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Research Article

Difficult Embryo Transfers during ART: Anticipating Them by Highlighting Female Risk Factors

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Abstract

Background: Difficult embryo transfer during *in vitro* fertilization is responsible for low pregnancy and live birth rates. This study aims to identify possible female risk factors to anticipate a difficult embryo transfer.

Methods: A retrospective, case–control, monocentric study was conducted at the Saint-Etienne University Hospital from January 2014 to December 2020. Cases were defined as couples who had a first difficult embryo transfer during a fresh cycle; controls were randomly selected couples without history of difficult transfer. An easy transfer is defined when it is smooth and atraumatic, and difficult if greater resistance occurs, which requires the use of a firm catheter and/or a pozzi tenaculum forceps.

Patient's data included epidemiological characteristics, cause of infertility, gestity, number of caesarean sections, surgical antecedent, uterine malformation; biological data: number of oocytes retained, number of embryos transferred, quality of the embryos transferred… Data were studied with univariate and multivariate analysis.

Results: Our study population consists of 230 cases and 690 controls. Endometriosis (OR: 2.35), tubal infertility (OR: 1.60) and the presence of a uterine malformation (OR: 5.37) were associated with an increased risk of difficult fresh embryo transfer in the multivariate analysis.

Conclusion: This study identified female risk factors that could be anticipated. A prospective study may be carried out to validate these data and to verify whether corrective measures are effective in improving clinical pregnancy and live birth outcomes.

Keywords: IVF; Embryo transfer; Risk factors; Endometriosis; Tubal infertility

Abbreviations: ART: Assisted reproductive Technologie; IVF: *In Vitro* fertilization; cIVF: Conventional *In Vitro* Fertilization; ICSI: Intracytoplasmic Sperm Injection; CPR: Clinical Pregnancy Rate ; LBR: Live Birth Rate; GnRH: Gonadotropin-Releasing; FSH: Hormone Follicle Stimulating Hormone; HCG: Human Chorionic Gonadotropin; BLEFCO: Biologistes des Laboratoires de L'étude de la Fécondation et de la Conservation de L'oeuf; KPI: Key Performance Indicator

Introduction

Embryo transfer is the crucial last step in an *In Vitro* fertilization (IVF) cycle. Despite the apparent simplicity of embryo transfer, difficult transfers are frequent and have been shown to significantly decrease the pregnancy rate [1-3]. According to the patient's age, the type of treatment - conventional *In Vitro* Fertilization (cIVF) and Intracytoplasmic Sperm Injection (ICSI) - the number of transferred embryos, the difficulty of embryo transfer is an independent factor for predicting pregnancy [4]. This led to a recommendation in 2010, issued by the Evidence Based Guideline to ensure that the transfer goes as smoothly as possible. In 2015, the American Society for Reproductive Medicine wrote a guideline based on a review of the literature on the

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way embryo transfer should be performed. They recommend the use of abdominal ultrasound guidance, removal of cervical mucus, use of a flexible transfer catheter, placement of the embryo in the upper or middle part of the uterus, more than 1 cm from the fundus and immediate ambulation after completion of the transfer procedure [5].

Even though all these recommendations are followed, there are still difficult cases for embryo transfer we are currently unable to predict. Anticipating them would make it possible to implement corrections upstream of the transfer, make the transfer easier than expected and finally have the best pregnancy outcome. We know that there are anatomical causes such as tortuosity of the cervical canal or pronounced anteversion of the uterus [6], but many other factors could still influence the quality of the transfer and, to our knowledge, have not yet been studied, such as the patients' surgical and gynaecological history and the causes of infertility.

This study aims to identify risk factors (among women predisposition) for difficult embryo transfers that could be highlighted before the first transfer attempt.

Materials and Methods

Study Design and Population

In the field of public health, a retrospective case–control study design is the most valuable type of study to identify risk factors. That is why this type of study was purchased.

A retrospective case–control study was conducted at the University Hospital of Saint-Etienne. The study included all patients undergoing Assisted Reproductive Technology (ART) with fresh embryo transfer following conventional *In Vitro* Fertilization (cIVF) and Intracytoplasmic Sperm Injection (ICSI) between January 2014 and December 2020. For each transfer, the quality of the transfer and pregnancy occurrences were collected. Transfers without mention of the quality were excluded.

