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Original Research

Unique Implantation Window as a Possible Reason of Embryo Transfer Failure. Retrospective Analysis

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Abstract

Background: To explore the predictive value of endometrial receptivity and pregnancy outcomes using pipelle biopsy examination during the luteal phase of the menstruation cycle in infertile women. We also compare the importance of this factor with other potential causes of infertility. **Methods**: This retrospective study included 279 women with repeated implantation failures. All patients were examined and treated at the Intersono *in vitro* fertilization (IVF) center. To determine the cause of the implantation failure, an implantation window (IW) was defined for all patients included in this retrospective cohort. Examinations were performed by three-fold aspiration biopsies of the endometrium during the luteal phase of the cycle as this period is when the endometrium is most receptive for implantation. Analysis of the tissue was done by scanning electron microscopy of endometrial tissues and, based on results obtained, an endometrial preparation protocol was individualized for the next attempt. Statistical analyses were performed using Microsoft Excel (Office 365) and RStudio software v. 1.4.1106. **Results**: According to the results obtained, 206 women included in this study displayed a unique IW (74%), and 73 women had a standard IW period (26%). Patient characteristics, screening indicators, previous IVF treatment details, IVF cycle characteristics, as well as number, quality, and stage of embryos transferred were comparable between the two groups. Clinical pregnancy rates of 59.2% (95% confidence interval (CI) 52.5–65.8) versus 57.5% (95% CI 46.1–68.6) ($p = 0.80$), and live birth rates of 50.7% (95% CI 43.9–57.6) versus 47.9% (95% CI 36.6–59.4) ($p = 0.49$) in the unique and standard IW groups, respectively, did not show significant differences. **Conclusions**: Unique IW is one of the underlying causes of implantation failures. The personalization of an endometrial preparation protocol is a method to improve IVF outcomes.

Keywords: endometrial receptivity; implantation window; pipelle biopsy; implantation failure; electron microscopy of endometrial tissue

1. Introduction

Infertility is a pressing problem in both medicine and society. Sociodemographic and economic conditions contribute to a negative trend within the reproductive age population in Europe [1]. *In vitro* fertilization (IVF) is the most commonly used method of infertility treatment; however, IVF does not guarantee pregnancy. According to currently available evidence, 60% of couples who sought medical help in specialized institutions must undergo a second attempt, and some require three or more IVF procedures [2]. Embryonic factors represent approximately one third of reasons for IVF failure, with the remainder due to implantation problems [3–5]. There are well-characterized morphological and molecular markers of implantation but the full dynamics of the process, as well as the relative importance of each step in the process, remain unclear [6].

One of the important factors in IVF failure is a lack of synchronicity between endometrial maturation and embryo development as this can lead to decreased endometrial receptivity and a lack of implantation. Endometrial receptivity is a complex process that provides the embryo with the opportunity to attach, invade, and further develop, culminating in a new individual and continuation of the species [7]. The time period when the endometrium is receptive to blastocyst implantation is termed the implantation window (IW). During this period, the plasma membrane of the endometrial epithelium lose microvilli and the apical surfaces of the cells form a dome-shaped protrusion termed pinopodes [8]. Pinopodes formation during the luteal phase [9] is a major indicator of endometrial readiness for embryo implantation, and an assessment of this condition has been proposed as one of the markers of endometrial receptivity [10–13]. In a standard IW, pinopodia are formed 6 or 7 days following luteinizing hormone (LH) surge with day 0 being the peak day of LH levels before ovulation. The formation of pinopodia earlier or later than LH + 6/7 days can result in failed embryo implantation in IVF. The reason is a fixed day of embryo transfer (ET), which is incorrectly timed in women with an irregular IW.

2. Materials and Methods

All patients of retrospective cohort were examined according to the order of the Ministry of Health of Ukraine 787, local clinical protocol "Recurrent implantation fail-

