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Pain during embryo transfer is independently associated with clinical pregnancy in fresh/frozen assisted reproductive technology cycles

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Abstract

Aim: To assess whether pain experienced during embryo transfer (ET) is associated with the chance of clinical pregnancy in assisted reproductive technology cycles.

Methods: This was a prospective observational study of 284 women conducted between July 2011 and January 2014. Women under 40 years undergoing *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles followed by fresh/frozen ET were recruited. Pain was measured using a 100-mm visual analogue scale. Several factors relating to both pain and also the nature of the ET procedure were recorded: use of vulsellum, uterine contractility, depth of ET, duration of ET catheter insertion, urgency of micturition, psychological profile tests, salivary α-amylase and salivary cortisol. Primary outcome was the achievement of clinical pregnancy.

Results: Women who experienced pain during ET had a significantly lower clinical pregnancy rate compared with women who did not (42.2% vs 53.8%; $P = 0.03$). Non-pregnant women also had significantly higher pain scores compared with pregnant women (10.3 vs 6.4; $P = 0.01$). Pain was independent of >20 variables relating to (i) the nature of the ET procedure; (ii) psychological testing; and (iii) potential confounding factors inherent to IVF/ICSI. On binary logistic regression analysis, pain was an independent predictor for the chance of clinical pregnancy (OR, 0.59; 95%CI: 0.37-0.94; $P = 0.03$).

Conclusions: Pain during ET is independently associated with the chance of clinical pregnancy. The underlying mechanism could involve factors other than nature of the ET and the psychological state of the patient, and warrants further investigation.

Key words: assisted reproductive technology, embryo transfer, infertility.

Introduction

There is increasing evidence linking psychological stress to infertility. Historically, it has long and anecdotally been debated that adoption may increase fertility,[1–3] although this remains highly controversial.[4,5] More recently, studies focusing on couples undergoing assisted reproductive technology (ART) have also reported a correlation between stress and outcome,[6–12] while others failed to identify any robust correlation.[13–15]

In the last few years, there has also been an interest in the assessment of stress biomarkers, such as salivary α-amylase (sAA) and salivary cortisol, as surrogates for psychological stress in women with infertility.[16–19] Within the broader literature, it is of great interest to note that sAA in particular has been associated both with psychosocial stress,[20–22] including specific anxiety scores, such as the State–Trait Anxiety Inventory (STAI) score,[23] and also physical stress/painful stimuli, with a strong correlation with the visual analogue scale (VAS) for

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pain.[24–26] It is therefore evident that stress, anxiety and pain, along with their respective biomarkers, are intertwined.[27]

Within ART, the embryo transfer (ET) procedure can potentially involve stress, anxiety and pain in combination. To date, the research focus around ET has been only with regard to the assessment of technical factors such as cervical preparation,[28] difficult ET,[29] use of vulsellum,[30] time of catheter insertion,[31] type of catheter use,[32] embryo deposition location,[33] ultrasound guidance,[34] bed rest following procedure,[35] and others.[36] The subjective experience of pain during ET, however, which may be a reflection of both physical and psychological stress, has not been examined.

At Prince of Wales Hospital, Hong Kong, there was the clinical impression that women undergoing ET experienced varying degrees of distress, irrespective of the difficulty of the procedure. In addition, it appeared that women who tolerated the procedure very well had a more favorable clinical outcome. The aim of this study was therefore to examine the relationship between pain perception during ET and the chance of clinical pregnancy, while also correlating the findings with (i) the nature and characteristics of the procedure; and (ii) baseline psychological tests and stress biomarkers.

