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CASE REPORT *Infertility*

What a difference two days make: "personalized" embryo transfer (pET) paradigm: A case report and pilot study

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ABSTRACT: Embryo implantation requires that the blastocyst will attach during the receptive stage of the endometrium, known as window of implantation (WOI). Historically, it has been assumed that the WOI is always constant in all women. However, molecular analyses of endometrial receptivity demonstrates a personalized WOI (pWOI) that is displaced in one out of four patients suffering from recurrent implantation failure (RIF) of endometrial origin and illustrates the utility of a personalized endometrial diagnostic approach. Here, we report a clinical case of successful personalized embryo transfer (pET) after four IVF and three oocyte donation failed attempts in which different embryo transfer strategies were attempted. This case report is complemented by a pilot study of 17 patients undergoing oocyte donation and who suffered failed implantations with routine embryo transfer (ET) but were then treated with pET after the personalized diagnosis of their WOI.

Key words: personalized embryo transfer / window of implantation / recurrent implantation failure / assisted reproduction

Introduction

Implantation of the blastocyst requires embryo-endometrial synchronization within an optimal time-frame in which the endometrial 'window of implantation' (WOI) must be the mirror of the implanting blastocyst. The master regulators for the acquisition of endometrial receptivity are estradiol and progesterone. Progesterone receptors (PR-A and PR-B) and estrogen receptors (ERα and ERβ) are expressed in the human endometrium at the epithelial and stromal compartment. ER and PR signaling during implantation are executed by juxtacrine, paracrine and autocrine factors orchestrated by various growth factors, cytokines, lipid mediators, homeobox transcription factors and morphogens (for review see Cha *et al.*, 2013). CD56 bright uterine NK cells are also implicated in the control of the invasion phase and vascular remodeling occurs in the placental bed, although these clinical actions are not yet fully understood. Understanding the 'molecular clock' at play, regardless of the presence or absence of an embryo, during the WOI could help to identify biomarkers of endometrial receptivity useful to create an objective personalized diagnostic test for this function.

Recurrent implantation failure (RIF) is a poorly characterized major alteration that prevents the final success of the assisted reproductive process (Margalioth *et al.*, 2006; Simon and Laufer, 2012). It is defined as three or more failed IVF cycles in which one or two morphologically high-grade embryos were transferred. It is a distressing clinical and personal situation that forces the majority of patient drop-outs and second opinions, as well as the addition of empirically unproven and sometimes harmful protocols to our treatments.

The causes of RIF can be grouped into several main clinical categories: pathological alterations of the endometrial cavity such as hyperplasia, submucous myomas/polyps, endometritis and synechiae that can be found in 18–27% of cases (Demirol and Gurgan, 2004); hydrosalpinx (Zeyneloglu *et al.*, 1998); an increased incidence of embryonic chromosomal abnormalities (Rubio *et al.*, 2013); and lifestyle or other causes such as hereditary and acquired thrombophilia (Penzias, 2012).

All of the pathological issues indicated above can be corrected, but the underlying implantation problem can still remain. Interestingly, the obvious fact that successful implantation requires synchrony between the embryo and the receptive endometrium has not yet been clinically addressed (Ruiz-Alonso *et al.*, 2012). The main reason is the lack of objective methods for the personalized diagnosis of endometrial receptivity during the infertility work-up. Furthermore it is assumed that the WOI is constant in time in all women, including RIF patients (Ruiz-Alonso *et al.*, 2013).

Here, we report a clinical case of successful personalized embryo transfer (pET) after four IVF and three oocyte donation failed attempts in which different embryo transfer strategies were attempted. This case report is complemented by a pilot study.

Table I Clinical outcome for total ET before ERA (purple) and total pET after ERA (green).

