): subgroup (a), which consisted of 56 cases for which no niche could be detected during ultrasound examination, and subgroup (b), consisting of 14 cases for which a niche was identified. In subgroup (a), pregnancy occurred in 32 cases (57.1%), while in subgroup (b), nine cases (64.3%) resulted in pregnancy. No statistically significant difference was found between the two subgroups in terms of pregnancy outcome. In subgroup (a), chemical pregnancy occurred in four cases (12.5%), clinical pregnancy in 28 cases (87.5%), and miscarriage in five cases (15.6%). In subgroup (b), chemical pregnancy occurred in two cases (22.2%), clinical pregnancy in seven cases (77.8%), and miscarriage in two cases (22.2%).

Discussion

For the past 25 years, the global rate of CS has been increasing [19]. It is crucial to assess the long-term effects of this trend on women's health. The underlying causes of reduced fertility and unfavorable pregnancy outcomes after prior CS have been extensively researched, revealing a significant decrease in the likelihood of a subsequent pregnancy after a previous CS [20-22]. Many factors could detrimentally impact future fertility following CS. These may lead to post-surgical complications such as intrauterine adhesions, CS niche, or the effects of uterine rupture, in addition to intra-abdominal adhesions, Fallopian tube dysfunction, and uterine abnormalities induced by the cesarean scar [20]. However, the relationship between CS and the outcomes of ART has remained ambiguous, with conflicting findings reported [9-12]. Therefore, we conducted this study to examine the influence of prior delivery mode on ICSI outcomes.

Our findings align with the 2022 results of Bayram et al. [23], who found that the potential negative effects of CS can be mitigated when a euploid frozen ET is performed, following the exclusion of intracavitary fluid. Similarly, Diao et al. [8] proposed in 2021 that a previous CS without a niche does not impair pregnancy outcomes after IVF or ICSI relative to a previous NVD. Our results also concur with the research conducted by Patounakis et al. [9] and Zhang et al. [10], who determined that a previous CS does not influence embryo implantation and pregnancy outcomes in IVF cycles.

In contrast, in 2021, two systematic reviews and meta-analyses conducted by Riemma et al. [11] and Zhao et al. [7] indicated that a history of CS was associated with significantly reduced CPR and LBR, as well as an increased MR, after ART compared with women with previous vaginal deliveries. This could be attributed to the diminished contractility of the fibrotic tissue and myometrium at the scar site, which can lead to an accumulation of intracavitary fluid. In addition, findings have indicated that a scarred area at the site of a previous CS typically exhibits significantly reduced vascularization, decreased leukocyte infiltration, and delayed endometrial maturation compared to a non-scarred uterus. This suggests that regulation of endometrial receptivity may be impaired following CS [24]. These findings could potentially account for the decreased pregnancy and implantation rates observed in women with a prior CS. Similar findings have been reported by multiple retrospective clinical studies, indicating that the LBR and CPR in women with previous CS are lower than in those with previous vaginal delivery [6,25,26].

A major strength of our study lies in its prospective nature, as most studies assessing the impact of CS on ICSI outcomes have been retrospective. Another advantage is that we matched cases in both groups by age, body mass index, and ovarian reserve, further enhancing the validity of the findings. However, the present study does have limitations. To obtain more reliable data, the sample size should be expanded. Additionally, we did not gather detailed information about the category of CS (emergency vs. elective) [27], the type of CS incision, or the influence of previous CS on LBR. Therefore, further research is required to clarify these aspects.

In conclusion, the mode of previous delivery did not significantly impact pregnancy outcomes following frozen-thawed ET. Our findings may be explained by the fact that transfers only took place in frozen (more physiological) cycles, rather than fresh ones, once intracavitary fluid had been eliminated [23]. As the patients in the CS group had a history of only one previous CS, another possible explanation could be that the risk involved is minimized relative to multiple CS procedures.

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Conflict of interest

No potential conflict of interest relevant to this article was reported.

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Author contributions

Conceptualization: MSEAR. Data curation: HMAAM. Formal analysis: MAEES. Methodology: HMAAM. Project administration: MSEAR. Visualization: MAEES. Writing-original draft: SAG. Writing-review & editing: SAG.

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