Published in: Journal of Assisted Reproduction & Genetics (2003), vol.20, iss.8, pp.347-350
Status: Postprint (Author's version)

Twin Pregnancy Obtained with Frozen-Thawed Embryos after In Vitro Maturation in a Patient with Polycystic Ovarian Syndrome

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ABSTRACT:

Purpose: A twin pregnancy was obtained in a patient with polycystic ovary syndrome after the transfer of three in vitro maturation-derived day 3 embryos that has been frozen and thawed.

Methods: The patient had received mild hMG stimulation followed by hCG injection. After culture for 24-48 h, mature oocytes were fertilized by ICSI. Embryos were cultured until day 3; supernumerary embryos were cryopreserved using a slow protocol.

Results: Among 15 nonatretic oocytes, 9 matured, 8 were fertilized. Four embryos were transferred but they did not implant. The subsequent transfer of three frozen-thawed embryos resulted in the delivery of two healthy girls.

Conclusions: These results indicate that a pregnancy could be obtained with in vitro maturation-derived day-3 frozen-thawed embryos.

KEY WORDS: Embryo cryopreservation; in vitro maturation; polycystic ovarian syndrome.

INTRODUCTION

In vitro fertilization (IVF) treatment requires gonadotropin stimulation of the ovaries to increase the number of oocytes retrieved, the number of embryos available and the pregnancy rate. Ovarian stimulation has numerous disadvantages with the most important being the risk of ovarian hyperstimulation syndrome (OHSS). Women with polycystic ovarian syndrome (PCOS) have a particularly high risk of developing OHSS (1). Although many strategies are used to prevent OHSS, none is universally successful. The only reliable way to prevent OHSS would be to avoid ovarian stimulation. In vitro maturation (IVM) of immature oocytes retrieved from unstimulated ovaries is an assisted reproduction technique being accorded increasing attention.

Although early IVM success rates were relatively low, recent studies have reported much higher pregnancy rates (2,3), but the clinical pregnancy rate remains lower than that expected for conventional IVF (2). Among the multiple reasons for this lower pregnancy rate, incomplete cytoplasmic maturation, inadequate culture conditions or asynchrony between embryo development and endometrial receptivity can be suspected. This lower implantation rate means that more embryos must be transferred to maintain a comparable pregnancy rate, but such transfers expose the patient to high risk of multiple pregnancies. Because more and more patients refuse to be exposed to this higher risk, we propose limiting the number of embryos transferred and freezing the others.

We describe here a patient in whom a term twin pregnancy was achieved after transfer of frozen-thawed embryos obtained after IVM. Before the study, approval was obtained from the institutional review board of the Centre Hospitalier Universitaire of Liège.

CASE REPORT

A 29-year-old woman suffering from secondary infertility was referred to the department for ovulation induction. Three pregnancies had previously been obtained in 1996, 1997, and 2000, but all of them ended spontaneously, for no known reason, between 5 and 12 weeks of amenorrhea. The menstrual cycles were irregular, ranging from 40 to 60 days, and the luteinizing hormone (LH) level was 21.2 mlU/mL; serum testosterone concentration was normal. Transvaginal ultrasonography showed that both ovaries were enlarged

[1] First two authors have equally contributed and there is no conflict of interest in reference to the submitted material for any of the authors.

(43 and 30 x 19 mm) with stromal hyperplasia and contained >10 follicles of 2-8 mm in diameter. PCOS was diagnosed and ovulation induction was proposed. Mild stimulation was performed but unfortunately the stimulation cycle had to be cancelled because of multifollicular development. IVM protocol was proposed.

A contraceptive pill (Marvelon; Organon, Brussels, Belgium) was taken for 20 days. Four days later transvaginal ultrasonography detected >15 follicles of <10 mm in diameter in both ovaries. For the next 4 days, the patient received 150 IU of human menopausal gonadotropin (hMG; Humegon, Organon). On day 9, ultrasonography confirmed the absence of a dominant follicle. On day 10, 5000 IU of human chorionic gonadotrophin (hCG; Pregnyl, Organon) were administered and oocytes were collected 36 h later. Transvaginal ultrasonography showed the follicles to be <10 mm in diameter and they were aspirated (-7.5 kPa) using a specially designed needle (K-OPS-1235-Wood; Cook, Queensland, Australia).