Methods

Patient recruitment

Women undergoing fresh or frozen *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles at a tertiary university ART unit at the Prince of Wales Hospital, Hong Kong, between July 2011 and January 2014 were invited to participate in this study. Exclusion criteria for participation included: (i) age ≥40 years; (ii) abnormal uterine cavity; (iii) presence of hydrosalpinx; (iv) presence of genital tract infection; (v) repeated implantation failure; and (vi) cycle cancelation. The study was approved by the Ethics committee of the hospital (Joint Chinese University of Hong Kong–New Territories East Cluster Clinical Research Ethics Committee registration number CRE 2011.303) and was part of a large prospective cohort trial spanning more than 2 years dedicated to comprehensively assessing several factors inherent to the ET procedure. Two of these factors, namely uterine contractility and air bubble deposition, are being reported separately for the sub-group of patients undergoing fresh ET cycles only (Chung *et al.* and Saravelos *et al.*, unpubl. data, 2015). Patients provided written informed consent upon enrolment into the study.

Stimulation protocol

Women received a standard protocol for ovulation induction. For the long downregulation protocol, Buserelin nasal spray (Suprecur, Hoechst, Germany) 600 μg daily was used for at least 14 days from the mid-luteal phase of the preceding cycle. For the short protocol, Ganirelix or Cetrorelix subcutaneous injection (Cetrotide, Merck Serono, Germany) 0.25 mg daily was used from day 6 of stimulation (or alternatively when estradiol (E2) > 800 pmol/L or the leading follicle was >14 mm) until the day of human chorionic gonadotropin (hCG) trigger.

Ovarian stimulation was achieved using human menopausal gonadotropins (Pergonal, Serono, Aubonne/Switzerland) or recombinant follicle-stimulating hormone (rFSH; Gonal-F, Serono, Switzerland; or Puregon, Organon, Holland) at doses ranging from 150 to 450 IU/day. Ovarian response was monitored on transvaginal ultrasonography and serum E2 measurements from stimulation day 6 onwards. When three or more follicles ≥18 mm in diameter were seen, 5000 IU of hCG (Profasi, Serono, Switzerland) was given and transvaginal oocyte retrieval was performed 36 h later followed by either IVF or ICSI.

All embryos were transferred on day 3. Embryos were considered as good quality when they had no apparent morphological abnormalities, consisted of at least seven cells and had <10% fragmentation. Surplus embryos were cryopreserved following patient consent. Subsequent frozen ET was performed in either natural or hormone replacement treatment (HRT) cycles. Progesterone (Crinone gel 8% daily, Merck Serono, or Endometrin 100 mg BD, Ferring, Switzerland) was given as luteal phase support 3 days prior to ET in frozen HRT cycles and after the ET in fresh cycles and natural frozen cycles.

Embryo transfer procedure

All ET were performed under ultrasound guidance using a 3D/4D General Electric Voluson 730 Expert series ultrasound machine and an RAB4-8 L, 4.0–8.0 MHz 3D/4D probe (GE Medical Systems Kretztechnik, Austria). Patients were encouraged to have a moderately full bladder on the day of the procedure. They were placed in a dorsal recumbent position, and the cervical mucus was cleared through a Cusco speculum using a sterile cotton wool stick soaked in culture medium. Embryos were loaded into an atraumatic Cook K-jet SIVF embryo transfer catheter (Cook Medical, IN, USA). They were then transferred into the middle of the uterine cavity under ultrasound guidance, aiming

for approximately 10–20 mm from the fundus. When the air bubbles could be clearly visualized within the uterine cavity, the catheter was returned to the embryologist to confirm that there were no retained embryos.

In order to systematically assess and control for the inherent variations in the ET procedure, along with several patient characteristics, the following factors were recorded: (i) operator performing the procedure; (ii) urge of urinary frequency (mild, moderate, severe); (iii) length of time the outer and inner catheter was inserted into the cervical-uterine cavity; (iv) use of vulsellum; (v) uterine contractility following the ET (/min), assessed in the mid-sagittal plane on 3-min recording followed by analysis in a four-fold accelerated format; and (vi) depth of ET, where high transfer corresponded to air bubbles seen <15 mm from the fundus, and low transfer corresponded to air bubbles seen >15 mm from the fundus, in accordance with the majority of studies.[33,37,38]

Following transfer, the patients rested for up to 60 min and were then discharged. Pregnancy test was subsequently performed in 2 weeks, followed by an ultrasound 4 weeks after the procedure to confirm clinical pregnancy (presence of a gestational sac with or without contents). The pregnancy rate of the study cohort was defined as the number of clinical pregnancies achieved divided by the number of ET performed.

