

## Importance of embryo transfer duration in human assisted reproduction techniques

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### Abstract

*The most important step in in vitro fertilization (IVF) is the transfer of the embryos (ET) in the uterine cavity. Stimulation of the cervix or uterus during the procedure causes uterine contractions and possibly expulsion of the embryos. The catheter for embryo transfer can also be an element of local distress. The aim of the present pilot study was to assess the influence of the time the catheter remains inside in the uterine cavity on the final outcome of the IVF procedure. The prospective two-center study excluded patients with difficult ET procedure. The pregnancy was confirmed by ultrasound (embryonic cardiac activity present) 6 weeks after the procedure. Patients were divided into two groups (catheterization < 120 seconds or > 120 seconds). Data were processed and statistically analyzed applying Fisher's exact test. 122 cycles of IVF were analyzed. Average ages of patients included in the study was 34.6 years, the average number of transferred embryos was 2.18 and 36 % of the patients achieved a clinical pregnancy (29% of group A and 43% of group B). The statistical analysis indicated no significant differences between the two groups in term of pregnancy rate ( $p > 0.05$ ).*

**Keywords:** IVF, embryo transfer time, pregnancy

### 1. Introduction

The birth of Louise Joy Brown in 1978, the first baby conceived with IVF, was a milestone for assisted reproduction and marked the beginning of fundamental researches in this field. Beginning with the first in vitro fertilization (IVF) of human oocytes, biologist R.G Edwards and gynecologist P. Steptoe have spent 19 years in this field of research, before they have achieved this outstanding result (L. JIANG [1]). Since then multiples studies were conducted all over the world (J.D. BIGGERS [2]). The 15<sup>th</sup> European IVF-monitoring (EIM) report, presents the results of treatments involving assisted reproductive technology (ART) initiated in Europe during 2011. From a total of 361 972 ART cycles, performed a population of 285 million inhabitants, the clinical pregnancy rates per aspiration and per transfer for all IVF cycles were stable, 29.1% and 33.2% respectively (M.S. KUPKA et al [3]). The success of IVF depends on numerous factors: causes of infertility, embryo quality, endometrial receptivity and the embryo-transfer procedure itself. The ET is the final step in an IVF cycle which, despite its simplicity, can adversely impact the IVF result (F. GHAFARI et al [4]).

The first description of embryo transfer was published in 1984 by Edwards R. (R.G. EDWARDS [5]). In contrast to other steps of ART procedures, ET technique remained relatively unchanged for decades (I. HALVAEI et al [6]). According to Schoolcraft W.B., several variables play a role in the success of a transfer, including catheter type, atraumatic technique and the use of ultrasound guidance. Further research concluded that uterine contractions decrease was associated with better implantation and pregnancy rates (W.B. SCHOOLCRAFT et al [7]). Latest data suggests that the mere presence of the catheter in the uterine cavity could increase uterine contractions (W.B. SCHOOLCRAFT [8]). This concept represents the reference point of our research. Therefore, the main objective of our study was to investigate whether the duration of the embryo transfer catheter's presence has had any influence on pregnancy outcomes.

## 2. Materials and Methods

Our prospective two-center observational study was conducted in the Assisted Human Reproduction Center of Gynecology I Clinic from Cluj-Napoca and in the Center for Assisted Reproduction "Zygota Mureş" between 01.11. 2015 and 01. 04. 2016. Inclusion criteria were: age of patients 25-35, cycles where at least one good quality embryo (grading 1/2) was transferred and fresh ultrasound guided embryo transfer. Exclusion criteria were the following: uterine cavity abnormalities (diagnosed by hysteroscopy or saline infusion sonography), frozen thawed embryo transfers, difficult transfers (blood on the catheter or bleeding after the ET), endometrial thickness <7 mm, lack of follow-up. Selected patients have undergone controlled ovarian stimulation followed by either IVF or intracytoplasmic sperm injection (ICSI). All selected patients were treated according to standard long ovarian stimulation protocol: down regulation was achieved using triptorelyne acetate (Gonapeptyl<sup>®</sup>), 0,1 mg/day beginning on day 21 of menstrual cycle followed by ovarian stimulation with recombinant FSH (Puregon<sup>®</sup>/ Gonal F), starting with 225 UI/day. After day 5 of stimulation, follicular growth was assessed by transvaginal ultrasound. Doses were adjusted according to ovarian response. Endometrial thickness was also assessed during ultrasound. Ovulation was induced using 5000 UI HCG (Pregnyl<sup>®</sup>/ Ovitrelle) when three follicles reached 20 mm. After 35 hours, oocytes were collected and processed according to each center's internal protocol in agreement to IVF/ICSI standards. The embryo-transfer protocol included the following steps (following standard international recommendations): ultrasound guided embryo-transfer with instruction of patients to maintain a full bladder for proper ultrasound visualization, preparation of cervix with HEPES medium (4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid), use of soft catheters (Cook<sup>®</sup> Medical) with trial transfer done for all patients. Catheters were loaded with embryos flanked by air bubbles in 30 µl media and embryos were gently injected. After the transfer, the embryologist checked for retained embryos. If there were no retained embryos, the catheter was withdrawn. The time between catheter insertion and withdrawal was exactly monitored. All patients followed intravaginal progesterone treatment (Lutinus<sup>®</sup>) 100 mg/day after transfer and rested for 2 hours on the premises. Clinical pregnancy rates were assessed after 6 weeks by transvaginal ultrasound (gestational sac with embryo cardiac activity in the uterine cavity).

