Births After Transfer of Zona-Free Blastocysts in Oocyte Donation Cycles

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Purpose: Oocyte donation is a well-established method of assisted reproduction for women with irreversible infertility and with previous implantation failures after in vitro fertilization. Although the pregnancy rates are very high, sometimes implantation does not occur even after various attempts. We report the first two cases of transfer of zona-free blastocysts in oocyte donation cycles that developed to normal pregnancies and births.

Methods: The patients had undergone three previous standard oocyte donation cycles with failure of implantation. Endometrium preparation was performed after suppression of the pituitary function, with E₂ valerate and Progesterone at the day of oocyte retrieval. Normally fertilized embryos were cultured in Earle's culture medium until Day 3 and in S2 medium until Day 5. For each patient, the zonae of two fully expanded blastocysts were enzymatically removed with 0.5% pronase. Zona-free blastocysts were transferred for the patients 2 h later.

Results: On Day 12 after transfer, pregnancies were confirmed with elevated serum levels of β hCG. A gestational sac with a foetal heart beat was seen by ultrasound 15 days later, in each patient. Normal healthy babies were born at 38 and 39 weeks of pregnancy.

Conclusions: This is the first report of successful pregnancies and births after oocyte donation and transfer of zona-free blastocysts in human. It not only shows the feasibility of the treatment but also opens a new alternative for the patients with repetitive implantation failure after OD cycles.

KEY WORDS: Blastocysts; oocyte donation; zona-free.

INTRODUCTION

Oocyte donation (OD) is a well-established method of assisted reproduction for women with irreversibly infertility, and since the first report (1) many couples have achieved pregnancies with this technique worldwide. The first indication for OD was premature ovarian failure, and subsequently, the indications were expanded to physiologic menopause, poor responders to ovarian hyperstimulation for in vitro fertilization (IVF), and women with previous implantation failures after IVF. For the latter, OD is the ultimate opportunity for getting pregnant.

The pregnancy rates in cycles of OD are very high, that is, ~50% per cycle (2). The main reason for these results is the age of the donor patients, usually <30 years old. Therefore, many women who could not get pregnant with their own oocytes can be offered this technique, with higher probability of pregnancy than with conventional IVF cycles. However, sometimes implantation does not occur even after various attempts of OD cycles. In these cases, it is important to offer other alternatives to the couples who had experienced repetitive frustrations after implantation failures either with their own or with donated oocytes. Few advances have been published recently for patients with poor results after OD cycles.

Although the hatching of blastocysts is essential for implantation, the real mechanism and incidence in vivo remains unknown. The rates of blastocysts hatching in vitro ranges from 10% (3) to 20% (4). It is possible that the endometrium might play a role in the in vivo hatching process, increasing its incidence and improving the implantation rates. Therefore, we might assume that implantation failure can be related to the difficulty of the blastocysts to hatch in

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vivo because of an endometrial and not an embryonic cause.

A normal pregnancy after transfer of zona-free blastocysts in IVF (5) cycle, after eight implantation failures in standard IVF-ET cycles, have been reported recently. The authors suggest that the removal of the zona pellucida might increase the success of IVF because of better implantation. Therefore, we report the first two cases of transfer of zona-free blastocysts (due to previous implantation failures) after Day 3 embryo transfer in OD cycles, that developed to normal pregnancies and births.

MATERIAL AND METHODS

The two couples were reported to OD program due to four implantation failures after conventional IVF cycles. Women were 40 and 41 years old with normal regular menstrual cycles of 28 days during the last 10 years. Infertility evaluation showed normal endocrine profile, normal tubes, and endometrium. Semen evaluation was normal in both cases (8). After three more implantation failures, this time with donated oocytes, the couple agreed to transfer zona-free blastocysts after cycle of OD.

Endometrial Preparation

For both patients, treatment started on Day 2 of the cycle with subcutaneous administration of 3.6 mg of gonadotrophin releasing hormone analogue (GnRHa, Goserelin, Zoladex, Zeneca, Brazil) for suppression of the pituitary function. To confirm pituitary suppression, serum estradiol (E_2) levels were determined and endovaginal ultrasound (Tosbee, Toshiba, Japan) were performed 10 days later. As E_2 concentration was less than 30 pg/ml and the ultrasound showed an endometrial thickness of less than 3 mm, patients were considered ready to start treatment.

Endometrial preparation was achieved using hormonal replacement to mimic a natural cycle. Initially E_2 valerate (Climene, Schering, Brazil) was used to induce endometrial maturation by oral administration of 2 mg/day from Day 1 to 5, 4 mg/day from Day 6 to 10, and 6 mg/day from Day 11 onwards. After 16 days, patients were considered ready to receive embryos if E_2 level was higher than 300 pg/ml and the ultrasound showed an endometrial thickness of more than 8 mm. At the day of oocyte

retrieval, 100 mg/day of natural Progesterone was started intra-muscularly (I.M.).