Evaluation of the Transfer Quality

At the fertility center of Saint-Etienne, the transfers are classified into three categories: easy noted as A, correct noted as B and difficult noted as C. An easy transfer (A) is defined when it is smooth and atraumatic, correct (B) when a slight difficulty in passing the cervix occurs or when the flush is not seen with ultrasound. Embryo transfer is considered difficult (C) if greater resistance occurs, which requires the use of a firm catheter and/or a pozzi tenaculum forceps.

Cases were defined as patients who had a difficult fresh embryo transfer (C). If the couple had multiple difficult fresh embryo transfers, only the data at the time of the first difficult transfer were used to assess the risk factors for difficult transfers. Controls were defined as patients who had had an easy fresh embryo transfer (A) and had never had any fresh difficult transfer (C). They were selected by simple randomization; 3 controls were selected for 1 case.

The transfer is always performed by two gynaecologists: one performs the transfer itself, and the other guides the patient by performing an ultrasound. The gynaecologist performing the transfer classified it as easy (A), correct (B), or difficult (C), as described earlier.

We performed a preliminary analysis of our results for our Key Performance Indicator that determine Clinical Pregnancy Rate (CPR) and Live Birth Rate (LBR) for each group of transfer (A/B/C). A total of 3704 embryo transfers were analysed, of which 2392 (64.6%) were easy, 1025 (27.7%) were correct, and 287 (7.7%) were difficult. The characteristics at the time of the transfers are listed in Table 1. A difficult transfer (C) was associated with low CPR (26.8% vs 39.9% for an A transfer p=0.001) and LBR (21.6% vs 31.1% for an A transfer p<0.001) (Table 1). The results of this preliminary analysis concur with the literature [1-3,7,8].

Stimulation Technique

For each IVF attempt, we listed the stimulation protocol used.

In a Gonadotropin-Releasing Hormone (GnRH) antagonist protocol, patients receive a daily injection of Follicle Stimulating Hormone (FSH, recombinant or urinary) from the third day of the cycle. During the stimulation, injections of antagonist is added. In the case of the short agonist protocol, patients receive a daily injection of GnRH agonist (Decapeptyl 0.1mg ™, Ipsen Pharma, France) from the first day of the cycle, then stimulation by injections of FSH from the third day of the cycle is added. In the case of the long agonist protocol, ovarian function is put to rest by a unique injection of GnRH agonist (Decapeptyl 3mg ™, Ipsen Pharma, France) or a daily injection of de Decapeptyl 0,1 mg; after an ultrasound examination and biological control of ovarian function, stimulation starts with injections of FSH.

Table 1: Characteristics and outcomes of all embryo transfers.

Characteristics	Group A N=2392	Group B N=1025	Group C N=287	p-value
Number of embryos transferred, N(%)	2 392(64.6%)	1 025(27.7%)	287(7.7%)	
ART rank, Mean (± SD)	2.0(±1.3)	2.0(±1.3)	2.0(±1.4)	0.81[1]
Biological data				
Oocytes/pick up, Mean (± SD)	11.0(±6.9)	10.8(±7.0)	10.6(±6.9)	0.44 [1]
Cleaved embryos/pick up, Mean (± SD)	5.9(±4.6)	5.9(±4.5)	5.6(±4.3)	0.67[1]
Number of embryos transferred, N(%)				0.12[2]
1	779(32.6%)	323(31.5%)	72(25.1%)	
2	1 332(55.7%)	574(56.0%)	173(60.3%)	
3	281(11.8%)	128(12.5%)	42(14.6%)	
ART technique, N(%)				0,067[2]
IVF	406(17.0%)	206(20.1%)	57(19.9%)	
ICSI	1 986(83.0%)	819(79.9%)	230(80.1%)	
Day of embryo(s) transferred, N(%)				0,64[3]
Day 2-3	2 175(91.0%)	936(91.4%)	266(92.7%)	
Day 5-6	214(9.0%)	88(8.6%)	21(7.3%)	
Clinical pregnancies, n (Clinical pregnancy rate), N(%)	954(39.9%)	337(32.9%)	77(26.8%)	0.001[2]
Live births (live birth rate), N(%)	745(31.1%)	237(23.1%)	62(21.6%)	<0.001[2]
Early pregnancy loss, n (%)	179(18.8%)	88(26.1%)	13(16.9%)	0.057[2]
Ectopic pregnancy, n (%)	17(1.8%)	5(1.5%)	1(1.3%)	1[3]
Medical interruption of pregnancy, N(%)	8(0.8%)	4(1.2%)	0(0%)	0.76[3]

[1]Wilcoxon rank sum test; [2]Pearson's Chi-squared test; [3]Fisher's exact test.
ART: Assisted Reproductive Technologies; IVF: In Vitro Fertilization; ICSI: Intra-Cytoplasmic Sperm Injection

Table 2: Characteristics of cases and controls, Univariate analysis of factors affecting embryo transfer quality.

