Article

# Flushing the endometrium prior to the embryo transfer does not affect the pregnancy rate

After his residency training in Obstetrics and Gynecology at the Akdeniz University, Turkey, Dr Ozgur completed a 3-year fellowship in Reproductive Endocrinology and Infertility at Tygerberg Hospital, South Africa and Jones Institute, Norfolk, USA. He returned to Akdeniz University in 1997 and established the IVF unit at the university. Two years later, Dr Ozgur founded Antalya IVF, the largest IVF centre in southern Turkey. His major areas of interest are assisted reproduction, andrology and hysteroscopic surgery. He has published extensively in these fields, with over 30 articles published in renowned international journals.

*Dr K Ozgur*

M Berkkanoglu[1], M Isikoglu, M Seleker, K Ozgur
Antalya IVF Centre, Halide Edip Cad. No. 7, 07080, Antalya, Turkey
[1]Correspondence: Fax: +90 242 345 47 47; e-mail: mberkkan@hotmail.com

## Abstract

This study aimed to determine whether direct flushing of endometrial cavity with culture media just after cervical irrigation at the time of embryo transfer has any effect on pregnancy rates. A total of 240 women were prospectively randomised; one group of patients (group 1) underwent intrauterine direct flushing of endometrial cavity with culture media just after cervical irrigation at the time of embryo transfer as detected by transabdominal ultrasound and the other group of patients (group 2) had cervical flushing but did not undergo intrauterine flushing. Pregnancy (positive human chorionic gonadotrophin) rates were 57.5% and 62.9% for group 1 and group 2, respectively. Clinical pregnancy (positive fetal heart rate) rates were 45.2% and 51.4% for group 1 and group 2, respectively. Implantation rates were 20.0% and 21.2% for group 1 and group 2, respectively. Ongoing pregnancy (>12 weeks of pregnancy) rates were 47.9% and 47.2% for group 1 and group 2, respectively. There were no significant differences in pregnancy rates, clinical pregnancy rates, implantation rates and ongoing pregnancy rates between the two groups. In conclusion, even direct flushing of media into the uterine cavity neither improves nor adversely affects the pregnancy rate.

***Keywords:*** *embryo transfer, endometrium, flushing media, implantation, pregnancy rate*

## Introduction

Embryo transfer techniques are important determinants of success rates. Ultrasound guidance and new catheter materials have been used for embryo transfer (Letterie *et al.*, 1999; Corolu *et al.*,. 2000). Recently, the clinical details of embryo transfer have gained more attention. Irrigation and aspiration of cervical mucus have also become an integral part of embryo transfer techniques (McNamee *et al.*, 1998). However during irrigation and aspiration of cervical mucus, there is always a risk of reflux of media into the endometrial cavity that could result in dilution of the endometrial fluid and change of endometrial microenvironment. A recent study has indicated better pregnancy rates in cases where the endometrium was accidentally flushed prior to the transfer (Letterie *et al.*, 2003). Therefore this study aims to determine whether direct flushing of the endometrial cavity with culture media just after cervical irrigation has any effect on pregnancy rate.

## Materials and methods

### Patients and protocols

The protocol was approved by the institutional ethical committee. All patients gave informed consent. 240 women who were less than 40 years of age were included in the study. Patients were randomized at initiation of stimulation by a computer-generated list. 120 women were taken as group 1 (study group) and 120 women were taken as group 2 (control group). After excluding the cases in both groups associated with difficult transfer, uterine anomalies and unintentional reflux of media at the time of cervical irrigation, a total of 181 women remained in the study. 73 women (group 1) underwent intrauterine flushing and 108 women (group 2) did not undergo intrauterine flushing.