Pain scores

Following the ET, the patients were asked to rate the pain of the procedure on a 100-mm VAS, a validated and widely used method for pain assessment,[39] where '0' is no pain and '100' is the worst possible pain. The assessment was performed by a research assistant after the operating clinician had left the procedure room, to avoid any bias from the patient reporting lower (or higher) scores in the presence of the operator. Given that there is no standardized categorization (i.e. cut-offs for mild, moderate and severe pain) for the degree of pain according to the VAS,[40,41] moderate pain was considered as any score above the 85th centile of reported scores, in accordance with the methodology of previous studies.[40] Therefore, pain score 0 was considered as no pain; 1–19, discomfort/mild pain; and ≥20, moderate pain. This method takes into account the nature of the ET procedure, which is not considered a particularly painful procedure, while allowing categorization of patients into reasonably sized groups.

Psychological tests and biomarkers

A subset of women enrolling in the study agreed to complete the Chinese translation versions of the Beck Depression Inventory (BDI), the General Health Questionnaire (GHQ), and the STAI on the day of recruitment. The BDI is a multiple-choice self-report inventory widely used for measuring the severity of depressionl;[42] the GHQ is a screening tool to identify minor psychiatric disorders;[43] and the STAI is a self-report inventory that measures anxiety by assessing both state and trait anxiety.[44] Women agreeing to the baseline psychological testing completed the forms on the day of oocyte retrieval in a ward separate to that of the operating procedure. Saliva for sAA and cortisol was collected after the forms were completed. Preparation, collection, analysis and reporting was performed according to the published recommendations.[45]

Statistical analysis

All data were entered and analyzed using SPSS for Windows (version 22; SPSS, Chicago, IL, USA). Continuous parametric data are expressed in mean ± SD, non-parametric data as median (range) and categorical data as number and percentage (%). Continuous data were compared using either the independent Student's *t*-test or the Mann–Whitney *U*-test (according to the variable distribution) and categorical data were compared using chi-squared test with contingency tables (linear by linear association for 3 × 2 tables or Pearson chi-squared/Fisher's exact test for 2 × 2 tables according to the minimum expected count). Binary logistic regression analysis (enter and backward likelihood ratio [LR] methods) were used to identify the factors independently related to chance of clinical pregnancy. Two-sided $P < 0.05$ was considered as statistically significant.

Results

Of 287 eligible women recruited, 284 enrolled in the study following exclusion of two women with no viable embryos and one woman who elected to withdraw from the study. At the completion of the study a total of 360 cycles were included in the final analysis, of which 287/360 (79.7%) were fresh and 73/360 (20.3%) were frozen. Overall 175/360 cycles (48.6%) resulted in a clinical pregnancy, and 185/360 (51.4%) did not result in a clinical pregnancy. Comparison between these two groups against a series of variables relating to patient and treatment characteristics was performed to ascertain significant differences (Table 1). Of the variables, the following were significantly different between the two groups: age, E2 on day of trigger, progesterone (P4) on day of ET, number of good quality embryos

