Embryo Transfer with Controlled Injection Speed to Increase Pregnancy Rates: A Randomized Controlled Trial

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Key Words
Embryo transfer · IVF · Embryo transfer technique · Pump-regulated embryo transfer

Abstract
Background/Aims: Catheter injection speed affects depth and placement of the embryo into the uterine cavity and is shown to be highly variable in, and between, subjects in a manually performed embryo transfer. In an effort to standardize the injection speed during embryo transfer, we developed an automated transfer pump: the pump-regulated embryo transfer (PRET) device. In this randomized controlled trial, we aimed to investigate if standardization of the injection speed and pressure with this PRET results in a better controlled positioning of the transferred embryo(s).

Methods: Five hundred ninety-nine in-vitro fertilization/intracytoplasmic sperm injection/frozen-thawed embryo transfer cycles were randomly assigned to the PRET or manual transfer. Positioning of the embryo(s) into the uterine cavity was measured with ultrasound.

Results: The PRET device generates a significantly smaller variance of the positioning of the embryo(s) into the uterine cavity. This resulted in an ongoing pregnancy rate of 21% in the PRET versus 17% in the manual (p = 0.22) transfer group; frozen-thawed embryo transfers resulted in 17.5 versus 10.9% (p = 0.097), respectively.

Conclusion: The PRET results in better controlled positioning of the embryo(s), and it also gives the opportunity to standardize embryo transfer. Whether the PRET may positively influence pregnancy rates, needs to be investigated in a multicenter trial.

Introduction

The embryo transfer is one of the crucial steps that influences pregnancy rates in in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) treatment [1–3]. Patients reaching the stage of embryo transfer have successfully completed important early key stages of the treatment such as ovarian stimulation, oocyte retrieval, fertilization and embryo development. Although about 85% of all couples undergoing IVF or ICSI treatment reach the stage of embryo transfer, only one-third actually becomes pregnant at the end of a treatment cycle (fresh cycles: most recent ESHRE European register: 2009 [4]). Various factors concerning embryo transfer have been described to explain those limited embryo implantation rates: the type of catheter used for the transfer [5–7];
including the relevance of a soft distal part on the catheter [8, 9], the presence of blood or bacteria on the catheter [10, 11]; the experience of the physician [12] and the use of ultrasound guidance [13–16]. Much of the inefficiency of the embryo implantation may therefore be derived from the embryo transfer technique [3]. Despite its relevance, the technique used in embryo transfer has received little attention and progress until recent years [2, 3]. Several studies [13, 17–23] have demonstrated that the depth of the embryo placement into the uterine cavity, as a result of the embryo transfer technique, should be considered an important factor concerning pregnancy rates. Our research group pointed out that despite standardization of the transfer by protocol, the final position of the transferred air bubbles including the embryo(s) was not accurately predictable because of dependency of the injection speed which depends on the force used to press the plunger, the resistance of the plunger and a possible uterine resistance during transfer [24].

This injection speed affects the depth and placement of the embryo in the uterine cavity and is shown to be highly variable in, and between subjects, in a manually performed embryo transfer with a syringe [25]. In an effort to standardize the injection speed during embryo transfer, we developed an automated device that generates a standardized injection speed, the pump-regulated embryo transfer (PRET) device, in co-operation with the Delft University of Technology (fig. 1). This device enables a better controlled positioning of the embryo(s) inside the uterine cavity, extensively described by Groeneveld et al. [25]. The results of this in vitro study strongly indicated that the PRET device generates a reliable and reproducible injection speed and pressure and, therefore, brings new possibilities for further standardization and potential optimization of the embryo transfer procedure, compared to the manually performed embryo transfer. The study also demonstrated that even after standardization, there was still a large variation in injection speed and pregnancy rates in laboratory technicians.

Given the results of the study of the injection speed in vitro by Groeneveld et al. [25], we conducted a randomized controlled trial to investigate whether this standardization of the injection speed and pressure results in a better controlled positioning of the transferred embryo(s) in vivo. Both the safety of clinical use and effect on pregnancy rates were investigated. The aim of our study was to investigate whether the PRET device results in a more controlled positioning of the embryo(s), compared to the manually performed embryo transfer.