ID	Age	IVF cycles pre-ERA	OD cycles pre-ERA	WOI at ET	ERA result WOI at ET	N° of cycles pre-ERA with ET	Mean of embryos per transfer [range]	IR (%)	Clinical pregnancy	Ongoing pregnancy rate
1	35	0	3	P+5	PRE-R	3	2 [2]	0/6 (0)	No	No
2	32	0	1	P+4	PRE-R	1	2 [2]	0/2 (0)	No	No
3	42	2	3	P+4	PRE-R	2	2 [2]	1/4 (25)	Biochemical	No
4	39	0	1	P+5	PRE-R	1	2 [2]	0/2 (0)	No	No
5	38	0	3	P+4	PRE-R	3	2 [2]	1/6 (17)	Clinical abortion	No
6	43	0	1	P+5	PRE-R	1	2 [2]	0/2 (0)	No	No
7	43	2	3	P+5	PRE-R	2	2 [2]	0/4(0)	No	No
8	46	0	2	P+5	PRE-R	2	2 [2]	0/4(0)	No	No
9	46	0	8	P+5	PRE-R	3	1.3 [1-2]	0/4(0)	No	No
10	35	0	2	P+5	PRE-R	1	2 [2]	0/2 (0)	No	No
11	47	0	1	P+5	POST-R	1	2 [2]	0/2 (0)	No	No
12	36	0	2	P+5	PRE-R	2	2 [2]	1/4 (25)	Clinical abortion	No
13	41	0	4	P+5	PRE-R	3	1 [1]	1/3 (33)	Biochemical	No
14	40	0	6	P+5	PRE-R	6	1.8 [1-2]	2/11 (18)	Clinical abortion	No
15	40	0	2	P+5	PRE-R	2	2 [2]	0/4(0)	No	No
16	40	0	2	P+5	PRE-R	2	2 [2]	1/4 (25)	Clinical abortion	No
17	49	0	1	P+5	PRE-R	1	1 [1]	0/1 (0)	No	No
Total patients										
17	40.7	0.2 ±0.7	2.6 ±1.9		100% NR	2.1 ±1.3	1.8 [1-2]	11% (7/65)	19% (7/36)	0% (0/7)

ID	Age	IVF cycles pre-ERA	OD cycles pre-ERA	pWOI at pET	ERA result pWOI at pET	N° of cycles post-ERA with pET	Mean of embryos per transfer [range]	IR (%)	Clinical pregnancy	Ongoing pregnancy rate
1	35	0	3	P+6	R	2	1.5 [1-2]	1/3(33)	Yes	Babyat home
2	32	0	1	P+7	R	1	2 [2]	1/2(50)	Biochemical	No
3	42	2	3	P+6	R	2	2 [2]	2/4 (50)	Yes	Babyat home
4	39	0	1	P+7	R	1	2 [2]	0/2 (0)	No	No
5	38	0	3	P+7	R	1	1 [1]	0/1 (0)	No	No
6	43	0	1	P+7	R	1	2 [2]	1/2(50)	Yes	Yes
7	43	2	3	P+7	R	1	2 [2]	2/2(100)	Yes	Babyat home
8	46	0	2	P+7	R	1	2 [2]	1/2(50)	Yes	Yes
9	46	0	8	P+7	R	1	1.5 [1-2]	0/2 (0)	No	No
10	35	0	2	P+6	R	1	2 [2]	0/2 (0)	No	No
11	47	0	1	P+7	R	1	1.5 [1-2]	1/1 (100)	Yes	Babyat home
12	36	0	2	P+4 .5	R	1	2 [2]	1/2(50)	Biochemical	No
13	41	0	4	P+5.5	R	1	1 [1]	1/1 (100)	Yes	Yes
14	40	0	6	P+7	R	2	2 [2]	1/4 (25)	Yes	Yes
15	40	0	2	P+7	R	1	2 [2]	1/2(50)	Yes	Yes
16	40	0	2	P+7	R	1	2 [2]	1/2(50)	Biochemical	No
17	49	0	1	P+7	R	1	1 [1]	0/1 (0)	No	No
Total patients										
17	40.7	0.2 ±0.7	2.6 ±1.9		100% R	1.2 ±0.4	1.8 [1-2]	40% (14/35)	60% (12/20)	75% (9/12)

In order to make comparable ET vs pET we only have consdered as ET the previous cycles to ERA with the same conditions than pET (hormone replacement therapy cycle and oocytes from donation) but with different dayforthe embryotransfer. The last row in each table reflects the total valuesfor the 17 patients.

ET: cycles pre-ERAinwhich embryos were transferred at day non receptive by ERA; pET: cycles post-ERA in which embryos were transferred at day receptive by ERA; WOI at ET: day in which the transfer were performed in ET cycles; WOI at pET: day in which the transfer were performed in pET cycle; NR: non receptive; OD: ovum donation; IR: implantation rate. ERA, endometrial receptivity array test; ET, embryo transfer; pET, personalised embryo transfer; WOI, window of implantation.

Case report

In December 2008, a 39-year-old woman attended our reproductive clinic reporting two consecutive IVF failures. Each IVF procedure involved the transfer of two good quality embryos that failed to implant. The routine infertility assessment was normal (partner karyotypes, semen analysis including FISH, and biochemical, coagulation and immunological parameters in the female partner). Hysteroscopy revealed a sub-septate uterus. Surgical correction achieved normal morphology.