Characteristics	Controls N=690	Cases N=230	p-value
Women's			
Age (years), Mean (± SD)	32.9(±4.9)	33.3(±5.3)	0.32[1]
BMI (kg/m²), Mean (± SD)	23.7(±4.8)	24.0(±5.3)	0.89[1]
Smokers, N(%)	133(*19.6%*)	44(*19.6%*)	>0.99[2]
Partner's			
Age (years), Mean (± SD)	35.7(±6.1)	37.0(±7.5)	0.10[1]
BMI (kg/m²), Mean (± SD)	25.4(±3.9)	25.3(±4.2)	0.39[1]
Smokers, N(%)	251(*38.7%*)	93(*42.3%*)	0.35[2]
Gestity Parity, N(%)			<0.001[2]
G0P0	342(*49.6%*)	149(*64.8%*)	
G≥1P0	121(*17.5%*)	37(*16.1%*)	
G≥1P≥1	227(*32.9%*)	44(*19.1%*)	
Vaginal birth antecedent, N(%)			<0.001[2]
0	514(*74.5%*)	201(*87.4%*)	
≥1	176(*25.5%*)	29(*12.6%*)	
Cesarean delivery history,N(%)			0.94[3]
0	644(*93.3%*)	215(*93.5%*)	
≥1	46(*6.7%*)	15(*6.5%*)	
Chronic medical disease, N(%)	65(*9.4%*)	31(*13.5%*)	0.081[2]
Psychological illness, N(%)	13(*1.9%*)	5(*2.2%*)	0.79[3]
Uterine malformation, N(%)	9(*1.3%*)	10(*4.4%*)	0.012[3]
Surgical antecedent, N(%)	291(*42.2%*)	111(*48.3%*)	0.11[2]
Laparotomy	7(*1.0%*)	5(*2.2%*)	0.19[3]
Endo-uterine gesture	117(*17.0%*)	40(*17.4%*)	0.88[2]
Cervical surgery	16(*2.3%*)	5(*2.2%*)	0.90[3]
Gynecological laparoscopy	168(*24.4%*)	71(*30.9%*)	0.051[2]
Laparoscopy (other)	106(*15.4%*)	32(*13.9%*)	0.59[2]
Duration of infertility, Mean (± SD)	3.9(±2.2)	4.6(±2. 8)	<0.001[1]
Cause of infertility, N(%)			
Female			
Female cause only	303(*43.9%*)	129(*56.1%*)	0.001[2]
Tubal	116(*16.8%*)	50(*21.7%*)	0.092[2]
Ovulatory	128(*18.6%*)	40(*17.4%*)	0.69[2]
Endometriosis	96(*13.9%*)	61(*26.5%*)	<0.001[2]
Uterine	10(*1.5%*)	14(*6.1%*)	<0.001[3]
Ovarian	93(*13.5%*)	41(*17.8%*)	0.11[2]
Cervical	14(*2.0%*)	5(*2.2%*)	>0.99[3]
Male cause only	183(*26.5%*)	35(*15.2%*)	<0.001[2]
Idiopathic	124(*18.0%*)	32(*13.9%*)	0.16[2]
Sexual	6(*0.9%*)	2(*0.9%*)	>0.99[3]
Antecedent of intrauterine insemination, N(%)	245(*35.5%*)	84(*36.5%*)	0.78[2]
History of difficult intrauterine insemination, N(%)	1(*0.1%*)	11(*4.8%*)	<0.001[3]
History of difficult frozen embryo transfer, N(%)	10(*1.5%*)	16(*7.0%*)	<0.001[3]
ART rank, N(%)			0.002[2]
1	498(*72.2%*)	140(*60.9%*)	
2	114(*16.5%*)	45(*29.6%*)	
≥3	78(*11.3%*)	45(*19.6%*)	
Ovarian stimulation protocol, N(%)			0.024[3]
GnRH antagonist	341(*49.4%*)	92(*40.0%*)	
Long agonist	254(*36.8%*)	92(*40.0%*)	
Short agonist	83(*12.0%*)	43(*18.7%*)	
Semi natural	12(*1.7%*)	3(*1.3%*)	
ART technique, N(%)			0.67[2]
IVF	132(*19.1%*)	47(*20.4%*)	
ICSI	558(*80.9%*)	183(*79.6%*)	
Origin of sperm, N(%)			0.36[3]
Fresh (partner)	639(*92.6%*)	219(*95.2%*)	
Frozen (partner, ejaculated)	19(*2.8%*)	1(*0.4%*)	
Frozen (partner, testicular)	16(*2.3%*)	6(*2.6%*)	
Straws (donor)	16(*2.3%*)	4(*1.7%*)	
Hyperstimulation, N(%)	85(*12.3%*)	26(*11.3%*)	0.68[2]
Oocytes/pick up, Mean (± SD)	11.5(±6.8)	10.9(±6.8)	0.22[3]
Cleaved embryos/pick up, Mean (± SD)	6.1(±4.4)	5.6(±4.3)	0.11[3]
Nb of embryos transferred, N(%)			0.001[2]
1	261(*37.8%*)	63(*27.4%*)	
2	385(*55.8%*)	139(*60.4%*)	
3	44(*6.4%*)	28(*12.2%*)	
Existence of frozen embryos, N(%)	282(*40.9%*)	85(*37.0%*)	0.29[2]
Day of transfer, N (%)			0.87[3]
Day 2-3	653(*94.6%*)	217(*94.4%*)	
Day 5-6	37(*5.4%*)	13(*5.7%*)	
Operator N (%)			0,8
A	136(*19,7%*)	45(*19,6%*)	
B	144(*20,9%*)	51(*22,2%*)	
C	128(*18,6%*)	49(*21,3%*)	
D	150(*21,7%*)	43(*18,7%*)	
E	132(*19,1%*)	42(*18,3%*)	
Embryo's quality, N(%)			0.4[2]
1	498(*72.2%*)	152(*66.1%*)	
2	93(*13.5%*)	37(*16.1%*)	
3	88(*12.8%*)	31(*13.5%*)	