Table 1 Factors associated with clinical pregnancy

	Pregnant (n = 175)	Non-pregnant (n = 185)	P-value†
Age (years)	34.4 ± 3.3	35.1 ± 3.3	0.23
BMI (kg/m^2)	21.4 ± 2.6	21.2 ± 2.8	0.38
Years of infertility	5.3 ± 3.2	5.3 ± 3.3	0.83
Main cause of infertility			0.43
Ovulatory	7/175 (4.0)	9/185 (4.9)	
Tubo-peritoneal	85/175 (48.6)	101/185 (54.6)	
Male	59/175 (33.7)	43/185 (23.2)	
Endometriosis	10/175 (5.7)	13/185 (7.0)	
Unexplained	14/175 (8.0)	19/185 (10.3)	
Treatment protocol			0.84
Agonist	148/175 (84.6)	155/185 (83.8)	
Antagonist	27/175 (15.4)	30/185 (16.2)	
Cycle type			0.70
Fresh	141/175 (80.6)	146/185 (78.9)	
Frozen	34/175 (19.4)	39/185 (21.1)	
Fertilization method			0.78
IVF	113/175 (64.6)	122/185 (65.9)	
ICSI	62/175 (35.4)	63/185 (34.1)	
Baseline FSH (IU/L)	7.1 ± 1.6	7.4 ± 2.3	0.19
Baseline LH (IU/L)	2.6 ± 1.7	2.7 ± 1.7	0.64
E2 on day of hCG (pmol/L)	12 295 ± 6739	10 567 ± 6431	0.02
P4 on day of ET (nmol/L)	251 ± 162	213 ± 152	0.02
No. mature oocytes retrieved	7.3 ± 2.7	6.9 ± 2.7	0.13
No. top quality embryos transferred			<0.001
0	2/175 (1.1)	7/185 (3.8)	
1	38/175 (21.7)	71/185 (38.4)	
2	135/175 (77.1)	107/185 (57.8)	
Endometrial thickness (mm)	11.4 ± 0.3	11.2 ± 0.3	0.55
Contraction frequency (n/min)	2.1 ± 1.3	2.5 ± 1.3	<0.001
Vulsellum use			0.91
Yes	8/175 (4.6)	8/185 (4.3)	
No	167/175 (95.4)	177/185 (95.7)	
Pain at embryo transfer			0.03
Yes (score > 0)	68/175 (38.9)	93/185 (50.3)	
No (score = 0)	107/175 (61.1)	92/185 (49.7)	
Average pain score (0–100)	3 (0–80)	0 (0–80)	<0.01
	6.4 ± 12.2	10.3 ± 15.6	
STAI score‡	55.1 ± 10.0	54.8 ± 8.6	0.84
BDI score‡	7.8 ± 8.2	7.5 ± 7.4	0.83
GHQ score‡	1.9 ± 2.0	3.4 ± 3.5	<0.001
Salivary α-amylase (U/mL)‡	140.1 ± 116.0	120.3 ± 96.2	0.25
Salivary cortisol (nmol/L)‡	8.3 ± 3.6	8.2 ± 4.1	0.82

Data given as mean ± SD for parametric data, median (range) for non-parametric data and n (%) for categorical variables. †Student's t-test, Mann–Whitney U-test or χ^2 test. ‡n = 156. BDI, Beck Depression Inventory; BMI, body mass index; E2, estradiol; ET, embryo transfer; FSH, follicle-stimulating hormone; GHQ, General Health Questionnaire; hCG, human chorionic gonadotropin; ICSI, intracytoplasmic sperm injection; IVF, *in vitro* fertilization; LH, luteinizing hormone; P4, progesterone; STAI, State–trait Anxiety Inventory.

transferred, experience of pain during ET, pain score during ET, and GHQ score.

Pain experienced during ET

In total, pain was experienced by women in 199/360 (44.7%) of ET procedures, with a mean pain score of 8.4 ± 14.2. In women who achieved a pregnancy, only 68/175 (38.9%) reported pain, whereas in those who did not become pregnant 93/185 (50.3%) reported pain (P = 0.03). In addition, the mean pain score of pregnant women (6.4 ± 12.2) was significantly reduced (P = 0.01) compared with non-pregnant women (10.3 ± 15.6; Table 1). Equally, when comparing women with pain versus no pain, the implantation and pregnancy rates

were significantly lower for the pain group (29.2% vs 37.7% and 42.2% vs 57.8% respectively; both $P < 0.05$). This was also demonstrated when grouping women into no pain, mild pain and moderate pain groups (Fig. 1). Equally, on receiver operating characteristic (ROC) curve analysis of pain score as a potential predictor of clinical pregnancy, the area under the curve (AUC) was 0.572 (95%CI: 0.51–0.63; $P = 0.02$). When repeating the ROC curve for cycles including only good-quality embryos and low frequency of uterine contractions (≤2/min), the AUC was 0.661 (95%CI: 0.54–0.78; $P = 0.01$; Fig. 2).