Data consisting of patients age at the time of ET, number of retrieved oocytes, fertilized embryos, number of transferred embryos and pregnancy outcome was collected in a database using Excel<sup>®</sup>. Patients were divided into two groups, depending on the length of time that the catheter had spent in the uterus: group A < 120 sec, group B > 120 sec. The data was processed and statistically analyzed comparing the two groups parameters applying Fisher's exact test. *p value* of < 0.05 was considered statistically significant.

### 3. Results and discussion

One hundred ninety-two (192) cycles of IVF were performed during the time-frame of our study in the two centers. We analyzed 122 cycles of IVF. The average age of patients included in the study was  $34.6 \pm 4.86$  years (fig. 1).

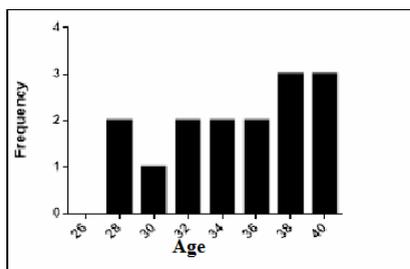


Figure 1. Patients age frequency ( $34.4 \pm 4.35$ , SE 1.12)

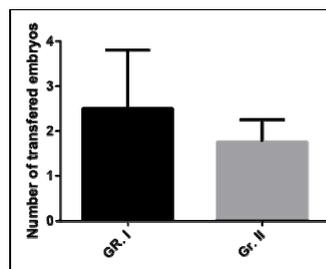


Figure 2. The average of transferred embryos

The mean age of patients who didn't achieve a pregnancy after the IVF and ET was 34.9 years versus 31.9 years, for the ones who became pregnant after the procedure. The average number of harvested oocytes was 9.27 ( $SD\ 5.193488$ ), with a maximum of 16 and a minimum of 3 oocytes in 43-year-old poor responder patient. The total number of oocytes obtained/ cycles was 779 which resulted in 513 embryos. Three hundred and ten were grade 1 embryos, 192 grade 2 and 11 embryos were grade 3 (excluded, to avoid this confounding factor). The average number of embryos transferred was  $2.18 \pm 0.64$ ,  $2.26 \pm 0.70$  for group A and  $2.18 \pm 0.55$ ,  $2.2 \pm 0.61$  for group B (fig. 2). Average embryo transfer time for patients in group A who achieved pregnancy was  $101.125 \pm 19.62$  seconds and  $100.725 \pm 16.00$  seconds for patients who didn't achieve pregnancy (fig.3). In group B,  $129.259 \pm 14.47$  seconds for the patient who achieved pregnancy group versus  $136.61 \pm 25.62$  seconds for the ones who did not.

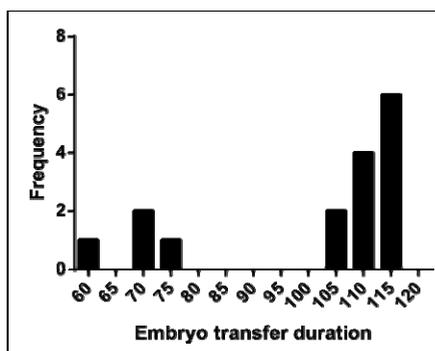


Figure 3. Embryo transfer time ( $101.125 \pm 19.62$ , SE 4.90)

The shortest embryo transfer time achieving pregnancy was 45 seconds. The longest one was 251 seconds long, but did not result in a pregnancy. From the 122 IVF cycles performed, the overall pregnancy rate was 36%. The clinical pregnancy rate was 29% in group A and 43% in group B, as seen in Table 1.

**Table 1.** Embryo transfer time and pregnancy rates

	<b>Total embryos transferred (e=122)</b>			
	<b>GROUP A (&lt;120 sec)</b>		<b>GROUP B (≥ 120 sec)</b>	
<b>Clinical pregnancy</b>	Yes 29% (n=17)	No 71% (n=42)	Yes 43% (n=27)	No 57% (n=36)
<b>Average embryo transfer time</b>	101.125 ±19.62	100.725 ±16.00	129.259 ±14.47	136.61± 25.62

The comparative statistical analysis, applying Fisher's exact test indicated no significant statistical difference between the two groups regarding the pregnancy rate ( $p>0.05$ ).