[1]Wilcoxon rank sum test; [2]Pearson's chi-squared test; [3]Fisher's exact test.
GnRH: Gonadotropin-Releasing Hormone; BMI: Body Mass Index; ART: Assisted Reproductive Technologies; IVF: In Vitro Fertilization; ICSI = Intra-Cytoplasmic Sperm Injection

Regardless of the type of protocol, all of them end with an injection of recombinant Human Chorionic Gonadotropin (HCG, Ovitrelle TM, Merck Lipha Sante, France) to trigger ovulation 36 h before oocyte pick up.

Embryo Quality

In our center, four-cell stage (day 2) embryo quality is assessed using the BLEFCO classification (Biologistes des laboratoires de l'étude de la fécondation et de la conservation de l'oeuf) [9], which divides embryos into 5 types according to blastomere number and regularity, degree of fragmentation, and the presence of multinucleation. In this study, type 1 and type 2 are considered good-quality embryos (carriers of at most one low-quality criterion), while types 3 to 5 are considered poor-quality embryos.

Blastocysts were classified according to the conventional criteria of Gardner and Schoolcraft [9]. For this study, B6, B5, B4, and B3 extensions with inner mass cell and trophectoderm AA, AB, and BA were considered good-quality embryos. The others were considered poor-quality embryos.

Using these definitions, we graded the quality of transferred embryos from 1 to 3: 1 when the transferred embryo(s) was/were considered as good-quality embryo(s), 3 when the transferred embryo(s) was/were considered as poor-quality embryo(s) and 2 when there was at least one good-quality embryo and one poor-quality embryo that were transferred.

ART Rank

The first fresh cycle for IVF permitting to obtain a new live birth defines a rank 1 in our study. It includes all the transfers of fresh and/or Frozen Embryos (FET) issued from this cycle. If all these transfers failed to obtain a live birth (more than 22 weeks of gestation and/or more than 500 g of weight birth), the next following fresh cycle is defined as a rank 2, etc…

Embryo Transfer Technique

The patient was placed in the gynaecological position, the cervix was exposed with a speculum, the external orifice was cleaned with a saline solution, and the mucus was removed with cotton pads. This procedure is the same for all the operators. An abdominal ultrasound was performed throughout the transfer. Two gynaecologists were present at each transfer: one was in charge of the transfer, and the other guided the patient with ultrasound.