At the set-up of the study, the number of patients was calculated using the centre's clinical pregnancy rate (45%) and expected

clinical pregnancy rate (65%) in flushing group, and an alpha value of 0.05 and a desired power of 0.800. The expected clinical pregnancy rate was taken as 65% because there was a 20% increase in the pregnancy rate in a previous study (Letterie *et al.*, 2003). According to these criteria, a sample size of 198 patients was calculated using Sigmastat for Windows, version 3.0.

## Technique of embryo transfer

The technique of embryo transfer was standardized and carried out by one examiner. All women underwent embryo transfer on day 2. Endometrial cavities of all patients were assessed with ultrasound and all embryo transfers were carried out under ultrasound guidance. The grading criteria for embryos were based on cell number, evenness of blastomeres, and the amount of fragmentation. Embryo quality was graded from 1 to 4 as follows: grade 1 embryo: no fragmentation with equal sized homogeneous blastomeres; grade 2 embryo: <20% fragmentation with equal sized homogeneous blastomeres; grade 3 embryo: 20–50% fragmentation with equal or unequal sized blastomeres; grade 4 embryo: >50% fragmentation with equal or unequal sized blastomeres (Balaban *et al.*, 2001).

Cervical canal irrigation was done in both groups before embryo transfer. Cervical canal was irrigated with culture media (IVF™-30, Vitrolife, Gothenburg, Sweden) solution using the outer sheath of a coaxial transfer catheter (Labotect embryo transfer catheter set, Labotect GmbH, Deisenhofen, Germany). The procedure was observed with transabdominal ultrasound to assess whether the media was accidentally flushed into the uterine cavity. If inadvertent endometrial flushing during cervical irrigation happened, the case was taken out of the study because the total amount of fluid that had entered the endometrial cavity could not be calculated. For women in group 1, after irrigating the cervical canal, the inner sheath was placed through the transfer catheter into the endometrial cavity. The endometrial cavity was then given 0.4 ml culture media. The procedure was observed with transabdominal ultrasound to assess whether the media was flushed into the uterine cavity. After intrauterine flushing, embryo transfer was performed by using another inner sheath.

## Statistical methods

Pregnancy rates [positive human chorionic gonadotrophin (HCG)], clinical pregnancy rates (positive fetal heart rate), implantation rates (ratio of gestational sacs with positive fetal cardiac activity to embryos transferred) and ongoing pregnancy rates (>12 weeks of pregnancy) were compared between groups by chi-squared analysis. Age, peak serum oestradiol concentrations, total gonadotrophin amount and number of embryos transferred were compared between groups by Student's *t*-test. $P < 0.05$ was considered statistically significant.

**Table 1.** Clinical data of (**a**) group 1 (flushing) and group 2 (no flushing) and (**b**) group 1A (cases with good prognosis and flushing) and group 2A (cases with good prognosis and no flushing).

| (**a**) | *Group* *1* | *2* |
|---|---|---|
| Sample size | 73 | 108 |
| Age (years)[a] | 31.3 ± 0.5 | 31.1 ± 0.5 |
| Total gonadotrophin (ampoules) | 47.8 ± 2.5 | 48.2 ± 2.2 |
| Peak serum $E_2$ (pg/ml) | 4452.9 ± 295.6 | 4287.9 ± 228.9 |
| No. of eggs | 14.4 ± 1.1 | 15.5 ± 1.1 |
| No. of embryos transferred | 3.4 ± 0.2 | 3.2 ± 0.2 |
| Clinical pregnancy rate (%) | 45.2 | 51.4 |
| Implantation rate (%) | 20.0 | 21.2 |
| Ongoing pregnancy rate (%) | 47.9 | 47.2 |
| (**b**) | *Group* *1A* | *2A* |
| Sample size | 59 | 82 |
| Age (years) | 31.1 ± 0.6 | 30.9 ± 0.6 |
| Total gonadotrophin (ampoules) | 45.6 ± 2.5 | 45.2 ± 2.4 |
| Peak serum $E_2$ (pg/ml) | 4021.7 ± 312.4 | 4186.1 ± 247.9 |
| No. of eggs | 14.8 ± 1.2 | 16.9 ± 0.9 |
| No. of embryos transferred | 3.9 ± 0.1 | 3.7 ± 0.4 |
| Clinical pregnancy rate (%) | 50.9 | 57.3 |
| Implantation rate (%) | 20.4 | 23.4 |
| Ongoing pregnancy rate (%) | 50.8 | 53.7 |

Values are means ± SEM.
There were no statistically significant differences when comparing group 1 with group 2 and group 1A with group 2A.