IVF, Embryo Development and Zona Removal

The oocytes of each patient were inseminated 5 h after oocyte retrieval with ~50,000 motile sperm/oocyte in 20- μ l drops of Earle's balanced salt solution (EBSS; Sigma, USA) with 10% of substitutive synthetic serum (Irvine, USA), covered with mineral oil (Sigma, USA), and incubated at 37°C under a gas phase of 5% CO_2 . Semen preparation was performed with the swim-up technique. On the following day, 17–19 h later (day 1), fertilization was confirmed by the presence of two pronuclei.

Embryos were cultured in 20 μ l of EBSS (Sigma) with 10% substitutive synthetic serum (Irvine, USA) at 37°C in a Petri dish (Falcon, BD, USA) under mineral oil (Sigma), in a gas phase of 5% CO_2 , until Day 3. The embryos were then transferred to a droplet of S2 (Scandinavia IVF, Sc) until Day 5.

For each patient, the zonae of the selected fully expanded blastocysts were enzymatically removed with 0.5% pronase in Earle's medium. Each blastocyst was put in pronase solution for 1–2 min at 37°C in 5% CO_2 . Zona-free blastocysts (Fig. 1) were then washed and incubated in S2 medium until transfer, performed 2 h later.

RESULTS

A total of eight oocytes were donated for each patient, and seven and six were fertilized. At Day 3 after insemination five eight-cell embryos were observed in each patient. Two days later, three embryos developed to fully expanded blastocyst stage in one patient and two embryos in the other patient. The zona of two fully expanded blastocyst were removed enzymatically for each patient without damage for the embryos. Both embryos were transferred in ~10 μ l of S2 medium with 30% substitutive synthetic serum. Transfer was performed 2 h later, as routinely, using Frydman catheter (CCD, France) and guided ultrasonographically (Tosbee, Toshiba, Japan).

On Day 12 after embryo transfer, pregnancies were confirmed with elevated serum levels of β HCG. A gestational sac with a foetal heart beat was seen by ultrasound in each patient 15 days later. Hormonal replacement was discontinued on the 12th week.

Fig. 1. Fully expanded blastocyst with the zona pellucida removed. Hoffman's optics (original magnification 800 $\times$).

Normal healthy babies were born at 38 and 39 weeks of pregnancy by caesarean section.

DISCUSSION

The success rate of OD treatment for repetitive implantation failure after IVF have been well demonstrated in the past years. In fact, Remohi *et al.* (2) have shown an accumulated conception rate of 95% and live birth of 89%, regardless of the cause of infertility and patient age. Although these results are very high, sometimes implantation does not occur even after repetitive OD cycles, with different donors. For these patients, the only option so far is to keep insisting in OD cycles until pregnancy is achieved.

Since the development of sequential culture medium (6) the transfer of blastocyst stage embryos became an interesting alternative for routine Day 3 embryo transfer. The initial results are very promising with high pregnancy rates. As we are transferring embryos in a later stage, we might be increasing implantation rates because of a better synchronization

with endometrial receptivity. Moreover, keeping embryos in culture for 2 more days we can improve our selection criteria for transfer.

The zona pellucida appears to have an important role in fertilization, in preventing dispersal of the embryonic cells, and in avoiding contact between the blastomeres and the foreign cells (7). However, it is essential for implantation that the blastocyst hatches from the zona pellucida. The mechanisms of hatching are still not fully understood, and it is possible that the endometrium might play a role in the *in vivo* hatching process. Therefore, we might assume that repetitive implantation failures can be related to the difficulty of the blastocysts to hatch *in vivo*, because of an endometrial and not an embryonic cause.

The report of a normal pregnancy achieved after transfer of zona-free blastocysts (5) opened a new opportunity for patients with repetitive implantation failures. The removal of the zona pellucida before transfer of the blastocysts might improve the implantation process, specially in cases where the endometrium is probably responsible for the absence of hatching *in vivo*.

In our report, we describe two cases in which the patients were previously submitted to three attempts of standard OD cycles with different donors. As the embryos developed from different sources of oocytes, we assumed that the implantation failures were not due to an embryonic cause. Therefore, we decided to transfer blastocyst stage embryos without the zona pellucida. The resulting pregnancies might show that the endometrium do play a role in the hatching process.

This is the first report of successful pregnancies and births after OD and transfer of zona-free blastocysts in human. It shows not only the feasibility of the treatment but also opens a new alternative for the patients with repetitive implantation failure after OD cycles. Moreover this technique might increase the success in routine OD cycles.

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