For embryo transfer, a flexible catheter (Ellios®, CDD, Paris, France) connected to a 5 cc syringe is used. The embryos are then loaded by a laboratory operator in a volume of 20 µL and the catheter is handed to the clinician who inserts it through the endocervical cervix and into the uterine cavity keeped at least 1 cm from the fundus. The embryos are injected into the uterine cavity, and then the catheter is slowly withdrawn. The speculum was gently removed after the biologist verified the absence of retained embryos. If the passage of the catheter is not possible through the cervical canal, there are several possible actions for the gynaecologist, according to the clinical aspect: use of a firm catheter with an outer sheath (TDT® set), use of pozzi tenaculum forceps, or both handsets. No mock transfer or systematic hysteroscopy was performed before the first transfer. The classification of the transfer quality A, B or C and all the data related to the embryo transfer are systematically recorded in the patient's medical file: the use of an Ellios® catheter or a firm catheter, pozzi tenaculum forceps, resistance into

Table 3: Multivariate analysis of factors affecting embryo transfer quality.

Characteristics	Odds Ratio	95% CI	p-value
Age (year)[a]	1.01	0.98, 1.05	0.5
Gestity [a]	0.87	0.71, 1.06	0.2
Parity [a]	0.31	0.18, 0.54	<0.001
Gestity * Parity [a]	1.28	1.07, 1.52	0.006
History of endo-uterine gesture	0.64	0.40, 1.00	0.05
Endometriosis	2.35	1.58, 3.50	<0.001
Ovarian cause	1.41	0.87, 2.29	0.2
Tubal cause	1.6	1.05, 2.45	0.028
Uterine cause			
No	1	—	
Uterine malformation	5.37	1.76, 16.3	0.003
Another cause	4.08	0.94, 17.7	0.061
Duration of infertility	1.12	1.04, 1.20	0.001
History of difficult frozen embryo transfer	4.1	1.70, 9.92	0.002
Cesarean delivery history			
0	1	—	
≥1	2	0.97, 4.15	0.061
Oocytes/pick up	1	0.97, 1.02	0.7
Day of transfer			
Day 2-3	1	—	
Day 5-6	0.89	0.43, 1.83	0.71

Model runned on imputed data with 920 observations
[a]Age, gestity and parity are discrete variable. Odds Ratio refer to a 1unit augmentation.
CI: Confidence Interval

the endocervical cervix and proper visualization by ultrasound.

Data Collection

Each patient's data was extracted from the MediFirst® software (France) and included:

Epidemiological data; for the women: gestity, parity, number of vaginal births, of Caesarean sections, existence of a chronical disease, psychological illness, surgical antecedent (laparotomy, intrauterine gesture: hysteroscopy or curettage, surgery of the cervix, laparoscopy due to a gynaecological cause and laparoscopy for another cause), uterine malformation; for the couple: age, body mass index (BMI), tobacco, cause of infertility, duration of infertility, transfer rank, history of intrauterine insemination, difficulty encountered during inseminations or Transfer of Frozen embryo (TEF).

Biological data: type of ovarian stimulation protocol, cIVF or ICSI, sperm origin, hyperstimulation (defined has more than 20 oocysts/retrieval), number of oocytes retained, number of embryos obtained, number of embryos transferred, existence of frozen embryos, quality of the embryos transferred, quality of the transfer, operators designed by A to E; pregnancy issue: live birth rate, defined as the number of deliveries that resulted in a live born neonate, and expressed per 100 embryo transfers and clinical pregnancy rate, defined as human Chorionic Gonadotropin (HCG) >100 UI/L 16 days after triggering the ovulation by recombinant HCG, expressed per 100 embryo transfers.

Ethical Aspects

An information notice was distributed to each patient in accordance with European Regulation No. 2016/679 on Data Protection. The study received the approval of the ethics committee of the Saint-Etienne University Hospital: IRBN 462017 CHUSTE".

Statistical Analysis

The collected variables were described by the mean and standard deviation when they were quantitative and by size and proportions when they were qualitative.

The first description addressed the transfer results of all patients undergoing assisted reproductive technology with fresh embryo transfer following cIVF/ICSI in the same cycle over the study period. The characteristics of the transfers rated A, B and C were compared.