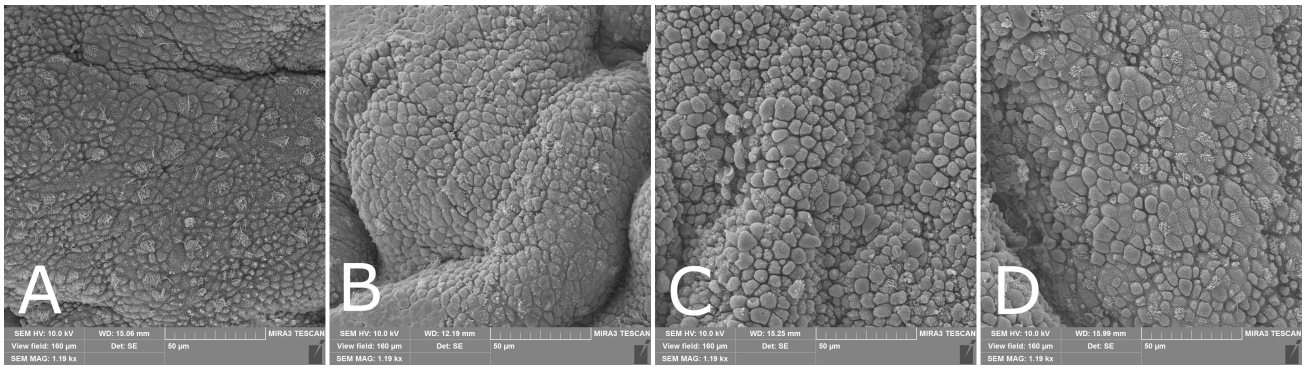

Fig. 1. Different patterns of pinopod expression according to electronic microscopy. Pinopod expression was scored as follows: (A) absence of pinopods; (B) pinopods starting to form; (C) formed pinopods; or (D) pinopod regression. Scale bars are provided for each micrograph.

ures" and the recommendations of the European Society of Human Reproduction and Embryology.

An artificial cycle was used for endometrium preparation and IW determination. Estrogen administration was started in day 2 or 3 of the cycle with oral estradiol valerate given at a dosage of 4 mg, and this dosage was increased to 6 mg daily in day 7 or 8 day of the cycle. Progestins were administered from day 13–15 of the cycle at a dosage of 400 mg daily given intravaginally after an endometrial thickness of over 7 mm was achieved. Endometrial samples were obtained by pipelle biopsy. Biopsies were conducted three times during the artificial cycle on 6, 8 and 10 of progestin administration.

Endometrial tissues were gently rinsed in phosphate-buffered saline (PBS) to remove blood and surface debris, and placed in fixative. A small portion of each specimen was fixed in 2.5% glutaraldehyde and, after several rinses in buffer, dehydrated by increasing concentrations of ethanol (25%/50%/75%) (KYIVSPYRT, Sumy, Ukraine). Specimens were transferred in absolute ethanol to a Samdri model 780-A Critical Point Dryer (Tousimis, Rockville, MD, USA), dried using liquid carbon dioxide (CRYOGENSERVICE, Kyiv, Ukraine), mounted onto aluminum scanning electron microscopy stubs (lot 16111, Ted Pella Inc. Redding, CA, USA), and sputter coated with gold:palladium alloy (50:50) to a thickness of 300 nm using a Gatan pecs 682 instrument (Gatan AMETEK., Pleasanton, CA, USA). Scanning electron microscopy (SEM) was performed using a Tescan Mira 3 LMU microscope (TESCAN ORSAY HOLDING, a.s., Brno – Kohoutovice, Czech Republic) . All SEM parameters, such as accelerating voltage, working distance, magnification, and view field, are provided in the micrographs.

Pinopods were defined as smooth apical protrusions from the surface epithelium without microvilli. Pinopod expression was scored as follows: A (absence of pinopods); B (pinopods starting to form); C (formed pinopods); D (pinopod regression) (see Fig. 1).

Table 1. Structure of infertility factors in retrospective groups (% (95% CI)).

Infertility factor	Group 1	Group 2	*p*
Tube-peritoneal	27.2 (21.3–33.5)	24.7 (15.5–35.1)	0.67
Chronic anovulation	25.7 (20.0–31.9)	35.6 (25.1–46.9)	0.13
Low ovarian reserve	15.5 (10.9–20.8)	19.2 (11.0–28.9)	0.49
Uterine	4.4 (2.0–7.6)	8.2 (3.1–15.6)	0.28
Immunological	9.7 (6.1–14.1)	11.0 (4.9–19.1)	0.77
Endocrine	6.8 (3.8–10.6)	12.3 (5.8–20.8)	0.20
Male	39.8 (33.2–46.6)	35.6 (25.1–46.9)	0.53
Unexplained	6.8 (3.8–10.6)	11.0 (4.9–19.1)	0.31

95% CI, 95% confidence interval.

An IVF program was re-attempted upon the next menstrual cycle, taking into account the individual characteristics of the IW. Patients were divided into two groups. The first group consisted of women who displayed a standard IW, specifically, those displaying pinopod formation (C) on the 6th day of progestin administration. The second group, defined as those with irregular IW, included patients who displayed pinopod formation either before or after the 6th day of progestin administration. We note that we failed to observe any patient when pinopods were absent in all three time samples.

Standard IW patients underwent a single embryo transfer (ET) on day 6 or 7 of progestin administration. Irregular IW patients had a double ET with the first transfer done on the first day when pinopods start to form (i.e., C), and a second transfer conducted two days later.

To optimize the statistical analysis of embryo quality assessment and its impact on the results of IVF programs, the classification of the Istanbul Consensus 2011 [14] was used. Statistically categorical (nominal) variables are presented as percentages and the 95% confidence interval was calculated using the Wald method. For particles which were less than 25%, or more than 75%, the confidence interval was calculated by the arcsin Fisher transformation method.