## Results

Pregnancy rates were 57.5% and 62.9% for group 1 and group 2, respectively. Clinical pregnancy rates were 45.2% and 51.4% for group 1 and group 2, respectively. Implantation rates were 20.0% and 21.2% for group 1 and group 2, respectively. Ongoing pregnancy rates were 47.9% and 47.2% for group 1 and group 2, respectively. There were no significant differences in the age, peak serum E2 concentration, total gonadotrophin amount, number of embryos transferred, pregnancy rates, clinical pregnancy rates, implantation rates and ongoing pregnancy rates between the two groups (**Table 1a**).

The data of patients with good prognosis, described as having more than three embryos transferred, at least one of which was a grade 1 embryo, were further analysed in both groups, and their data were represented as group 1A (flushing, and at least one grade 1 embryo transfer) and group 2A (no flushing, and at least one grade 1 embryo transfer). Pregnancy rates were 62.7% and 67.1% for group 1A and group 2A, respectively. Clinical pregnancy rates were 50.9% and 57.3% for group 1A and group 2A, respectively. Implantation rates were 20.4% and 23.4% for group 1A and group 2A, respectively. Ongoing pregnancy rates were 50.8% and 53.7% for group 1A and group 2A, respectively. However, no significant difference was found between these subgroups (**Table 1b**).

## Discussion

One unevaluated aspect of embryo transfer procedure is the impact of media flushing into the uterine cavity on pregnancy rates. Endometrial reflux during cervical irrigation at the time of embryo transfer is a frequent event, and one study has found a positive effect of accidental flushing on the clinical IVF outcome (Letterie *et al*., 2003). In addition, the direct effect of media on pregnancy rates has not been studied prospectively. Therefore this study proposed that the intentional flushing may also have a positive effect on the outcome parameters and can be used as part of clinical practice during transfer procedure. Thus, a new technique of intentionally flushing the endometrial cavity at the time of embryo transfer was employed.

The cross-talk between the embryo and endometrium is essential for allowing the embryo to implant and it is mediated by different kinds of cytokines and mediators during the implantation window. Cytokines including interleukin-1 (IL-1), tumour necrosis factor-α (TNF-α), leukaemia inhibitory factor (LIF), colony stimulating factor-1 (CSF-1) and integrins are all factors that have been shown to be important to implantation (Arici *et al*., 1995; Beier *et al*., 1998; Bulletti *et al*., 2005). Paracrine modulations may enable a molecular cross-talk between endometrial cells and blastocyst cells, contributing locally to endocrine embryo-maternal dialogue. Both the cytokines and their receptors are secreted or expressed by either the embryo or the endometrium in an increased manner during the implantation window. Although there are no data for day 2 embryos, secretion of embryonic IL-1□â seems to be the first response of the blastocyst to the receptive endometrium, inducing a second wave of cytokines in the endometrium such as LIF, IL-1 and others (Cross *et al*., 1994). By binding to their receptors, the cytokines induce molecular changes in the expression patterns of adhesion and antiadhesion molecules, such as integrin $\alpha_v\beta_3$, essential for attachment of the blastocyst (Simon *et al*., 1997). Therefore, the presence of a small amount of fluid in the endometrial cavity just before the embryo reaches the endometrial cavity may not affect the implantation. The interference of the fluid with the second wave of cytokine release by the endometrium does not seem to play a role at this stage of embryo development. However, as in the case of hydrosalpinges, the leakage of fluid through the uterine cavity may either wash out embryos (Bloeche *et al*., 1997) or may induce an increase in endometrial peristalsis (Ijland *et al*., 1999). The hydrosalpingeal fluid may also exert detrimental effects on the release of these cytokines or integrins by endometrium or embryo (Meyer *et al*., 1997). Therefore, the nature of the fluid and the duration of the presence of fluid seem more important.