After case and control selection, their characteristics were compared using Wilcoxon signed-rank tests for quantitative variables and Chi2 or Fisher's test for qualitative variables. The cases and control missing data were credited by multiple imputation [10].

To assess risk factors for difficult embryo transfers, a multivariate logistic regression was carried out. The variables included in the model were selected by bootstrap on 50 simulated imputed datasets from a selection of interest variables defined a priori (age of the patient and of the partner, patient's and partner's BMIs, tobacco for patient and partner, gestity, parity, number of vaginal deliveries, number of cesarean deliveries, uterine malformation, history of laparotomy, of intrauterine gesture, antecedent of cervix surgery, of laparoscopy (for a gynecological cause), history of intrauterine insemination, of difficult embryo transfer, ART rank, duration of infertility, number of oocytes/pick up, female cause of infertility, tubal cause, ovulatory cause, endometriosis, ovarian cause, cervical cause, male cause of infertility, idiopathic cause, number of transferred embryos and day of the transfer and quality of embryo transferred). Maternal age, number of caesarean sections, gestity and parity and their interactions together with the number of transferred embryos were forced into the model. The variables included in the final model were maternal age, gestity, parity and their interactions, history of intrauterine gesture, tubal cause of infertility, endometriosis, ovarian cause of infertility, uterine cause of infertility (including uterine malformation), duration of infertility, history of difficult frozen embryo transfer(s), number of caesarean sections, number of transferred embryos and day of the transfer and quality of embryo transferred.

All statistics were carried out using R [11] and tidyverse packages [12].

Results

A total of 690 controls and 230 cases were analysed.

Univariate results for patients and their partners' characteristics are summarized in Table 2. Age, BMI, smoking (both the patient and her partner) appeared to have no significant impact on the quality of embryo transfer.

Concerning the patient history, having a history of a chronic disease, a psychological illness or a surgical history do not seem to have an impact on the quality of the transfer. The nulliparous women had significantly more difficult embryo transfers, and the parity was lower in the difficult embryo transfer group (0.24 vs 0.42 p<0.001). The patients who had already had a vaginal birth had a better chance of having an easy embryo transfer (Table 2).

Concerning the causes of infertility, a female cause significantly increases the risk of having a difficult transfer (56% vs 44% p<0.001). When the cause is only due to the partner, there

is a significantly greater rate of easy transfers (27% vs 15% p<0.001). Regarding previous gestures due to the assisted reproductive technique, it appeared that the rate of patients with a history of intrauterine insemination is similar in both groups, but having already had a difficult intrauterine insemination significantly increased the risk of having another difficult embryo transfer (Table 2).

Nevertheless, in the univariate analysis, regarding the biological factors, there were no differences between the two groups regarding the assisted reproductive technique, the origin of the sperm, the number of hyperstimulation, the number of oocytes, the number of cleaved embryos per pick up, the existence of frozen embryos (for the supernumerary ones), the day of transfer and the embryo's quality. There was a greater rank in the difficult embryo transfer group, as well as a higher proportion when three embryos were transferred. The ovarian stimulation protocols were different (p=0.024).

In line with the findings of the univariate analysis, a multiple logistic regression analysis was performed to assess the relationship among values thought to be factors of a difficult embryo transfer outcome (Table 3). There is a significant association between a difficult embryo transfer and the presence of endometriosis, of a tubal cause of infertility and of having a uterine malformation as well as a history of difficult embryo transfer. There are other factors that appear to be more frequent in the control group, such as parity and having a history of endo-uterine gestures.

Discussion

Some authors described the role of the operator in the transfer results [6], and others have shown that when the technique was standardized, the results did not depend on the operator [13]. As our operators were similar in their performance as assessed by the Key Performance Indicator (KPI) controlled and validated each year and because two gynaecologists always performed the transfers, we did not notice an operator's influence (by standardizing the practice and avoiding some derivation of the procedure).

In this study, several factors that increase the risk of having a difficult embryo transfer were highlighted. The most important causes were endometriosis and tubal and uterine infertility. Parity, gestity and previous uterine gestures were found to facilitate embryo transfers.