Table 2. Structure of gynecological morbidity in retrospective groups (% (95% CI)).

Gynecological pathology	Group 1	Group 2	*p*
Pelvic inflammatory disease	26.7 (20.9–32.9)	27.4 (17.9–38.1)	0.91
Endometriosis. including	17.0 (12.2–22.4)	21.9 (13.3–32.1)	0.38
• Adenomyosis	10.2 (6.5–14.7)	9.6 (4.0–17.3)	0.88
• Endometrioma	3.4 (1.4–6.3)	6.8 (2.2–13.7)	0.29
• Other endometriosis	3.4 (1.4–6.3)	5.5 (1.5–11.8)	0.48
Uterine myoma	11.7 (7.6–16.4)	9.6 (4.0–17.3)	0.62
Endometrial polyp	10.7 (6.8–15.3)	15.1 (7.9–24.1)	0.35
Sexually Transmitted Infections	9.7 (6.1–14.1)	12.3 (5.8–20.8)	0.55
Polycystic ovary syndrome (PCOS)	8.7 (5.3–13.0)	11.0 (4.9–19.1)	0.59
Ovarian cysts	7.3 (4.1–11.2)	11.0 (4.9–19.1)	0.37
Müllerian duct anomalies (MDAs)	4.9 (2.4–8.2)	6.8 (2.2–13.7)	0.55
Benign Breast Disease	4.4 (2.0–7.6)	8.2 (3.1–15.6)	0.28
Endometrial hyperplasia	3.9 (1.7–6.9)	5.5 (1.5–11.8)	0.59
Asherman syndrome	1.0 (0.1–2.8)	1.4 (0.0–5.3)	0.79

95% CI, 95% confidence interval.

Table 3. Average values of the concentration of reproductive hormones in retrospective groups (Median (25%; 75%)).

Hormone	Group 1	Group 2	*p*
Anti-Mullerian hormone (AMH), ng/mL	2.0 (0.9; 3.9)	2.4 (0.9; 4.4)	0.89
Follicle-stimulating hormone (FSH), mIU/mL	7.0 (5.6; 9.4)	8.3 (5.8; 11.8)	0.39
Luteinizing hormone (LH), mIU/mL	6.0 (4.4; 7.6)	5.6 (4.4; 7.8)	0.97
Prolactin, ng/mL	14.1 (10.2; 20.3)	13.2 (9.9; 18.7)	0.56

The difference between groups of categorical (nominal) variables was studied using a frequency table and the presence of reliability established using the Pearson chi-square test. If the expected value in one from the cells of the frequency table was less than 5 then the exact one was used in a Fisher's test.

At the first stage of statistical processing, numerical variables passed checks for distribution normality using the Shapiro-Wilk test. According to the results of the normality test, the corresponding data normal distribution was presented in the form of M $\pm$ SD (where M is mean value and SD is the standard deviation). Data that did not correspond to the normal distribution was presented in the form of the median and quartile, Me (25%; 75%), where Me is the median (50th percentile), 25% is the first quartile (25th percentile) and 75% is the third quartile (75th percentile). To detect the validity of the difference between two groups we used either the t test for unrelated groups for data with normality distribution or a Mann-Whitney U-test. To test the significance of the difference between related groups, the *t* test for related groups was used or the Wilcoxon sign-rank test (i.e., Mann-Whitney-Wilcoxon text). Statistical correlation was investigated using the Spearman test in R.

Sample differences were considered significant at $p < 0.05$. Statistical calculations were carried out using Microsoft Excel (Office 365) (2KB4Y-6H9DB-BM47K-749PV-PG3KT, One Microsoft Way, Redmond, Washington, USA) and R Studio software v. 1.4.1106 (http://www.gnu.org/licenses/agpl-3.0-standalone.html).

3. Results

This study was carried out from 2012 to 2020 and included 279 women of reproductive age (34.1 years old, range 31.0 to 38.0) with primary 78.0% (95% confidence interval (CI) 71.0–82.5) and secondary 22.0% (95% CI 16.0–30.1) infertility. Based on the findings from scanning electron microscopy experiments, all patients were divided into two groups: Irregular IW (Group 1) composed of 206 women (74%); and standard IW (Group 2) composed of 73 women (26%). The dominant form of infertility, in both groups, was primary infertility. Specifically, 77.0% (95% CI 71.0–82.5) of cases in Group 1 displayed primary infertility, and a slightly higher, but statistically insignificant ($p = 0.48$), proportion of Group 2 patients displayed primary infertility (80.8%, 95% CI 71.1–89.0). We did not detect statistically significant differences in various infertility factors between these two groups (Table 1).