The present pilot study aimed to identify the relationship between the time spent by the embryo transfer catheter in the uterine cavity and the number of pregnancies achieved. Certainly, there are many factors involved in the success of assisted reproduction and the ET itself, but there are also some simple aspects that may change the outcomes. An atraumatic transfer is the goal, but what does trauma to the uterus really mean in this scenario? The use of tenaculum for grasping the cervix or any other manipulation during ET is not accepted anymore; since it was proved that it induces oxytocin release which triggers uterine contractions (P. LESNY et al [9]). All patients with difficult transfers were excluded but even in this scenario specialists in our centers avoid using a tenaculum. This effect could happen also in the event of touching the fundus of the uterus with the catheter. (C. DORN et al [10]). The negative effect of increased uterine contractions was also observed by Fanchin et al, with the occasion of digital recording of the uterus, using ultrasound frequency and direction of uterine contractions after ET, concluding that pregnancy and implantation rates decrease as the frequency of uterine contractions increase (R. FANCHIN et al [11]). The use of soft catheters was correlated with better pregnancy rates in several studies (A.M. ABOU-SETTA et al [12], W. BUCKETT [13]). Our centers are also using soft catheters, and, in the rare event of a very difficult transfer we, sometimes, use the firm ones. Mucus, if present, can interfere with depositing the embryos by obstruction of the catheter tip (L. MAINS et al [14]). The removal of the mucus from the cervix was associated in some trials with a slightly higher pregnancy rate (B.A. VISSCHERS et al [15]). This is the reason why we gently clean the cervix with HEPES medium (4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid). Blood on the tip of the catheter could be a witness of difficult transfer (V.T. GOUDAS et al [16]) and could impact implantation rates (R. ALVERO et al [17]). The decision to exclude difficult embryo transfers, including the ones where we saw blood on the catheter or where the patient witnessed bleeding after the procedure was also supported by the low pregnancy rates after difficult ET, published in the current literature (R. MANSOUR et al [18]).

Nowadays, when more and more reproductive aged couples postpone childbearing, the average age of women addressing ART procedures for conception increased from 35 years to 35.9 years (G.M. CHAMBERS et al [19]). In our study the average age of patients was 34.9 years. These facts are similar with other published data. A well known fact is that fertility and the success rates of ART procedures decreases with age, also demonstrated in our study when seeing that the mean age of women who didn't achieve pregnancy was higher (34.9 versus 31.9 years). The pregnancy rates achieved per transfer was 36% which is comparable to the latest published data regarding success rates of 33.2% in Europe (M.S. KUPKA et al [3]). Our results, were consistent with this data, even considering the fact that the study was conducted in two different centers, with slightly different laboratory settings (distance from embryology lab to ET room).

Regarding the number of embryo's transferred, the mean number overall the 122 cycles was 2.18, meaning mostly double embryo transfers. The data published by The European IVF-Monitoring Consortium for the European Society of Human Reproduction and Embryology in January 2016 about Assisted Reproduction in Europe in 2011 states that in most cases (56.7%) a double embryo transfer occurred even though in some countries like Belgium, Finland and Sweden the trend towards a single embryo transfer is increasing (M.S. KUPKA et al [3]).

In the present study, the distribution of embryos transferred was mostly even between groups. So was the distribution of grading, but the problem of embryo grading heterogeneity persists. In a prospective sample-randomized, blinded study published in 2005 by Bendus et al, in which 35 day 3 embryos were independently graded by 26 different embryologists, indicated that there was an important inter-observer variability that could affect the success of IVF outcome (A.B. BENDUS et al [20]). To prevent the confounding effect of embryo grading in the future, we aim to develop this study and assess the impact of the time spent by the catheter during ET only on frozen thawed blastocysts. This will bypass the embryologist's decision on choosing the right cleavage stage embryo for transfer (W.B. SCHOOLCRAFT [8]). Also, by transferring them in a natural cycle we wish to avoid intense myometrial activity caused by the effect ovarian stimulation treatment. The only somewhat similar study we have found regarding the effect of the time spent by the catheter in the uterine cavity analyzed 100 women undergoing ET and randomized them into two groups: immediate removal of the catheter versus removal after a 30 second delay. There was no statistical difference between the two groups regarding the pregnancy rates (F. MARTINEZ et al [21]). One of the drawbacks of our study was evaluating the effect of the time spent by the catheter on the pregnancy outcome which is to far a reach, since there are so many other factors, as commented above, that could interfere with the success of IVF. The major drawback of our study was that the used embryos were in different developmental stages at the time of ET (day 3 to day 5 blastocysts). For future directions, we aim to find intermediate biochemical and ultrasound parameters in a thawed blastocyst transfer in order to evaluate the impact of the time spent by the catheter in the uterine cavity.

#### 4. Conclusion

The time spent by the catheter in the uterine cavity has no influence on pregnancy rates in fresh ET-IVF cycles. More research is needed to evaluate this aspect using intermediate parameters, after minimizing other confounding factors.

#### 5. Acknowledgements

IVF – in vitro fertilization; ET – embryo transfer; ICSI – intracytoplasmic sperm injection; ART – assisted reproduction technology.

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