Regarding the causes of infertility, in the univariate analysis, female causes in infertility were more frequent in the difficult embryo transfer group. In addition, the male causes were more frequent in the easy embryo transfer group. Furthermore, in the multivariate analysis, endometriosis and tubal and uterine causes were highlighted as risk factors. Having a uterine malformation is strongly associated with a difficult embryo transfer (the probability of having a difficult embryo transfer is 437% higher than in the control group), but the other causes among the uterine causes are not significant, perhaps due to a lack of statistical power. This could be explained by the impact of the malformation (didelphys, uni- or bicornuate) on the uterocervical angle. In addition, the presence of submucosal, intramural or cervical myomas may interfere with the entry or progress of the catheter in the cavity. Regarding the other causes of infertility, there are significantly more women with endometriosis and tubal causes in the group of difficult transfers. It can be due to the local inflammation created by endometriosis [14] or induced pain that makes gynecological examination difficult for women. Concerning tubal causes, a difficult transfer may be linked to an inflammatory disease if the patient has a history of upper genital tract infection or endometriosis. It would be interesting to classify them according to the initial mechanism of the tubal lesions; however, these hypotheses could not be tested due to an insufficient number of patients in this subgroup.

Regarding the ART history of the patient, a history of difficult intrauterine insemination was found to be significantly associated with difficult embryo transfer. In the univariate analysis, cases have a significantly higher rank, more embryos transferred, a longer duration of infertility and a greater use of agonist protocols. All these elements are linked and can be explained by the transfer policy of the center: repeated failures make the rank increase and result in longer infertility and a greater number of transferred embryos.

The greater the rank, the longer the infertility and the greater number of embryos. Regarding the stimulation protocol, the agonist is used as a second line because it is harder and longer to perform, and it requires more injections. However, it is difficult to know whether these elements are the cause or the consequence of the greater difficulty in transferring. Indeed, we have once again confirmed that a difficult transfer leads to a decrease in the clinical pregnancy rate, which can lead to repeated failures and an increased number of attempts.

Concerning the gynecological and obstetrical history, parity has an impact on the quality of the transfer; each delivery increases the chances of obtaining an easy transfer, and it tends to be the same with the gestity and with the history of intrauterine gestures. This could be explained by the fact that the cervix was dilated by the delivery or the intervention. In contrast, there is a trend to see more patients with a history of caesarean section deliveries in the case group; however, this trend is not significant in the multivariate analysis. It would be interesting to perform an analysis based on the indication of the caesarean section: before or during labor, to see if the effect is due to the gesture itself or if it is related to the absence of prior opening of the cervix.

In the study reporting by Larue *et al.* [6] concerning the anatomical causes of difficult embryo transfer, there was not any difference between the easy versus difficult embryo transfer groups regarding the age of the patient, the causes of infertility (unknown, male, ovulatory, other), parity, history of caesarean delivery, or history of conization or curettage. Tubal causes were significantly lower in the difficult group. However, their population was smaller (151 and 155 patients in each group), and the causes of infertility were less detailed. The first limitation of the study is the case–control design. Selection bias might have occurred despite the randomization initially performed to select the couples in the control group. It could also have a bias of reporting, including history (medical and surgical) and the difficulty of the previous intrauterine insemination. Concerning the collected data, there might be a lack of power for the history of surgery of the cervix since it was found only in 21 patients. This type of surgery might impact the difficulty of the transfer. It would be interesting to go further in the study of chronic medical diseases and psychological illnesses to see in detail if some have more impact than others: are there more difficult transfers in patients with depression or anxiety or in patients with a chronic inflammatory pathology? To answer these questions, an exhaustive collection of the data is needed.

The results of the study at stake in this article show that the presence of endometriosis, a tubal or uterine cause of infertility, and a history of difficult intrauterine insemination or frozen embryo transfer are risk factors for difficult fresh embryo transfer. This is the first study to bring together so many female factors and assess their impacts on the quality of the transfer.

Aiming at improving the pregnancy rate by decreasing the difficult embryo transfer rate, it is possible to identify these different factors before any transfer and act on them. Multiple procedures on the cervix are possible before the attempts as a mock transfer to select the catheter that will be the best for the patient, as shown by Mansour *et al*. [15]. Hysteroscopy with cervix dilatation is also possible [16,17].

Furthermore, in another study, hysteroscopy was suggested to these patients, along with cervical dilatation and a mock transfer, and the results were analysed in terms of transfer quality and clinical pregnancy rate.