**Materials and Methods**

**Patients**

A total of 599 embryo transfer cycles were enrolled into this non-blinded randomized controlled trial (fig. 2). Patients were recruited from August 2010 until February 2013 at the IVF center of the VU University Medical Center in Amsterdam, The Netherlands. Patients starting their IVF/ICSI or frozen-thawed embryo transfer treatment cycle were asked to participate and were also allowed to participate more than once. Patients participating in other IVF/ICSI-related research trials, patients with intra-uterine pathology and surrogacy or egg donation cycles were excluded. Ethical approval was obtained from the Institutional Review Board of the VU University Medical Center, Amsterdam, The Netherlands. The trial was registered in the Dutch National Trial Registry (trial registration number NTR1638).
Stimulation Protocols

IVF/ICSI stimulation protocols were carried out as previously described [26]. Briefly, standard long or short GnRH agonist (tripotrelin; Decapeptyl®; Ferring, Denmark) or short GnRH antagonist (Cetorelix; Cetrotide®; Merck Serono, Germany) protocols were used. Ovarian hyperstimulation was performed with individually determined dosages of recombinant FSH (Gonal-F®; Merck Serono, Germany or Puregon®; MSD, USA) or highly purified human menopausal gonadotropin (Menopur®; Ferring, Denmark). Treatment cycles were monitored using vaginal ultrasonography and serum estradiol determinations. A minimum of 1 follicle of >17 mm or at least 3 follicles of ≥16 mm were required to subcutaneously administer 10,000 IU of human chorionic gonadotropin (hCG; Pregnyl®; Organon, The Netherlands) or 6,500 IU recombinant chorioc gonadotropin (Ovitrelle®; Merck Serono, Germany). Oocyte retrieval was performed 36 h after administration of hCG. The luteal phase was supported by intravaginally administered progesterone (200 mg thrice a day; Uterogestan®; Besins Health Care Belgium) from the day of oocyte retrieval.

Frozen-thawed embryo transfer cycles were mainly performed in a natural cycle. Women without or with a very irregular cycle were assigned to an artificial cycle. Follicle growth was monitored by transvaginal ultrasonography. In a spontaneous cycle, hCG was administered when a follicle reached a size of at least 17 mm in combination with an adequate endometrial thickness of at least 6 mm. In an artificial cycle, estradiol valerate (Progynova®; Bayer Schering Pharma, Germany) was administered in an increasing dosage scheme from 2 to 6 mg/day (orally). After reaching an adequate endometrial thickness of at least 6 mm, 100 mg of progesterone (Uterogestan®; Besins Health Care Belgium) was administered intravaginally thrice a day, and estradiol valerate was decreased to 4 mg/day. Six days after hCG injection (spontaneous cycle) or after starting the progesterone administration (artificial cycle), the embryo transfer was carried out.

Laboratory

IVF or ICSI was performed according to the laboratory’s routine insemination procedures as previously described by Vergouw et al. [26]. In short, on the day of oocyte retrieval (day 0) oocytes

Fig. 2. CONSORT flow diagram of assignment, treatment and analysis of patients/transfer cycles.
were placed in a fertilization medium (Sage®; Quinn’s advantage protein plus fertilization medium, Cooper Surgical USA). During the fertilization check, 16–18 h after insemination, IVF zygotes were transferred into 25-μl pre-equilibrated medium drops of cleavage medium (Sage®; Quinn’s advantage protein plus fertilization medium, Cooper Surgical USA). ICSI oocytes were placed directly into 25-μl pre-equilibrated cleavage medium drops after injection. The embryos were individually cultured under oil in incubators at 37°C with 5% CO₂ and atmospheric O₂ concentration. Embryo development was checked daily at 25–27, 44–48 and 68–72 h after insemination. In the morning of day 3 after oocyte retrieval, embryos were transferred into a new culture dish with blastocyst medium (Sage®; Quinn’s advantage protein plus blastocyst medium, Cooper Surgical, USA). On the same day, early in the afternoon (73–75 h after insemination), the embryo transfer was carried out.