In approximately half of patients in both groups, specifically, 52.2% (95% CI 45.4–59.1) in Group 1 and 47.9% (95% CI 36.6–59.4) in Group 2 ($p = 0.53$), patients were found to possess a combination of several infertility factors. Gynecological pathology was detected in the majority of women in both retrospective groups, 75.3% (95% CI 68.7–81.3) of cases in Group 1 and 75.0% (95% CI 64.4–84.3) cases in Group 2 ($p = 0.84$). The frequency of gynecological pathologies were not statistically different between the two groups (Table 2).

The levels of key hormones, such as follicle-stimulating hormone (FSH), luteinizing hormone (LH),

Table 4. Average values of thyroid function indicators concentrations in retrospective groups (Median (25%; 75%)).

Indicator	Group 1	Group 2	*p*
Thyroid-stimulating hormone (TSH), mIU/L	1.8 (1.3; 2.5)	1.7 (1.3; 2.2)	0.62
Thyroid peroxidase antibody (TPOAb), IU/mL	8.8 (1.0; 19.5)	8.9 (1.3; 16.8)	0.05
Antithyroglobulin antibody (ATG), IU/mL	1.9 (0.2; 10.0)	5.0 (0.3; 10.1)	0.70

Table 5. The structure of gynecological pathology revealed by the results of hysteroscopy (% (95% CI)).

Histological conclusion	Group 1	Group 2	*p*
Endometrial polyp, glandular-fibrous type	45.0 (30.1–60.4)	75.0 (47.9–94.3)	0.048
Endometrial polyp, glandular type	27.5 (15.0–42.2)	0.0 (0.0–7.8)	0.01
Chronic endometritis	25.0 (13.0–39.4)	8.3 (0.01–29.6)	0.12
Endometrial hyperplasia	2.5 (0.0–9.5)	16.7 (1.9–41.8)	0.20
Hypoplastic endometrium	2.5 (0.0–9.5)	0.0 (0.0–7.8)	0.32

95% CI, 95% confidence interval.

anti-Mullerian hormone (AMH), and prolactin fluctuated widely in both groups, but none of these hormone levels showed statistically significant differences when the groups were compared to each other (Table 3).

A thyroid function screening was done by determining thyroid-stimulating hormone (Table 4), thyroid peroxidase antibody (TPOAb), as well as antithyroglobulin antibody (ATG) levels in peripheral blood. Elevated levels of TPOAb were detected in 13.0% (95% CI 8.0–19.2) of Group 1 patients and somewhat less often in Group 2 patients (11.9%, 95% CI 4.0–23.3) (p = 0.84). Increased levels of ATG were observed in approximately the same proportion of patients of both groups, specifically, in 4% in Group 1 (95% CI 0.8–5.0) and 4.4% in Group 2 (95% CI 0.3–7.7) (p = 0.89).

To further examine potential factors leading to implantation failure, patients in both groups underwent antiphospholipid screening. Lupus anticoagulant levels in both groups were 1.1 $\pm$ 0.1 (p = 0.43), indicating no risk of antiphospholipid syndrome. The screening test of patients in Group 1 was 34.8 $\pm$ 0.4 s and 33.4 $\pm$ 0.7 s in Group 2 (p = 0.06). The results of the confirmatory test were distributed in a similar way and resulted in an average of 32.2 $\pm$ 0.2 s in Group 1 and 31.6 $\pm$ 0.6 s in Group 2 (p = 0.39). Cardiolipin and beta-2-glycoprotein antibodies (IgG) levels also displayed extremely small variability. Specifically, Group 1 patients showed 1.6 (1.6; 1.6) and Group 2 patients showed 1.6 (1.6; 2.0) (p = 0.06) for cardiolipin antibodies, and Group 1 showed 1.4 (1.4; 1.4) and Group 2 showed 1.4 (1.4; 1.4) (p = 0.30) for beta-2-glycoprotein antibodies.

To examine and potential pathology of the uterine cavity as a factor in implantation failure, we noted that patients of both groups commonly underwent hysteroscopy, specifically 88.9% (80.7–95.1) in Group 1 and 95.2% (82.3–100.0) in Group 2 (p = 0.29). Other pathological changes were observed in 62.5% (50.4–73.9) of Group 1 cases and 60.0% (38.3–79.8) of Group 2 cases (Table 5).

Partner spermograms were also evaluated. We observed no significant difference in sperm concentrations between patients of both groups. Specifically, 17.1 (2.2; 31.6) and 18.2 (11.4; 33.2) million sperm/mL (criteria World Health Organization (WHO) 2004) (p = 0.61) were measured in Group 1 and Group 2 partners, respectively. The average value of the assessment of the spermatozoa morphological characteristics were 13.0 (4.0; 24.6) for Group 1 and 11.0 (3.9; 24.2) for Group 2 (criteria WHO 2004) (p = 0.69). Teratospermia was detected in more than half of the partners of both groups when evaluating spermograms (p = 0.99). The proportion of oligospermia in the partners of Group 1 women (22.2% of cases (15.2–30.2)) was significantly higher (p = 0.01) than the proportion of oligospermia measured in the partners of Group 2 women (7.0 (1.4–16.4)) (Table 6).