After counseling, the couple underwent two more IVF cycles each involving the transfer of two blastocysts, first in a 'fresh' cycle and then in a 'frozen' cycle using her natural cycle. As these were unsuccessful, the next step was oocyte donation. Donated oocytes and sperm from the recipient's partner were used to create embryos for transfer. In a first attempt, two Day-3 embryos were transferred in a hormone replacement therapy (HRT) cycle following 2 days of progesterone replacement (P+2). After a further failure, a new attempt was performed by transferring two good quality Day-3 embryos in a natural cycle, 3 days after ovulation triggered by hCG administration. Since this was also unsuccessful, a third oocyte donation cycle was performed with two good quality Day-5 blastocysts transferred in an HRT cycle after 5 days of progesterone (P+5).

Despite RIF with different oocyte sources, our patient remained committed to achieve pregnancy. The underlying problem was likely to be of endometrial origin since three oocyte donation cycles were unsuccessful. A molecular diagnosis of her WOI was established with the endometrial receptivity array test (ERA®). ERA is based on the expression of 238 genes related to the endometrial receptivity gene signature derived from endometrial biopsy (Díaz-Gimeno *et al.*, 2011). Its accuracy and consistency has been demonstrated (Díaz-Gimeno *et al.*, 2013). This test identifies a 'personalized' WOI (pWOI) in women with RIF guiding the time-frame for personalized embryo transfer (pET) (Ruiz-Alonso *et al.*, 2013).

An endometrial biopsy was collected on day P+5 in an HRT cycle, identical to that in previous failed embryo transfer attempts, revealing that her endometrium was pre-receptive. A 2-day displacement of her WOI was diagnosed and this determined the need to repeat the ERA test in a further HRT cycle after 7 days of progesterone replacement (P+7). This exercise achieved a receptive endometrium appropriate for pET. Then, pET at P+7 with two blastocysts was performed in her next cycle and a twin pregnancy was achieved. The pregnancy was uneventful. She was delivered by Cesarean section at 36 weeks' gestation of two healthy boys (2780 g, and 2840 g).

A pilot study followed, with 17 patients undergoing oocyte donation after one to six previous implantation failures. In all these patients, the same approach of diagnosing their pWOI was performed and then pET, instead of routine embryo transfer, was implemented resulting in normalization of their reproductive outcome (Table I). For the comparison of the pET clinical outcomes with the previous cycles, we have considered as embryo transfer just the previous cycles in which the conditions were the same as for pET (oocyte donation and HRT cycle) but with different days for the embryo transfer: the day for the embryo transfer was diagnosed later as non-receptive by ERA and the day for the pET was the day diagnosed as receptive by ERA.

Written consent to publish this case report was obtained from the patient. The pilot study was approved by Ethics Committee of the *Instituto Valenciano de Infertilidad* (IVI), Valencia, Spain (n.25/02/2006).

Discussion

Modern genomic and bioinformatics permits stratified or personalized medicine. Personalized medicine in ART is still in its infancy but the vast majority of clinicians agree that 'one size does not fit all'. In the ovarian stimulation field, we are guided by anti-Müllerian hormone (AMH) levels and antral follicle count (AFC) as the main parameters to tailor gonadotrophin dosage. But no objective diagnostic test is routinely available for the endometrial factor. Therefore, we assume that the endometrial WOI is always there whenever the embryo is ready to be transferred. This case report shows how the personalization of the embryo transfer timing can make a difference after several previous IVF and oocyte donation failures. Moreover, the pilot study presented here demonstrates the different clinical outcomes when comparing routine embryo transfer (ET) on a day in which the endometrium was diagnosed as non-receptive (i.e. pre- or post-receptive) versus a personalized embryo transfer (pET) at the time where the endometrium was diagnosed to be receptive. Therefore, this case and the pilot study identifies for the first time the diagnosis of the personalization of the endometrial factor to improve reproductive outcome. Previously, we described a personalized 'WOI' that is displaced in one out of four patients suffering from RIF of endometrial origin (Ruiz-Alonso *et al.*, 2013) and we illustrated the utility of a personalized endometrial diagnostic approach. The consequences of this new paradigm is that individual physiological variation in endometrial receptivity has hitherto been attributed to unknown endometrial pathology, in turn deemed as causal in RIF. The real problem has been the absence of a reliable endometrial diagnostic tool to guide pET. This exciting finding is now being explored in an international RCT (The ERA as a diagnostic guide for personalized embryo transfer. ClinicalTrials.gov Identifier: NCT01954758) to determine the utility of this endometrial diagnostic intervention in the work-up for reproductive care. We hope it will make a difference.

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Authors' roles

N.G. and A.P. cared for the patient; M.R.-A. did the genetic analysis; M.R.-A., N.G., A.P. and C.S. wrote the report.

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Conflict of interest

M.R.-A. is an employee of IVIOMICS S.L. C.S. and A.P. are co-inventors of the patent GENE EXPRESSION PROFILE AS AN ENDOMETRIAL RECEPTIVITY MARKER licensed to IVIOMICS S.L.

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