In women under the age of 40 years, our center’s protocol dictates to transfer 1 embryo in the first 2 cycles. Therefore, a single embryo transfer was predominantly performed. The morphological choice of which embryo(s) to transfer was based on the laboratory’s routine procedures, both in fresh and frozen-thawed embryos [26]. Each embryo was morphologically assessed by combining the number and regularity of blastomeres and the degree of fragmentation. A score is given to each embryo combining the fragmentation and the irregularity of the blastomeres. Scores went from 1 to 4, with 1 being no fragmentation, regular blastomeres up to score 4 with >50% fragmentation and irregular blastomeres. A score 1 is preferred above score 2 and the early cleavage score were used for the embryo selection. Supernumerary good quality embryos with at least 8 cells and <20% fragmentation were exclusively frozen on day 4. Depending on the pre-freezing quality and on the number of transferred embryos in the fresh cycle, 1–3 embryos were frozen in 0.5-ml straws (CBS, L’Aigle, France). The embryo-freezing media consisted of human tubal fluid (HTF) with Hepes (Lonza, Belgium) and 10% human serum albumin (HSA) and dimethyl sulfoxide (DMSO; Sigma Aldrich, Germany). Embryos were frozen using a standard ‘slow freezing’ protocol as described earlier by Vergouw et al. [27]. All remaining poor quality embryos were checked for blastocyst formation on day 6 before they were discarded.

The thawing procedure was as follows. Embryos were thawed for 2–3 min at room temperature after removing the straws from the liquid/vapor nitrogen storage. Thawed embryos were incubated in a series of decreasing DMSO media solutions (1.25, 1.0, 0.75 and 0.375 mol/l DMSO in HTF/Hepes with 10% HSA). The final step was a rinse in DMSO-free HTF/Hepes/HSA media. Embryos were assessed after thawing by routine morphological criteria and subsequently cultured for 20–24 h in individual 25-μl media drops. Prior to embryo transfer, the embryos were assessed again by routine morphological criteria. The embryo with the fastest development and best morphology was selected for transfer. Only compaction stage embryos or blastocysts were selected for transfer. No assisted hatching was performed.

Embryo Transfer

In our center, we perform standard abdominal ultrasound during the transfer of embryos; therefore, our staff is well trained in this. Patients were instructed to come with moderately filled bladder to improve abdominal ultrasound view. The patient was positioned in the lithotomy position and the cervix was exposed using a trepat speculum. No anesthetic or sedative was used. The mucus in the cervical canal was removed with a cotton swab. The outer catheter of the Cook catheter (K-JETS-70190-SIVF; Cook IVF, Eight Miles Plains, Queensland, Australia) was placed by the physician under guidance of abdominal ultrasonography. Then, the inner catheter was loaded with the embryo(s) by the laboratory technician or embryologist using the ‘3-drop technique’ as previously described by van Weering et al. [9], either by manual transfer or with the help of the PRET device. The embryo(s) was/were separated by a bubble of air from a preceding and following drop of medium, and was inserted through the outer catheter.

The inner catheter was then properly placed with the tip of the inner catheter positioned at approximately 1 cm from the endometrial fundus, which was determined by abdominal ultrasound. Distances E and F were given by the physician who inserted the catheters. Distance D was measured using abdominal ultrasound shortly before the laboratory technician expelled the embryo(s) into the uterine cavity using manual transfer technique or the PRET device (embryo(s) were separated by a bubble of air from a preceding and following drop of medium). The transfer procedure was visible on the ultrasound, and then, additional measurements like distances B and C were measured immediately (freeze ultrasound image) after the transfer; distance A was therefore calculated. Consequently, the physician then removed the inner and outer catheters, and the laboratory technician checked for any retained embryo(s) by flushing both catheters with 0.5 ml of the remaining medium. The trepat speculum was removed, and the patient was allowed to immediately stand up.