Table 6. The structure of partner spermogram conditions (% (95% CI)).

Condition	Group 1	Group 2	*p*
Teratospermia	58.1 (49.1–66.9)	58.1 (43.3–72.3)	0.99
Asthenospermia	51.3 (42.3–60.3)	46.5 (32.0–61.4)	0.59
Normospermia	22.2 (15.2–30.2)	30.2 (17.6–44.6)	0.32
Oligospermia	22.2 (15.2–30.2)	7.0 (1.4–16.4)	0.01
Aspermia	9.4 (4.8–15.3)	4.7 (0.5–12.9)	0.26
Hypospermia	4.3 (1.4–8.7)	9.3 (2.6–19.7)	0.30
Cryptospermia	2.6 (0.5–6.2)	0.0 (0.0–2.2)	0.08
Hyperspermia	1.7 (0.2–4.9)	0.0 (0.0–2.2)	0.16

95% CI, 95% confidence interval.

Previously used assisted reproductive technology (ATR) methods determined that intrauterine insemination was occurred in a significantly (p = 0.01) higher proportion of patients in Group 2 (28.2% (18.4–39.1)) compared to Group 1 (11.9% (7.7–16.9)). The two retrospective groups did differ significantly (p = 0.03) in terms of the number of previous IVF attempts, but the median value of this indicator was 1 attempt (1; 11) for Group 1 women and 2 attempts (1; 6) for Group 2 women.

To measure controlled ovarian stimulation (COS) the GnRH antagonist IVF/ICSI (intracytoplasmic sperm injec-

Table 7. Ovarian stimulation IVF/ICSI protocols. The structure of application in retrospective groups (% (95% CI)).

Type of protocol	Group 1	Group 2	*p*
GnRH-antagonist protocols with rFSH	74.5 (67.2–81.2)	80.4 (67.9–90.5)	0.39
GnRH-antagonist protocols with aromatase inhibitors	16.8 (11.2–23.2)	15.2 (6.4–26.9)	0.80
GnRH-agonist COS protocol	7.4 (3.8–12.1)	6.5 (1.3–15.4)	0.84
Natural cycle (NC)	2.0 (0.4–4.9)	0.0 (0.0–2.1)	0.09

IVF/ICSI, *in vitro* fertilization/intracytoplasmic sperm injection; 95% CI, 95% confidence interval; rFSH, Recombinant follicle stimulating hormone; COS, controlled ovarian stimulation.

Table 8. Endometrium stimulation protocols. The structure of application in retrospective groups (% (95% CI)).

Type of the protocol	Group 1	Group 2	*p*
GnRH agonist-HRT protocol	72.3 (64.7–79.4)	65.1 (50.4–78.5)	0.38
Programmed/artificial cycle (AC)	35.5 (27.8–43.5)	46.5 (32.0–61.4)	0.20
GnRH antagonist COS protocols	1.4 (0.1–4.0)	0.0 (0.0–2.2)	0.84
Modified natural cycle (mNC)	0.7 (0.0–2.8)	0.0 (0.0–2.2)	0.52
GnRH-agonist COS protocol	0.7 (0.0–2.8)	0.0 (0.0–2.2)	0.32
Natural cycle (NC)	0.0 (0.0–0.7)	0.0 (0.0–2.2)	-

95% CI, 95% confidence interval; HRT, hormone replacement treatment; COS, controlled ovarian stimulation.

Table 9. Individualized endometrial stimulation protocols. The structure of application in retrospective groups (% (95% CI)).

Type of the protocol	Group 1	Group 2	*p*
GnRH agonist–HRT protocol	91.5 (87.3–95.0)	88.6 (80.1–94.9)	0.50
Programmed/artificial cycle (AC)	7.5 (4.3–11.6)	10.0 (4.1–18.1)	0.32
GnRH antagonist COS protocols	0.5 (0.0–2.0)	1.4 (0.0–5.6)	0.32
Natural cycle (NC)	0.5 (0.0–2.0)	0.0 (0.0–1.4)	-

95% CI, 95% confidence interval; HRT, hormone replacement treatment; COS, controlled ovarian stimulation.

tion) protocol was used in both retrospective groups—in Group 1, 74.5% (67.2–81.2) of cases and in Group 2, 80.4% (67.9–90.5) of cases. The values obtained for ovarian stimulation IVF/ICSI protocols conducted in each retrospective group is given in Table 7.

ET on the COS cycle was performed in less than half of the patients in both retrospective groups, specifically, 47.2% (39.1–55.4) in Group 1 and 43.9% (29.2–59.1) in Group 2 ($p = 0.67$). The remaining the patients underwent frozen embryo transfer (FET).