Some centers perform these procedures routinely for all patients in the context of IVF. In his study, Borkar et al. highlighted that there was no benefit in performing a mock transfer in a population with no particular history [18]. It would therefore be even more important to carry out this study in a targeted population to avoid subjecting potentially unsuitable examinations to the entire IVF population of a department.

In the context of *In Vitro* Fertilization (IVF), embryo transfer is the climax of the whole process. It is a key step. Difficult transfers are associated with a decrease in the chances of pregnancy. The identified risk factors for a first difficult embryo transfer were endometriosis and uterine and tubal causes of infertility. Given these results, it is possible to offer patients with identified risk factors possible corrections before any transfer, such as a mock transfer, hysteroscopy or cervical dilatation. A prospective study may be carried out to validate these data and to verify whether corrective measures are effective in improving clinical pregnancy and live birth outcomes.

References

1. Kava-Braverman A, Martínez F, Rodríguez I, Álvarez M, Barri PN, Coroleu B. What is a difficult transfer? Analysis of 7,714 embryo transfers: the impact of maneuvers during embryo transfers on pregnancy rate and a proposal of objective assessment. Fertil Steril. 2017; 107: 657-663.e1.
2. Spitzer D, Haidbauer R, Corn C, Stadler J, Wirleitner B, Zech NH. Effects of embryo transfer quality on pregnancy and live birth delivery rates. J Assist Reprod Genet. 2012; 29: 131-5.
3. Phillips JAS, Martins WP, Nastri CO, Raine-Fenning NJ. Difficult embryo transfers or blood on catheter and assisted reproductive outcomes: a systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2013; 168: 121-8.
4. Tomás C, Tikkinen K, Tuomivaara L, Tapanainen JS, Martikainen H. The degree of difficulty of embryo transfer is an independent factor for predicting pregnancy. Hum Reprod. 2002; 17: 2632-5.
5. Practice Committee of the American Society for Reproductive Medicine. Electronic address: ASRM@asrm.org, Practice Committee of the American Society for Reproductive Medicine. Performing the embryo transfer: a guideline. Fertil Steril. 2017; 107: 882-96.
6. Larue L, Keromnes G, Massari A, Roche C, Bouret D, Cassuto NG, et al. Anatomical causes of difficult embryo transfer during in vitro fertilization. J Gynecol Obstet Hum Reprod. 2017; 46: 77-86.
7. Listijono DR, Boylan T, Cooke S, Kilani S, Chapman MG. An analysis of the impact of embryo transfer difficulty on live birth rates, using a standardised grading system. Hum Fertil (Camb). 2013; 16: 211-4.
8. Spandorfer SD, Goldstein J, Navarro J, Veeck L, Davis OK, Rosenwaks Z. Difficult embryo transfer has a negative impact on the outcome of in vitro fertilization. Fertil Steril. 2003; 79: 654-5.
9. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod. Proceedings of the an expert meeting. The Istanbul consensus workshop on embryo assessment Oxf Engl 2011. 2011; 26: 1270-83.
10. van Buuren Sv, Groothuis-Oudshoorn K. mice: multivariate Imputation by Chained Equations in R. J Stat Softw. 2011; 45: 1-67.
11. R: the R Project for Statistical Computing n.d. [cited Apr 7, 2021] Available from: https://www.r-project.org/.
12. Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, et al. Welcome to the Tidyverse. J Open Source Softw. 2019; 4: 1686.
13. van Weering HGI, Schats R, McDonnell J, Hompes PGA. Ongoing pregnancy rates in in vitro fertilization are not dependent on the physician performing the embryo transfer. Fertil Steril. 2005; 83: 316-20.
14. Donnez J, Donnez O, Orellana R, Binda MM, Dolmans MM. Endometriosis and infertility. Panminerva Med. 2016; 58: 143-50.
15. Mansour R, Aboulghar M, Serour G. Dummy embryo transfer: a technique that minimizes the problems of embryo transfer and improves the pregnancy rate in human in vitro fertilization. Fertil Steril. 1990; 54: 678-81.
16. Aubriot FX. [Difficult embryo transfer: what can be done in practice?]. Gynecol Obstet Fertil. 2003; 31: 157-61.
17. Arora P, Mishra V. Difficult embryo transfer: A systematic review. J Hum Reprod Sci. 2018; 11: 229-35.
18. Borkar A, Shah A, Gudi A, Homburg R. Outcome of mock embryo transfer before the first IVF cycle: A randomized control trial. Int J Reprod Biomed. 2020; 18: 951-60.