Intervention

Patients were assigned to the manual embryo transfer (control) technique or transfer using the PRET device (intervention). In a manually performed embryo transfer, the inner catheter was loaded and unloaded by the laboratory technician using a simple syringe; in a PRET, the syringe was replaced by the PRET device. The PRET device is developed to perform the embryo transfer in a standardized and reproducible way; the technical aspects of the PRET device were previously described by Groeneveld et al. [25]. The inner catheter is connected to the PRET device and loaded by the laboratory technician using the device’s scroll-wheel interface. To expel the embryo(s), the laboratory technician pushes the eject button on the top of the device, causing the embryo to be placed inside the uterine cavity with a constant pressure and speed. In both manual and PRET transfers, Cook catheters (K-JETS-70190-SIVF; Cook IVF, Eight Miles Plains, Queensland, Australia) were used. Both the PRET device and the syringe made no contact with the embryo(s). Embryo transfer including the performed measurements (fig. 3) during abdominal ultrasound were performed by 2 research physicians using a standardized protocol.

Objectives and Outcomes

The primary end point of the study was the accuracy of the embryo positioning (better controlled positioning), determined by distance between the inner catheter and the transferred embryo.
(transferred distance: distance A) and the distance between embryo and the endometrial fundus measured during the embryo transfer procedure (distance B; fig. 3).

Secondary end points were clinical outcomes, the ongoing pregnancy rate (OPR), defined as an intact intrauterine pregnancy confirmed by ultrasound at 12 weeks of gestational age. Other secondary end points were clinical pregnancy rate (the confirmation of at least 1 gestational sac visualized by ultrasound), implantation rate (number of gestational sacs on ultrasound scan per embryo transferred), miscarriages, extra-uterine gravidity (EUG) and twin pregnancies.

Randomization, Blinding and Treatment Allocation
Randomization was performed by an independent person and checked by the physician and laboratory technician, just prior to the embryo transfer procedure, using serially numbered, opaque, sealed envelopes, which was generated by computerized tables. Physicians, laboratory technicians and patients were not blinded.

Recruitment and Consent
Patients were informed about the study at the beginning of their treatment by their physician, through an information letter and a meeting. Informed consent was given and could be withdrawn at any moment. If participation was refused, a manual embryo transfer was performed by using a simple syringe.

Sample Size Calculation
To our knowledge, no studies are available concerning the positioning of the embryo into the uterine cavity using a pump device, making it unable to conduct a power calculation on our primary outcome. Since the depth of embryo placement is hypothesized to be an important factor in improving pregnancy rates, the overall aim of a better controlled positioning of the embryo into the uterine cavity with the PRET device is to improve pregnancy rates, our secondary outcome, on which the power calculation was based on. An increase in the OPR of 8% was considered to be clinically relevant. To prove that a PRET yields an 8% increase in the OPR, 1,200 embryo transfers were required (power 80% and alpha 0.05). A planned interim analysis was performed after 600 embryo transfers.

Statistical Analysis
Statistical analysis was performed using SPSS 20.0 software for Windows (SPSS Inc., Chicago, Ill., USA). We performed an intention-to-treat analysis with data from all randomized cycles. Results are expressed as the mean ± SD or n (%). Student’s t tests, chi-square tests and Fisher’s exact tests were used where appropriate. Generalized estimating equations (GEEs) method was used to correct for participation of patients in multiple transfer cycles. Unadjusted and adjusted (for age, BMI, duration of infertility and embryo quality) ORs were calculated. All treatment outcomes are presented for all included embryo transfers (total) and for fresh and frozen-thawed embryo transfers separately. A value of p < 0.05 was considered statistically significant.