In the study by Letterie *et al*. (2003) there was an increase of nearly 20% in pregnancy rate in cases with endometrial reflux during cervical irrigation, although it was not statistically significant. The data presented here, based on prospective randomized manner, have not shown the positive effect of the intentional flushing on the clinical outcome. There could be two explanations regarding the difference in the clinical outcomes of this study and the previous study. First, the accidental flushing in the previous study may have occurred due to the anatomy of the genital tract and this may have had no effect on the uterine peristalsis or accessibility of the cavity. It may show a self-selection process of easier transfers being more likely to have reflux during cervical irrigation. However the endometrium was flushed in all study cases and this may have interfered with the uterine peristalsis in some cases. Second, this study transferred day-2 embryos while the previous study transferred day-3 embryos. Day-2 embryos may respond differently than day-3 embryos to the flushed endometrium and this could explain the contradictory results.

In the study some patients were excluded after randomization if there was difficult transfer or inadvertent endometrial flushing during cervical irrigation or uterine anomalies, including small submucosal myomas or polyps that had not been diagnosed before but were noticed at embryo transfer. All cases with inadvertent flushing were excluded from the study because the total amount of fluid that entered endometrial cavity could not be calculated. The cases with difficult transfer or uterine anomalies were also excluded because the media flushed into the uterine cavity could not be observed adequately with transabdominal ultrasound.

In conclusion, data from this study show that even direct flushing of media into the uterine cavity neither improves nor adversely affects the final outcome, the pregnancy rate. This technique has not shown any further benefit for embryo transfer, but it can be understood that unintentional flushing during cervical irrigation, even direct flushing of the endometrial cavity, does not adversely affect the pregnancy rate. In addition, obtaining flushed endometrial secretion at the time of embryo transfer could be a valuable tool for assessment of the implantation chances of the embryos. The first biochemical analyses of proteins in human uterine secretions were performed by flushing the uterine lumen after hysterectomy more than two decades ago (Beier *et al*., 1973). Today, methodological improvements in the analysis of minute fluid volumes have made it possible to investigate samples of protein as small as 60–80 µg. To reveal changes in uterine secretion could lead to a greater accuracy in

diagnosing either normal or deficient endometrial performance (Beier *et al*., 1998; Li *et al*., 1993a, b; Beier-Hellwig *et al*., 1994). There could be cases with molecular abnormalities in the endometrium, such as in cases with recurrent implantation failure, particularly when transferred embryos are good quality (Urman *et al*., 2005). Obtaining flushed endometrial secretion in such cases would provide very useful clues. In another study it has been shown that endometrial secretion aspiration prior to embryo transfer does not reduce implantation rates and protein content in endometrial fluid has been evaluated for protein pattern analysis to assess endometrial receptivity during treatment cycles (van der Gaast *et al*., 2003).

However, since different stages of development may act differently, flushing the endometrial cavity at later stages, including the blastocyst stage transfer, needs to be explored with prospective clinical studies.

## References


Arici A, Engin O, Attar E, Olive DL 1995 Modulation of leukemia inhibitory factor gene expression and protein biosynthesis in human endometrium. *Journal of Clinical Endocrinology and Metabolism* **80**, 1908–1915.

Balaban B, Urman B, Isiklar A *et al*. 2001 The effect of pronuclear morphology on embryo quality parameters and blastocyst transfer outcome. *Human Reproduction* **16**, 2357–2361.