Evaluating the structure of the endometrial preparation IVF protocols noted that the most commonly applied type was a GnRH agonist hormone replacement treatment (HRT) protocol. This was observed in 72.3% (64.7–79.4) of Group 1 patients and 65.1% (50.4–78.5) in Group 2 patients ($p = 0.38$). Over a third of patients in Group 1 (35.5% (27.8–43.5)) and almost half of patients in Group 2 (46.5% (32.0–61.4)) followed a programmed/artificial cycle (AC) ($p = 0.20$). No patients in either group underwent FET in natural cycle (NC) (Table 8).

The average thickness of the endometrium at the start of progesterone administration, 9.1 $\pm$ 0.2 mm in Group 1 patients and 9.7 $\pm$ 0.4 mm in Group 2 patients, was found to be not significantly different ($p = 0.16$).

In the majority of patients in both groups, ET was performed on the 5th day of embryo cultivation (blastocyst stage). Specifically, these conditions applied to 90.8% (85.8–94.9) of Group 1 cases and 87.0% (75.8–95.0) of Group 2 cases ($p = 0.48$). On the 2nd day, ET was performed in 3.9% (1.4–7.6) of Group 1 cases and 13.0% (5.0–24.2) of Group 2 cases ($p = 0.08$), on the 3rd day in 10.5% (6.1–15.8) of Group 1 cases and 8.7% (2.4–18.4) in Group 2 cases ($p = 0.72$). On the 6th day, ET was conducted in 1.3% (0.1–3.7) of Group 1 cases and 4.3% (0.4–12.1) of Group 2 cases ($p = 0.34$). We did not detect any case of performing ET on the first day of cultivation, and noted only one patient in Group 1 where this procedure was performed on the 4th day ($p = 0.32$).

IVF with donor oocytes was performed in a significantly ($p = 0.03$) higher proportion of patients in Group 1 (13.8% (8.9–19.6)) compared to Group 2 (4.5% (0.5–12.6)). The two retrospective groups did not differ significantly ($p = 0.05$) in terms of the cycle number when own oocytes were utilized. Moreover, the average values of transferred embryos between each of the retrospective groups did not differ ($p = 0.93$) and were 2 (1; 2) for Group 1 and 2 (1; 2) for Group 2.

As a result of IVF attempts, biochemically detectable pregnancy was registered in 6.4% (3.2–10.5) Group 1 cases

Table 10. Cumulative structure of embryo quality which were transferred in the individualized endometrial stimulation cycle (% (95% CI)).

Embryo quality	Group 1	Group 2	*p*
		Embryo 1	
Good	56.6 (49.5–63.6)	47.8 (36.2–59.6)	0.39
Fair	39.7 (32.8–46.7)	49.3 (37.6–61.0)	0.32
Poor	3.7 (1.5–6.7)	2.4 (0.0–9.1)	0.15
		Embryo 2	
Good	26.3 (15.8–38.4)	35.7 (22.1–50.6)	0.30
Fair	61.4 (48.5–73.5)	61.9 (46.9–75.8)	0.08
Poor	12.3 (5.1–22.0)	2.4 (0.0–9.1)	0.01
		Embryo 3	
Good	0.0 (0.0–22.2)	0.0 (0.0–69.0)	0.16
Fair	75.0 (28.0–99.9)	100.0 (31–100.0)	0.26
Poor	25.0 (0.1–72.0)	0.0 (0.0–69.0)	0.59

95% CI, 95% confidence interval.

and 4.2% (0.4–11.6) of Group 2 cases ($p > 0.52$). Missed abortions were subsequently observed in 4.1% (1.3–8.3) and in 5.7% (0.6–15.7) in Group 1 and 2 patients, respectively ($p > 0.71$). No pregnancy was prolonged until 12 weeks of gestation.

Based on the SEM analysis, we determined that the average value of optimal day for ET in Group 1 was 8.2 $\pm$ 0.1 days and in Group 2 this indicator was 6.3 $\pm$ 0.1 days, and this differed significantly between the two retrospective groups ($p = 0.001$). This analysis also highlighted that the variability of IW was wide in Group 1 patients with this window extending from the 4th day of progesterone administration until the 11th day in these patients.

The dominant protocol in both retrospective groups was the GnRH agonist–HRT protocol as it was used in 91.5% (87.3–95.0) of Group 1 cases and 88.6% (80.1–94.9) of Group 2 cases ($p = 0.05$). In 7.5% (4.3–11.6) of patients in Group 1 and 10.0% (4.1–18.1) of patients Group 2, programmed/AC was applied ($p = 0.32$). The natural protocol and GnRH antagonist COS protocols were not used in any of the patients in either of the retrospective groups (Table 9).

The thickness of the endometrium on the first day of progesterone administration in the two retrospective groups was not significantly different ($p = 0.35$), averaging 8.8 $\pm$ 0.1 mm in Group 1 and 9.0 $\pm$ 0.2 mm in Group 2.