Results
A total of 599 embryo transfers (in 453 patients) were randomly assigned to a manual embryo transfer (294 cycles for 225 patients) or a PRET (305 cycles for 228 patients; fig. 2) and included for analysis. One randomized embryo transfer was not carried out and therefore excluded from the analysis. Baseline characteristics of the study...
The primary end point, the accuracy of the embryo positioning, is shown in figure 4. The positioning of the embryo transfer is illustrated by the distance of the embryo from the endometrial fundus (distance B) and the distance of the embryo from the inner catheter (distance A). The distance of the embryo from the endometrial fundus (distance B) is not significantly different (9.4 ± 3.2 mm after PRET vs. 9.8 ± 3.8 mm after manual ET, p = 0.23), but the variance of the PRET compared with the manual embryo transfer shows a significantly smaller variance (Levene’s test p = 0.023; results not illustrated). The distance covered by the embryo from the inner catheter to the cavity, the transferred distance of the embryo (distance A), is not significantly different (0.40 ± 3.9 mm after PRET vs. 0.47 ± 5.4 mm after manual embryo transfer, p = 0.88). But the variance of the distances measured is significantly different (Levene’s test p = 0.000; fig. 4).

Clinical secondary outcomes for both groups are described in figure 5. For the total group (fresh and frozen-thawed embryos combined), 64 ongoing pregnancies (21.0%) were accomplished after a PRET compared to 50 (17.0%), after a manually performed embryo transfer (p = 0.22, number needed to treat [NNT] 25). GEE analysis showed an OR of 1.27 (95% CI 0.87–1.85, p = 0.21). The adjusted OR was 1.36 (95% CI 0.91–2.02, p = 0.14).

Subgroup analysis showed that after fresh embryo transfer, 35 ongoing pregnancies (25.2%) were achieved after a PRET versus 34 (23.1%) after a manually performed embryo transfer (p = 0.69, NNT 49). GEE analysis showed an OR of 1.10 (95% CI 0.66–1.85, p = 0.71). The adjusted OR was 1.02 (95% CI 0.57–1.81, p = 0.95).

Frozen-thawed embryo transfers resulted in 29 (17.5%) ongoing pregnancies after a PRET versus 16 (10.9%) after a manually performed embryo transfer (p = 0.097, NNT 15). GEE analysis showed an OR of 1.70 (95% CI 0.89–3.21, p = 0.11) after a PRET-assisted transfer.

Regarding safety, no differences were observed in the number of miscarriages, extra-uterine gravidities and multiple (twin) pregnancies. The number of retained embryos was similar in both study groups: n = 3 in the manual ET group and 2 in the PRET group.

**Discussion**

In this randomized controlled trial, the clinical use and safety of a new method to standardize the injection speed (and pressure) during embryo transfer by using the PRET device was investigated and compared with the manually performed embryo transfer. A better controlled (smaller variance) positioning of the embryo(s) was visualized by ultrasound after embryo transfer with the PRET device compared to the manually performed embryo transfer. This result strengthens the in vitro results of the standardized and reproducible injection speed of the PRET device as earlier published by our research group [25].

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**Table 1. Patient baseline characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Manual embryo transfer (n = 225)</th>
<th>Pump-regulated embryo transfer (n = 228)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years</strong></td>
<td>35.6±3.9</td>
<td>35.0±4.4</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>23.52±4.26 (n = 221)</td>
<td>23.79±4.42 (n = 221)</td>
</tr>
<tr>
<td><strong>Primary infertility</strong></td>
<td>98 (43.6)</td>
<td>100 (43.9)</td>
</tr>
<tr>
<td><strong>Duration of infertility, months</strong></td>
<td>40.4±26.5 (n = 224)</td>
<td>39.4±30.0 (n = 227)</td>
</tr>
<tr>
<td><strong>Medical cause of infertility</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>103 (45.8)</td>
<td>93 (40.8)</td>
</tr>
<tr>
<td>Tubal</td>
<td>20 (8.9)</td>
<td>35 (15.3)</td>
</tr>
<tr>
<td>Unexplained</td>
<td>61 (27.1)</td>
<td>49 (21.5)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>27 (12.0)</td>
<td>25 (11.0)</td>
</tr>
<tr>
<td>Other</td>
<td>13 (5.8)</td>
<td>24 (10.5)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (0.4)</td>
<td>2 (0.9)</td>
</tr>
</tbody>
</table>