Beier HM, Beier-Hellwig K 1973 Specific secretory protein of the female genital tract. *Acta Endocrinologica Supplementum* **180**, 404–425.

Beier HM, Elger W, Hegele-Hartung C *et al*. 1991 Dissociation of corpus luteum, endometrium and blatocyst in human implantation research. *Journal of Reproduction and Fertility* **92**, 511–523.

Beier-Hellwig K, Sterzik K, Bonn B *et al*. 1994. Hormone regulation and hormone antagonist effects on protein patterns of human endometrial secretion during receptivity. *Annals New York Academy of Sciences* **734**, 143–156.

Beier HM, Beier-Hellwig K 1998 Molecular and cellular aspects of endometrial receptivity. *Human Reproduction Update* **4**, 448–458.

Bloeche M, Schreiner T, Lisse K 1997 Recurrence of hydrosalpinges after transvaginal aspiration of tubal fluid in an IVF cycle with development of serometra. *Human Reproduction* **12**, 703–705.

Bulletti C, Flamigni C, de Ziegler D 2005 Implantation markers and endometriosis. *Reproductive BioMedicine Online* **11**, 464–468.

Corolu B, Carreras O, Veiga A *et al*. 2000 Embryo transfer under ultrasound guidance improves pregnancy rates after in vitro fertilization. *Human Reproduction* **15**, 616–620.

Cross JC, Werb Z, Fisher SJ 1994 Implantation and the placenta: key pieces of development puzzle. *Science* **266**, 1508–1518.

Ijland MM, Hoogland HJ, Dunselman GA *et al*. 1999 Endometrial wave direction switch and the outcome of in vitro fertilization. *Fertility and Sterility* **71**, 476–481.

Letterie G, Marshall L, Angle M 2003 Intrauterine reflux of media during cervical irrigation at embryo transfer. *Fertility and Sterility* **79**, 1444–1445.

Letterie G, Marshall L, Angle M 1999 A new coaxial catheter system with an echo dense tip for ultrasonographically guided embryo transfer. *Fertility and Sterility* **72**, 266–268.

Li TC, MacKenna A, Roberts R 1993a The techniques and complications of out-patient uterine washing in the assessment of endometrial function. *Human Reproduction* **8**, 343–346.

Li TC, Dalton C, Hunjan KS *et al*. 1993b The correlation of placental protein 14 concentrations in uterine flushing and endometrial morphology in the peri-implantation period. *Human Reproduction* **8**, 1923–1927.

McNamee PI, Huang TT, Carwile AH *et al*. 1998 Significant increase in pregnancy rates achieved by vigorous irrigation of endocervical mucus prior to embryo transfer with Wallace catheter in an IVF–ET program. *Fertility and Sterility* **70** (Suppl. 1), 228.

Meyer WR, Castelbaum, AJ, Somkuti S *et al*. 1997 Hydrosalpinges adversely affect markers of endometrial receptivity. *Human Reproduction* **12**, 1393–1398.

Simon C, Gimeno MJ, Mercader A *et al*. 1997. Embryonic regulation of integrins beta 3, alpha 4, and alpha 1 in human endometrial epithelial cells in vitro. *Journal of Clinical Endocrinology and Metabolism* **82**, 2607–2616.

Urman B, Yakin K, Balaban B 2005 Recurrent implantation failure in assisted reproduction: how to counsel and manage. A. General considerations and treatment options that may benefit the couple. *Reproductive BioMedicine Online* **11**, 371–381.

Van der Gaast M, Beier-Hellwig K, Fauser BCJM *et al*. 2003 Endometrial secretion aspiration prior to embryo transfer does not reduce implantation rates. *Reproductive BioMedicine Online* **7**, 105–109.


*This study was presented at the 60th Annual meeting of the ASRM, Philadelphia, Pennsylvania, 16–20 October, 2004.*

*Received 24 November 2005; refereed 11 January 2006; accepted 20 April 2006.*