The presence or absence of pain during ET was also examined against a series of 28 potentially relevant variables relating to patient characteristics, ART treatment and the nature of the procedure (Table 2). There were no significant differences in any of the variables except one: the use of vulsellum, which occurred in 16/360 cases (4.4%). Of these 16 women, 12 (75%) reported pain, versus only 4/16 (25%) who reported no pain ($P = 0.01$). There was no difference, however, in the frequency of vulsellum use in the pregnant and non-pregnant groups (8/175, 4.6% vs 8/185, 4.3%, respectively; $P = 0.91$).

Regression analysis

All variables potentially affecting the chance of pregnancy were entered into a binary logistic regression model (non-pregnant = 0; pregnant = 1). Using both the enter and the backward LR methods the following variables were found to be associated with clinical pregnancy in the following order of significance: (i) number of good quality embryos transferred (OR, 2.34; 95%CI: 1.48–3.69; $P < 0.001$), (ii) uterine contractions following ET (OR, 0. 892; 95%CI: 0.829–0.959;

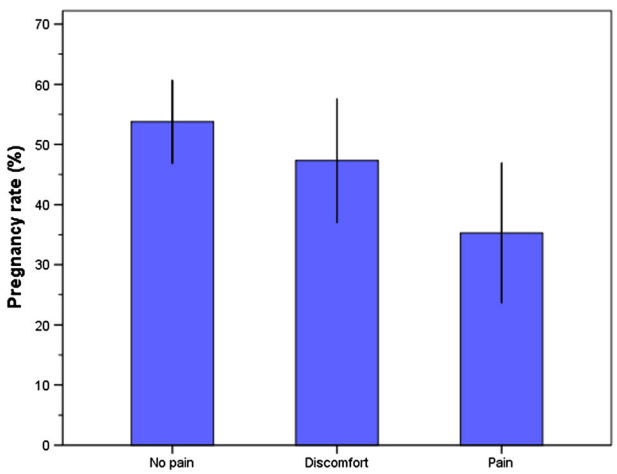

Figure 1 Pregnancy rate according to pain experienced at the time of the embryo transfer ($n = 360$). No pain group (pain score, 0; $n = 199$), discomfort/mild pain group (pain score, 1–19; $n = 93$), pain/moderate group (pain score ≥20; $n = 68$). $P = 0.009$ (chi-squared test; 3 × 2 [linear by linear association]; whiskers, 95%CI).