For continuous variables, mean ± SD are presented; for categorical variables, n (%) are presented. There were no significant differences in patient baseline characteristics.
## Table 2. Embryo transfer characteristics

<table>
<thead>
<tr>
<th></th>
<th>Manual embryo transfer</th>
<th>Pump-regulated embryo transfer</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>total (n = 294)</td>
<td>fresh (n = 147)</td>
<td>frozen (n = 147)</td>
</tr>
<tr>
<td><strong>Fertilization</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>method</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVF</td>
<td>234 (79.6)</td>
<td>111 (75.5)</td>
<td>123 (83.7)</td>
</tr>
<tr>
<td>ICSI</td>
<td>60 (20.4)</td>
<td>36 (24.5)</td>
<td>24 (16.3)</td>
</tr>
<tr>
<td><strong>Embryo transfer</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SET</td>
<td>6 (2.0)</td>
<td>4 (2.7)</td>
<td>2 (1.4)</td>
</tr>
<tr>
<td>DET</td>
<td>58 (20.1) (n = 289)</td>
<td>25 (17.2) (n = 145)</td>
<td>33 (22.9) (n = 144)</td>
</tr>
<tr>
<td><strong>Embryo quality</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TQE</td>
<td>169 (57.5)</td>
<td>125 (85.0)</td>
<td>44 (29.9)</td>
</tr>
<tr>
<td>MQE</td>
<td>97 (33.0)</td>
<td>17 (11.6)</td>
<td>80 (54.4)</td>
</tr>
<tr>
<td>PQE</td>
<td>28 (9.5)</td>
<td>5 (3.4)</td>
<td>23 (15.6)</td>
</tr>
<tr>
<td>Difficult embryo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>transfer</td>
<td>9.7±2.1 (n = 291)</td>
<td>10.5±2.0 (n = 146)</td>
<td>8.9±1.9 (n = 145)</td>
</tr>
<tr>
<td>Endometrial thickness, mm</td>
<td>7.4±1.3 (n = 282)</td>
<td>7.3±1.5 (n = 137)</td>
<td>7.4±0.97 (n = 145)</td>
</tr>
<tr>
<td>Uterine length, cm</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SET = Single embryo transfer; DET = double embryo transfer; TQE = top quality embryo; MQE = medium quality embryo; PQE = poor quality embryo; for continuous variables mean ± SD is presented; for categorical variables n (%) is presented.

For statistical comparison and in order to objectively quantify the embryo quality, embryos were subdivided into 3 groups: top quality embryos, medium quality embryos and poor quality embryos. The embryos are mostly classified according to all criteria of the Istanbul consensus 2011 [34]. Top quality fresh embryos: <10% fragmentation and 7-, 8-, 9- or 10 cells, compaction or morula. Top quality frozen-thawed embryos: blastocyst, expended, hatching or hatched blastocyst. Medium quality fresh embryos: 10–50% fragmentation and 7-, 8-, 9- or 10 cells, compaction or morula; or <10% fragmentation and 5- or 6-cells. Medium quality frozen-thawed embryos: compaction, early blastocyst. Poor quality fresh embryos: >10% fragmentation and 5- or 6-cells, or >50% fragmentation in <5-cells. Poor quality frozen-thawed embryos: 7-, 8-, 9-, 10-cells, morula or early compaction.
Promising and new development of a better controlled positioning. We found a non-statistically significant, however, clinically relevant difference in the OPR, in particular after the frozen-thawed embryo transfers.