Follicular fluid was collected in 15-mL culture tubes containing 3 mL of modified EBSS (EBSS with 100 IU/mL of penicillin G, 100 µg/mL of streptomycin sulfate and 0.2 mM sodium pyruvate; all made by Gibco BRL, and supplied by N.V. InVitrogen, Merelbeke, Belgium) with 5% SSS (serum supplement substitute from Irvine Scientific, supplied by Orange Medical, Brussels, Belgium) and 150 IU/mL of heparin (B. Braun Medical, Diegem, Belgium). Follicular fluid was then passed through a 70-µm pores filter (Emcon 15010/8000; MTG, Altdorf, Germany). The filter was washed with modified EBSS containing 5% SSS, and oocyte-cumulus complexes (OCC) were detected under the stereomicroscope. Oocyte maturity was determined by cumulus compaction and/or the presence or absence of germinal vesicle. Of the 17 oocytes obtained, two were atretic. Only the 15 nonatretic OCC were then transferred into the IVM medium (tissue-culture medium 199 (TCM-199) with glutamine from Gibco BRL supplemented with 0.075 IU/mL of recombinant follicle-stimulating hormone (r-FSH, Puregon; Organon Benelux, Brussels, Belgium), 0.5 IU/mL of hCG, 100 IU/mL of penicillin G sodium, and 100 µg/mL of streptomycin sulfate, 0.29 mM sodium pyruvate and 10% heat-inactivated patient's serum from the day of oocyte collection).

Fifteen oocytes were cultured for 24-48 h in 50-µL microdrops of IVM medium under oil (M-8410; Sigma Aldrich, Bornem, Belgium), at 37°C and in a 5% CO_2 humidified atmosphere. After 24 h of culture, eight (53.3%) oocytes had undergone germinal vesicle breakdown and were denuded with hyaluronidase (type IV, H-3884; Sigma Aldrich), revealing complete nuclear maturation before intracytoplasmic sperm injection (ICSI) with washed sperm; all were fertilized. In contrast, an additional oocyte that matured after 48 h of culture (1/15) was not fertilized after ICSI.

Thus, the maturation rate was 60% (9/15) and the fertilization rate was 88.9% (8/9). After 48 h of culture, the cleavage rate was 100%; two embryos were at the 7-cell stage, three at the 8-cell stage, and three at the compacted morula stage. Fertilization and cleavage cultures were performed with media from Cook (K-SIFM-100 and K-SICM-100, respectively). On day 12 of the cycle, four embryos (two 8-cell and two compacted morulae) were transferred, but no pregnancy ensued. The four surplus embryos were cryopreserved according to the previously described protocol (4).

Three months later, these embryos were thawed; three of them were at the compacted morula stage after overnight culture in blastocyst medium from Cook (K-SIBM-20). These three morulae were transferred on day 19 of an artificial cycle, combining the administration of 4 mg of estradiol valerate per day (Progynova; N.V. Schering-Plough, Diegem, Belgium) from day 1 to day 18 and 600 mg of micromized progesterone (Utrogestan; Besins International Belgium, Drogenbos, Belgium) administered vaginally (from day 16 until 12th week of amenorrhea). The pregnancy test was positive 15 days after embryo transfer (ET) and ultrasonography showed a bichorial biamniotic twin pregnancy. The patient vaginally delivered by two healthy girls at 33 weeks of gestation.

DISCUSSION

To the best knowledge this is the first pregnancy with babies born from cryopreserved day 3 embryos generated from ICSI into in-vitro-matured germinal vesicle-stage oocytes from a PCOS patient.

Veeck *et al.* (5) reported that immature human oocytes obtained during egg retrieval in an IVF program could be matured and fertilized in vitro, and among 30 ET, eight pregnancies were established. From unstimulated ovaries, Cha *et al.* (6) harvested immature human oocytes, which were matured and fertilized in vitro; five embryos transferred into a woman with premature ovarian failure resulted in the delivery of triplet girls. Trounson *et al.* (7) also achieved in vitro maturation and fertilization of immature oocytes recovered from patients with untreated PCOS.