In addition to modifying endometrial preparation protocol, medications and/or procedures were also used alter infertility factors in patients from both retrospective groups. For example, more than a quarter of patients in both groups have had intravenous infusion of immunoglobulins during IVF procedures. The proportion of such patients was 28.4% (22.4–34.8) in Group 1 and 23.9% (14.8–34.5) in Group 2 ($p = 0.46$). Administration of drugs that effect granulocyte growth factor was noted in 18 Group 1 patients (9.4% (5.7–14.0)) and 9 Group 2 patients (13.2% (6.3–22.3)) ($p = 0.41$). Three patients in Group 1 (1.6% (0.3–3.8)) and one patient in Group 2 (1.5% (0.0–5.7)) underwent endometrial plasma therapy ($p = 0.96$) . In more than half of the patients in both retrospective groups, the management plan also included anticoagulant therapy. In group 1 this indicator was 58.3% (51.5–64.9) of patients while 63.0% (51.7–73.7) of group 2 patients were treated in a similar manner ($p = 0.47$).

In the majority of cases in both retrospective groups, IVF was performed with the patient's own oocytes. In Group 1 this was 78.7% (72.8–84.1) of cases whereas this number was 81.2% (71.2–89.4) of Group 2 patients ($p = 0.66$). Preimplantation genetic testing for aneuploidy was performed in only 6 Group 1 patients (3.0% (1.1–5.8)) and 5 (6.9% (2.3–13.9)) Group 2 patients ($p = 0.22$).

The first ET was performed significantly later ($p = 0.001$) in Group 1 patients (7.3 $\pm$ 0.1 days) compared with Group 2 patients (6.4 $\pm$ 0.1 days). During the first ET, from 1 to 3 embryos were transferred to patients of both groups. Specifically, in Group 1 patients 69.8% received one embryo, 28.1% received 2 embryos, and 2.1% received 3 embryos. In Group 2 patients 39.2% received one embryo, 59.4% received two embryos, and 1.4% received 3 embryos.

A second ET was performed for Group 1 patients. Here, the transfer of two embryos was performed in 8.5% cases and transfer of three embryos did not occur for any patient in this group. Significant differences in embryo quality was detected only in the second embryo transferred. The percentage of poor quality embryos transferred was significantly higher in Group 2 patients (12.3% (5.1–22.0)) when compared to Group 1 patients (2.4% (0.0–9.1)), ($p = 0.01$) (Table 10).

Outcome of IVF cycles with an individualized endometrium preparation protocol in retrospective groups is presented in Table 11.

4. Discussion

Early reproductive failure is the most commonly encountered complication of pregnancy. Approximately 70% of embryos stop development prior to reaching viability [15], and over than 50% of pregnancies are lost through implantation failure [16]. Implantation is dependent on the developmental synchronization between the developing embryo and the endometrium. The IW is described as the time frame with maximal endometrial receptivity which is surrounded by a refractory endometrial status [17]. During the IW the endometrium is characterized by both receptivity and selectivity, which allows it to implant an embryo with developmental potential [18]. Therefore, the principal causes of unsuccessful implantation are an aneuploid embryo and/or dysfunction in either endometrial selectivity and/or receptivity. Recurrent implantation failure (RIF) is the term used when failure arises after serial IVF attempts; however, this definition is not unequivocally agreed upon in the medical and scientific community.

Table 11. Outcome of IVF cycles with individualized endometrium preparation protocol in retrospective groups (95% CI).

Rate	Group 1	Group 2	*p*
Pregnancy			
Chemical pregnancy rate	63.1 (56.4–69.6)	67.1 (56.0–77.4)	0.54
Clinical pregnancy rate	59.2 (52.5–65.8)	57.5 (46.1–68.6)	0.80
Abortion rate	9.2 (5.7–13.5)	16.4 (8.9–25.8)	0.01
Fetus quantity			
Single pregnancy rate	81.1 (73.2–88.0)	72.2 (56.7–85.4)	0.30
Multiple pregnancy rate (twins)	17.0 (10.5–24.7)	27.8 (14.6–43.3)	0.80
Multiple pregnancy rate (triplets)	1.9 (0.2–5.3)	0.0 (0.0–2.6)	0.14
Delivery			
Natural vaginal (%)	19.2 (11.3–28.7)	32.1 (16.5–50.3)	0.29
C–section (%)	80.8 (71.3–88.7)	67.9 (49.7–83.6)	0.20
Complications during pregnancy and delivery (%)	43.8 (33.1–54.7)	60.7 (42.3–77.7)	0.16
Live birth rate	50.7 (43.9–57.6)	47.9 (36.6–59.4)	0.49
Newborn characteristics			
Baby maturation rate	78.5 (68.8–86.8)	74.1 (56.2–88.5)	0.72
Height (cm)	50.1 ± 0.4	49.3 ± 0.8	0.48
Weight (g)	2942 ± 73.6	2901 ± 120.0	0.75
Gender:			
- Girls (%)	60.9 (50.5–70.9)	32.6 (19.5–47.1)	0.002
- Boys (%)	39.1 (29.1–49.5)	67.4 (52.9–80.5)	0.002

IVF, *in vitro* fertilization; 95% CI, 95% confidence interval.