In contrast to its relevance, the technique of the embryo transfer has remained relatively unchanged in years. Several studies recommend to ‘transfer the matter gently’ [18, 19, 21, 28, 29]. A more concrete advise about the speed of the transferred catheter load is only made by Eyten et al. [30], who concluded that the catheter should deliver the load gently over a period of ≥10 s. Also, there is still no consensus about the most optimal depth of embryo placement. In early times, it was reported to position the embryo(s) 0.5 cm from the endometrial fundus [31–33]. In later years, studies conclude to position the embryo(s) 2 cm from the endometrial fundus [13, 18, 19]. Most recently, Cenksoy et al. [17] reported that the optimal position of the air bubble seems to be a distance of <10 mm from the endometrial fundus, in accordance with Friedman et al. [34]. Rovei et al. [22] concluded that the embryo(s) must be replaced between 5 and 15 mm from the endometrial fundus. This is in line with previous results from our study group – the position of the transfer air bubbles after embryo transfer closer to the endometrial fundus resulted in higher pregnancy rates [24]. In embryo transfer using the ‘3-drop technique’, the air bubbles can be regarded as an indication of the position of the embryo(s) [35]. In the ‘3-drop technique’, used in our center, a drop of medium containing the embryo(s) is separated from a preceding and following drop of medium by an air bubble [9]. With ultrasonographic guidance at embryo transfer, it has become possible to visualize these air bubbles [36], and the air bubbles also often function as a marker for transfer content [15]. Of all embryo(s) that implant successfully, 81% does so in the area where the air bubbles were initially seen on embryo transfer [28]. It is demonstrated that the air bubbles did not move after immediate ambulation after transfer in 94.1% of the cases [37] and this is confirmed by our study group [38]. We agree there is a lack of knowledge about positioning of the embryo(s) during and after embryo transfer in the uterine cavity, independently using a simple syringe or a new device. However, nowadays the only way to visualize the content or movement of the transfer is the air bubble.

Pushing a simple syringe allows for a wide range of power and speed [25], which is mainly affected by the force used to press the plunger, but also the resistance in this plunger and the resistance to where force is applied to, that is, the uterine cavity. As far as we know, there are no data available on uterine resistance in embryo transfer.
Thereby, the pump device keeps the speed stable, independent of the pressure.

Concerning two important aspects in embryo transfer, speed and optimal placement, we composed a transfer protocol in our study center, which includes performing the manually embryo transfer ‘gently’ and the intention to place the embryo(s) approximately 1 cm from the endometrial fundus for both the PRET device and manually performed embryo transfer. The mean distance from the endometrial fundus is not different in both groups, and therefore, both methods were performed as accurately as possible.

Despite this comparable mean distance from the endometrial fundus, both this distance and the transferred distance of the embryo(s) (fig. 3 and 4) demonstrate a smaller variance of the embryo placement by using the PRET device compared to the manually performed embryo transfer. This can be interpreted as a better controlled placement, which allows us to do accessory research on the most optimal positioning of the transferred embryo(s) regarding improvement in implantation and thereby OPRs. Following this, as soon as the optimal position is found, this can directly be implemented in clinical practice. Since the device can adapt the catheter speed, the distance of optimal placement can easily be influenced.

The plotted figure of the transferred distance of the embryo (fig. 4) includes negative values. This can be explained by removing the catheter out of the uterine cavity. Even when this happens slowly and gently, the air bubbles including an embryo can be pulled back minimally because of negative pressure created as a result of removing the catheter. In these little distances (in mm), negative values can easily arise. This pulling back of the air bubble happened in both study groups. Since the distance of insertion of the inner catheter is not different in both groups, we can conclude that in both groups the embryo(s) are as precisely placed as possible.

Whether this better controlled positioning of embryo(s) with the PRET device results in an increase of the OPR needs to be researched in a larger study. In this current study, a non-significant difference in OPR was found for the total group (21.0% after PRET vs. 17.0% after the manually performed embryo transfer, NNT 25).