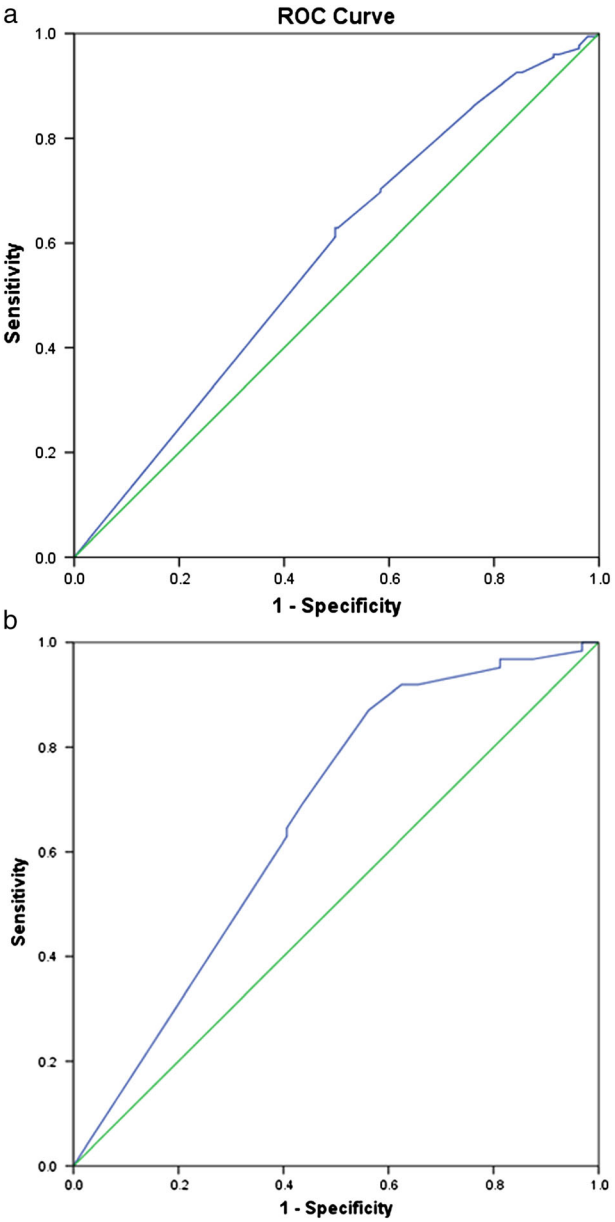

Figure 2 Receiver operating characteristic curve of pain score as a predictor of clinical pregnancy in (a) all cycles ($n = 360$), and (b) cycles with top embryo quality and low uterine contraction frequency (<2/min; $n = 129$). (a) Area under the curve (AUC), 0.572; 95%CI: 0.513–0.631; $P = 0.019$; (b) AUC, 0.661; 95%CI: 0.537–0.784; $P = 0.011$.

Table 2 Factors associated with pain during ET

	Pain (n = 161)	No pain (n = 199)	P-value†
Age	35.1 ± 3.2	34.7 ± 3.4	0.23
BMI	21.5 ± 2.8	21.1 ± 2.7	0.16
No. IVF cycles	1.4 ± 0.7	1.4 ± 0.8	0.68
Main cause of infertility			0.80
Ovulatory	8/16 (50.0)	8/16 (50.0)	
Tubo-peritoneal	79/186 (42.5)	107/186 (57.5)	
Male	50/102 (49.0)	52/102 (51.0)	
Endometriosis	12/23 (52.2)	11/23 (47.8)	
Unexplained	12/33 (36.4)	21/33 (63.6)	
Previous surgery			0.23
Uterine	4/13 (30.8)	9/13 (69.2)	
Adnexal	54/104 (51.9)	50/104 (48.1)	
Peritoneal	22/58 (37.9)	36/58 (62.1)	
None	81/185 (43.8)	104/185 (56.2)	
Cycle type			0.14
Fresh	134/287 (46.7)	153/287 (53.3)	
Frozen	27/73 (37.0)	46/73 (63.0)	
Treatment protocol			0.31
Agonist	139/303 (45.9)	164/303 (54.1)	
Antagonist	22/57 (38.6)	35/57 (61.4)	
No. follicles >15 mm	7.2 ± 2.9	7.0 ± 2.8	0.48
No. oocytes retrieved	10.4 ± 4.0	10.2 ± 3.9	0.50
No. embryos transferred			0.76
1	32/69 (46.4)	37/69 (53.6)	
2	129/291 (44.3)	162/291 (55.7)	
Endometrial thickness	11.2 ± 2.6	11.3 ± 2.7	0.77
Uterine contractility	2.2 ± 1.3	2.4 ± 1.3	0.26
E2 on day of ET (pmol/L)	4958 ± 3635	4648 ± 3726	0.43
P4 on day of ET (nmol/L)	242 ± 162	224 ± 154	0.30
Urge of micturition			0.10
Mild–moderate	98/202 (48.5)	104/202 (51.5)	
Severe	63/158 (39.9)	95/158 (60.1)	
Pulse rate post ET	79.7 ± 10.9	77.9 ± 11.1	0.11
SBP after ET	118.4 ± 13.9	116.8 ± 13.1	0.28
DBP after ET	75.1 ± 10.8	74.3 ± 10.6	0.47
Clinician performing ET			0.89
Clinician A	76/165 (46.1)	89/165 (53.9)	
Clinician B	73/168 (43.5)	95/168 (56.5)	
Other clinician	12/27 (44.4)	15/27 (55.6)	
Depth of ET			0.18
High (<15 mm from fundus)	108/228 (47.4)	120/228 (52.6)	
Low (>15 mm from fundus)	53/132 (40.2)	79/132 (59.8)	
Outer catheter (s)	157.9 ± 34.8	163.4 ± 132.0	0.62
Inner catheter (s)	52.2 ± 17.9	61.7 ± 130.2	0.90
Vulsellum use			0.01
Yes	12/16 (75.0)	4/16 (25.0)	
No	149/344 (43.5)	195/344 (56.5)	
STAI score‡	53.8 ± 8.4	56.1 ± 10.0	0.14
BDI score‡	6.8 ± 5.9	8.6 ± 9.2	0.14
GHQ score‡	2.4 ± 2.8	2.9 ± 3.1	0.36
Salivary α-amylase (U/mL)‡	137.1 ± 113.4	123.3 ± 99.9	0.43
Salivary cortisol (nmol/L)‡	8.4 ± 4.0	8.2 ± 3.8	0.79