Success of IVF treatment depends of many female factors such as age, hormonal levels, endometrial/uterine status and underlying conditions, embryo-related factors such as embryonic cleavage speed, euploidy, and previous implantations of sibling embryos. Male factors such as genetic disorders and external factors, for example, the performance of the laboratory and clinic, transfer policies and legal restrictions are also recognized.

This study sought to examine if endometrial receptivity is a factor for IVF success, and if so, if this factor be compensated for. Current factors in assessing endometrial receptivity include several markers such as endometrial thickness [19], endometrial volume [20,21], endometrial receptivity array [22,23], and markers evaluated in endometrial fluid aspirates such as urocortin, activin A, human decidua-associated protein (hDP), and interleukin-18 [24]. Further, cytokines, glycodelin, isoforms of leucine-rich alpha2-glycoprotein, cytokines leukemia inhibitory factor (LIF), tumor necrosis factor (TNF), interleukin-1β, TNF-α, interferon gamma-induced protein 10, and monocyte chemoattractant protein [25], have all been evaluated as potential markers by hysteroscopy [26,27]. But despite the large number of proposed markers, there is presently no single generally accepted universal marker(s) for assessing the ability of the endometrium to ensure embryo implantation.

The primary aims of this study were to explore the effect of endometrial receptivity factors on IVF outcome, and to compare the importance of these factors with other potential causes of infertility. Our results did not find a significant difference in IVF outcomes between the two retrospective groups studied. However, it should be underscored that a multipoint screening of patients was conducted and all observed deviations were either treated or compensated for including the displacement of the IW. This resulted in some positive outcomes at the same rate as the correction of other factors.

Study limitations apply to the retrospective nature of the study, patient heterogeneity, comprehension and diversity of screening methods, and differing laboratories and operators. The lack of standardized preimplantation genetic diagnosis (PDG) and preimplantation genetic diagnosis for aneuploidy (PGD-A), which are principal laboratory methods to exclude embryonic factors for RIF and help to clarify the role of IW assessment is also problematic, as are outdated classifications and metrics.

RIF can be improved upon by development and approval of clinical guidelines based on evidence-based medicine such as transvaginal ultrasound-guided ET [28]. Using this procedure, as well as advances in modern diagnostics derived from a better understanding of the underlying molecular biology [29], an improvement in treatment approaches is likely to improve outcomes. In the longer term, new diagnostic approaches based on artificial intelligence, such as embryo creation and selection [30], understanding neural networks which take into account all characters of the embryo and the mother [31], and treatment methods such as a robot surrogate mother [32] all promise to improve IVF outcomes.

5. Conclusions

Unique IW among women looking to conceive are one of the reasons for implantation failure. IW screening is important for patients with recurrent implantation failure when other reasons, especially embryonic factors, have been excluded as the source of RIF. The personalization of an endometrial preparation protocol is proposed as a method to improve IVF outcomes. Further prospective studies, including examining potential underlying genetic causes, are needed to clarify the role of IW assessment in the management of recurrent implantation failure.

Abbreviations

IVF, *in vitro* fertilization; IW, implantation window; LP, luteal phase; SEM, scanning electron microscopy; PCOS, Polycystic ovary syndrome; MDAs, Müllerian duct anomalies; AMH, Anti-Mullerian hormone; FSH, Follicle-stimulating hormone; LH, Luteinizing hormone; TPOAb, Thyroid peroxidase antibody; ATG, Antithyroglobulin antibody; WHO, World Health Organization; ART, Assisted Reproductive Technology; ICSI, intracytoplasmic sperm injection; COS, controlled ovarian stimulation; GnRH, Gonadotropin Releasing Hormone; rFSH, Recombinant follicle stimulating hormone; NC, Natural cycle; ET, embryo transfer; HRT, hormone replacement treatment; AC, artificial cycle; mNC, modified natural cycle; FET, frozen embryo transfer; RIF, Recurrent implantation failure; LIF, cytokines leukemia inhibitory factor; TNF, tumor necrosis factor.

Availability of Data and Materials

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

Author Contributions

OK and MM designed the research study. OK performed the research. MM, AT provided help and advice on design development and calculation analysis. OK analyzed the data. OK wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This is a retrospective study and all procedures that were part of the studies were carried out according to the standards of care in our institution. Therefore there was no need to review the study in the ethical committee. Patients' informed consent for all procedures was obtained.

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Conflict of Interest

The authors declare no conflict of interest. Andrea Tinelli is serving as one of the Editorial Board members and Guest editors. We declare that Andrea Tinelli had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Michael H. Dahan. "NanoMedTech Llc" is laboraroty which perform electronic microscopy of endometrium and provides record and images.

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