Our IVM results indicate that the majority of the oocytes mature within 24 h (8). In this case, of the nine in-vitro-matured oocytes, eight (88.9%) were obtained within 24 h and only one within 2 days. Even though hCG had been administered before oocyte retrieval, all the oocytes were immature. The role of hCG in stimulated cycles is to induce oocyte maturation and meiosis resumption before ovum pick-up. But in nonstimulated cycles, its role is still being discussed. Some studies have shown that hCG is not necessary for IVM cycles (9), but others showed that it improved the maturational and developmental competence of the oocytes and the subsequent pregnancy rate in patients with PCOS (10).

It has been shown that qualitative hardening of the zona pellucida occurs during oocyte culture, and that this change may reduce conventional fertilization rate (11,12). So ICSI is the best option for fertilization of IVM oocytes even when sperm parameters are not impaired. Our fertilization results were good (88.9%), so the problem of zona hardening was circumvented by ICSI.

The IVM results in terms of clinical pregnancy rate remain lower than those obtained with a classical IVF cycle. It is generally explained by poor cytoplasmic maturation. Cytoplasmic maturation includes organelles and cytoskeleton modifications, like the migration of the cortical granules against oolemma (13,14), and protein and RNA accumulation in the ooplasm (15,16). These molecules support embryo development until the 8-cell stage, when the embryo begins to use is own genetic material to synthesize needed molecules (17). When an immature oocyte is retrieved from its follicular environment, meiosis tends to resume spontaneously, even when cytoplasmic maturation is not complete, so embryos obtained after IVM probably have reduced developmental competence, because of this incomplete maturation (18,19). The use of FSH before immature oocyte pick-up is controversal. Wynn *et al.* (20) demonstrated in 1998 that mild FSH stimulation increased the number of cumulus-enclosed oocytes collected, resulted in fewer degenerated oocytes after culture, and markedly enhanced the number of oocytes completing meiosis to metaphase II (71.1% vs. 43.5%). Because the FSH receptor gene is synthesized exclusively by granulosa cells (21), the effects of FSH administration must be mediated through the cumulus cells. FSH promotes cumulus cell steroid production, oocyte RNA, and protein syntheses and might inhibit granulosa cell apoptosis (22).

Another explanation for the lower pregnancy rate after IVM cycles could be the quality of the endometrium: endometrial exposure to estrogen is very short, which probably results in low endometrial receptivity to implantation, so estrogen administration has been proposed. Indeed, based on donor-oocyte-recipient data, >6 days of estrogen priming are needed to prepare endometrium for implantation before ET (23). Trounson *et al.*'s endometrial preparation involved a daily administration of 2 mg estradiol valerate from the day of oocyte retrieval and 100 mg progesterone bid via intravaginal suppositories 48 h later (7). According to the literature, this treatment requires only 5 days of exogenous estrogen priming before ET. Russell *et al.* (24) proposed in 1997 mild follicular endometrial priming with estrogens. To potentially enhance uterine synchrony, Barnes *et al.* (25) proposed leaving the dominant follicle intact during immature oocyte collection and then inducing luteinization with hCG. hCG administration 36 h before oocyte recovery stimulates the initiation of in vitro maturation and may contribute to better synchronization of embryo and endometrium development. An alternative could be the cryopreservation of the embryos after the IVM cycle, and the subsequent thawing and ET during an artificial cycle.

CONCLUSIONS

In our patient, IVM was done after mild FSH stimulation and hCG injection. The transfer of frozen-thawed embryos during an artificial cycle resulted in a twin pregnancy, but the fresh transfer was unsuccessful.

Clinical application of the immature oocyte collection procedure has several advantages: ovarian stimulation is avoided and thus the risk of OHSS is nil; patient management is simplified and discomfort markedly reduced because only a few injections of fertility drugs, blood tests, and sonographic scans of the ovaries are needed. Thus, a treatment combining IVM and embryo cryopreservation could be proposed to the patients. The efficiency of this treatment should be evaluated later.

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