[1]Data given as mean ± SD for continuous variables and n (%) for categorical variables. †Student's t-test or χ^2 test. ‡n = 156. BDI, Beck Depression Inventory; BMI, body mass index; DBP, diastolic blood pressure; E2, estradiol; ET, embryo transfer; GHQ, General Health Questionnaire; IVF, *in vitro* fertilization; P4, progesterone; SBP, systolic blood pressure; STAI, State–trait Anxiety Inventory.

P = 0.002); (iii) age (OR, 0.738; 95%CI: 0.606–0.899; P = 0.003), and (iv) pain score during ET (OR, 0.977; 95%CI: 0.960–0.995; P = 0.01) (Table 3). Pain during ET was additionally assessed as a dichotomized variable (pain vs no pain) and was also found to be significant (OR, 0.594; 95%CI: 0.374–0.943; P = 0.027).

The regression model was also performed in a subset of women who had exclusively two good-quality embryos transferred (n = 242). This way the factors of embryo number and quality were simultaneously controlled for. In this case, the pain score ranked either first (enter method: AOR, 0.965; 95%CI: 0.942–0.989; $P < 0.01$) or second behind uterine contractions (backward LR method: AOR, 0.964; 95%CI: 0.942–0.987; $P < 0.01$), according to the method of the regression analysis.

Discussion

The present results are intriguing because they demonstrate that the experience and degree of pain during ET does indeed have a significant and independent correlation with the chance of clinical pregnancy.

The initial notion that pain may be purely a surrogate/confounding factor reflecting the difficulty of the procedure was refuted in this study. This is of particular importance because, although some studies report no association between the difficulty of the ET and the chance of pregnancy,[46] a recent meta-analysis based on five low-quality studies has suggested that a difficult ET may be associated with a lower clinical pregnancy rate.[29] The usual quantifiable variables reflecting procedure difficulty are the use of vulsellum,[47,48] and prolonged manipulation and insertion of the ET catheter.[49,50] Both of these factors, however, were controlled for in this study. In particular, although the use of vulsellum was associated with significantly more pain, it was not associated with reduced chance of pregnancy. In addition, the duration of inner and outer catheter insertion was not different for women with pain versus women with no pain (Table 3).

With regards to the second notion that experience of pain may be related to the psychological states of stress and anxiety, although this has been recognized within the non-ART context,[27,51] we did not find an association in the present study. More specifically, for women with pain versus women with no pain, the STAI, BDI and GHQ scores were similar. In addition, the biomarkers of sAA and salivary cortisol, which have been associated with stress and anxiety, were also not significantly different between the two groups (Table 3). When examining pregnant and non-pregnant groups, the STAI and BDI scores were not significantly different, but the GHQ score was significantly lower in pregnant versus non-pregnant women (Table 1). This is in accordance with some studies that have found an association between assessment of stress and anxiety, and ART outcome,[6–8] and may warrant further investigation. Despite this association, sAA and salivary cortisol were not significantly different for pregnant versus non-pregnant women (Table 1).