Since the improvement in OPR for the total group was lower than the expected 8% on which the sample size calculation was based on the OPR, it was decided to further investigate this. A clinically relevant improvement of 4% requires more than 2,300 embryo transfers to reach statistical significance. It is not desirable to extend this inclusion target in this current mono-center design. Therefore, we decided to discontinue the study and to perform subgroup analysis. A subgroup analysis showed that the OPR in particular showed a trend of significance toward a clinically relevant improvement, after frozen-thawed embryo transfer with the PRET device (17.5%) compared to the manually performed embryo transfer (10.9%, NNT 15).

This success might indicate that perhaps more vulnerable frozen-thawed embryos are more sensitive to a gentle injection speed (and/or pressure) or benefit more from exact positioning for implantation. Despite reproductive technologies, implantation is still less understood. Only 30% of the couples undergoing IVF/ICSI treatment who reach the stage of embryo transfer actually become pregnant. In the last few years, vitrification has improved, which make frozen-thawed embryo transfers more popular. Recent studies suggest that freeze-all strategy, in

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Fig. 5. Outcomes. The categorical variables are presented as % (n).

Embryo transfers
- Total (599)
  - Fresh (286)
  - Frozen (313)

Manual
- Total (294)
  - Fresh (147)
  - Frozen (147)

PRET
- Total (305)
  - Fresh (139)
  - Frozen (166)

Implantation rate
- Total: 21.3
  - Fresh: 26.9
  - Frozen: 15.7

Clinical pregnancy
- Total: 22.1 (65)
  - Fresh: 27.9 (41)
  - Frozen: 16.3 (24)

Ongoing pregnancy
- Total: 17.0 (50)
  - Fresh: 23.1 (34)
  - Frozen: 10.9 (16)

Implantation rate
- Total: 25.1
  - Fresh: 30.6
  - Frozen: 20.5

Clinical pregnancy
- Total: 26.6 (81)
  - Fresh: 32.4 (45)
  - Frozen: 21.7 (36)

Ongoing pregnancy
- Total: 21.0 (64)
  - Fresh: 25.2 (35)
  - Frozen: 17.5 (29)
which all embryos are cryopreserved and transferred at a later date in unstimulated cycles, leads to more pregnancies compared to the ‘fresh’ transfers [39–42]. Hypothesized is that successful implantation of an embryo depends on the endometrial receptivity (and microenvironment for embryo-maternal signaling) within the uterine cavity, during the peri-implantation period [43]. We hypothesize that the frozen-thawed embryos benefit more from placement using the PRET, maybe because of improved endometrial receptivity or the combination of both. It could be that the frozen-thawed embryos are more sensitive to gentle placing with the PRET; however, there are no data available on this subject, and therefore, this needs further research. Furthermore, since the overall OPR after frozen-thawed embryo transfer is lower compared to fresh embryo transfer, possibly more benefit from new techniques in improving the OPR can be expected.

Despite the insufficient study about the power to make conclusive statements regarding safety, we find it reassuring that the small number of EUGs and retained embryo(s) are equal in both groups. For those unwanted (side) effects, it was not possible to prove in the study protocol, but it is an important objective of this study and of great clinical relevance.

Overall, the OPR was lower than the overall results of our center. This can be explained by the fact that different study protocols are carried out in our academic IVF center. Most study protocols are performed in the first IVF/ICSI cycles, and women were not allowed to participate in the same cycle in our current study. Therefore, most patients had undergone former IVF/ICSI attempts that did not result in an pregnancy, leading to a negatively selected group of patients in this current study.

These current study results give the opportunity to create a well-designed following trial. To rule out bias as much as possible, patients will be included from their first treatment attempt, and time to pregnancy needs to be the primary outcome. In conclusion, this randomized controlled trial confirms our in vitro results of the PRET device, which generates a standardized injection speed and results in a reliable and better controlled positioning of the embryo(s) into the uterine cavity compared to the manually performed embryo transfer with a syringe. This study gives the opportunity to standardize and influence the last and important step of embryo transfer in IVF treatment, which may positively influence pregnancy rates, which needs to be researched in a multicenter trial. The PRET device is easy to implement and will not only be of great value in clinical practice but also with regard to future developments in the field of implantation studies.

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