To our knowledge, this is the first time that an independent correlation between pain experienced at the time of ET and chance of clinical pregnancy has been

Table 3 Significant indicators of clinical pregnancy†

	Wald	AOR	95%CI		*P*-value
			Lower	Upper	
Top-quality embryos transferred	13.418	2.339	1.484	3.685	<0.001†
Uterine contractility	9.465	0.892	0.829	0.959	0.002†
Age	9.083	0.738	0.606	0.899	0.003†
Pain score at embryo transfer	6.563	0.977	0.960	0.995	0.010†
Baseline FSH (IU/L)	0.963	0.940	0.831	1.063	0.326
Endometrial thickness (mm)	0.250	1.250	0.521	2.997	0.617
P4 on day of ET (nmol/L)	0.188	1.000	0.998	1.002	0.664
Baseline LH (IU/L)	0.156	0.973	0.848	1.116	0.693
E2 on day of hCG (pmol/L)	0.126	1.000	1.000	1.000	0.722
Years of infertility	0.021	1.005	0.936	1.079	0.886

†Logistic regression analysis (enter method; yes = 1, no = 0). When pain dichotomized (yes, no): AOR, 0.612; 95%CI: 0.385–0.971; P = 0.027. When only patients with good-quality embryos were transferred included, pain score ranked first: AOR, 0.965; 95%CI: 0.942–0.989; P = 0.005. †Also statistically significant in a backward likelihood ratio regression model. E2, estradiol; ET, embryo transfer; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone; P4, progesterone.

observed. It could be that pain during ET (i.e. pelvic pain), reflects a process of underlying inflammation and/or infection, such as chronic endometritis. Interestingly, chronic endometritis has in fact been reported to occur in >12% of unselected women with infertility[52,53] and in >30% of women with recurrent implantation failure.[54] Furthermore, women with chronic endometritis have significantly lower clinical pregnancy rates compared with patients without endometritis.[55] More recent studies of the Human Microbiome Project have also shown that vaginal microbiome on the day of ET may affect the clinical outcome in women undergoing ART,[56–58] re-igniting the interest of the role of infection in reproductive failure. The possible link between the increased perception of pain and inflammatory or infective processes, however, remains speculative; further good-quality prospective studies focusing on subclinical infections within the endometrium are required to confirm or refute the hypothesis.

Although this study was not geared towards assessing for potential inflammatory or infective markers in the genital tract, which could explain the variation in pain experience during ET, it has several other advantages. First, it controlled for >28 variables potentially relating to the reporting of pain by the patients; second, it controlled for baseline psychological state (by three different tests) and also sAA and salivary cortisol in a subgroup of patients, given that this was originally hypothesized to be potentially correlated. Third, the association was stringently analyzed on both serial bivariate analysis and also comprehensive logistic regression analysis including either all patients or subgroups of patients with only two good-quality embryos transferred. As a result, the association between the experience of pain and the achievement of clinical pregnancy does appear to be robust in this cohort of patients.

The limitations of this study lie potentially within aspects of the methodology and the generalizability. With regards to methodology, only a subset of patients underwent all three psychological tests and sAA and salivary cortisol sampling, although the numbers were sufficient for statistical analysis (n = 156). Furthermore, although the baseline psychological and biomarker testing was performed on the day of oocyte retrieval, similar to previously reported methodology,[59] in retrospect, we believe that a serial measurement before and after ET may be more insightful. Finally, given that the present study did not include inflammatory/infective markers, future studies could assess concurrently for vaginal, cervical and endometrial signs of infection and inflammation in these patients.

In conclusion, the present study has identified that women who experience pain during ET have a lower chance of clinical pregnancy. This appears to be independent of both the nature of the procedure and the psychological state and stress biomarkers of the patients. Potential alternative explanations for this relation, such as inflammatory/infective processes in the female genital tract, could be evaluated in future prospective studies.

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Disclosure

The authors declare no conflicts of interest.

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