CASE REPORT

Pregnancy after transfer of embryos which were generated from in-vitro matured oocytes

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In-vitro maturation of human oocytes is an important technique in assisted reproduction due to its potential for reducing the use of fertility drugs. We offered this technique as an alternative to cancelling the cycle to a patient who was at risk of ovarian hyperstimulation syndrome (OHSS) after treatment with gonadotrophin-releasing hormone analogue (GnRHa) and human menopausal gonadotrophin (HMG). The patient had 40 visible antral follicles with a maximum diameter of 13 mm and an oestradiol concentration of 14 000 pmol/l on cycle day 12. Immature oocytes were aspirated transvaginally under ultrasound guidance. Ten cumulus-enclosed oocytes were harvested and nine of them completed nuclear maturation to metaphase II after 48 h in culture. By 18 h after an intracytoplasmic sperm injection (ICSI) procedure, seven of these metaphase II stage oocytes displayed two distinct pronuclei and two polar bodies. All fertilized oocytes but one underwent cleavage; four of these were transferred 2 days later. Endometrial priming was initiated with 8 mg oestradiol valerate daily from the day of oocyte retrieval and 50 mg progesterone was injected i.m. daily starting 2 days after that. A single intrauterine sac was seen containing one fetus with positive fetal heart beat on ultrasound at 7 weeks of gestation. Unfortunately, the pregnancy ended at 24 weeks shortly after premature rupture of membranes; a live healthy-looking girl was delivered who died 18 days later.

Key words: embryo/human/hyperstimulation/in-vitro maturation/oocyte

Introduction

In mammals meiotic division of the oocyte is initiated during fetal life and is arrested at the diplotene stage of prophase I shortly before or after the birth. The oocyte remains under meiotic arrest until the ovulatory luteinizing hormone (LH) surge which stimulates the resumption of meiosis in the Graafian follicle (reviewed in Wassarman, 1988). It is also known that mammalian oocytes undergo spontaneous matura-

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with reduced aspiration pressure (100 mmHg). All the visible antral follicles were aspirated using a normal bevelled needle (Swemed®, Swemed Laboratories, International AB, Frolunda, Sweden). All the oocytes and some of the granulosa cell pieces from the follicular aspirate were collected and transferred into HEPES-buffered human tubal fluid (HTF-HEPES; Irvine Scientific, Santa Ana, CA, USA) with 10% synthetic serum substitute (SSS, Irvine Scientific) and washed twice. They were then co-cultured in 50 ml HTF (Irvine Scientific) supplemented with 10% SSS, 75 mIU/ml HMG and 500 mIU/ml HCG under pre-equilibrated mineral oil (R.E.Squibb and Sons Inc, Princeton, NJ, USA) in centre-well dishes (Falcon, #3037, Becton Dickinson Co., NJ, USA) for 48 h. Each drop contained five immature oocytes with 10–20 pieces of granulosa cell layers. At the end of the culture period, the cumulus was removed from the oocytes using a 160 mm diameter capillary pipette after exposure to 80 IU/ml hyaluronidase (Sigma, St. Louis, MO, USA) solution for 30 s. The stage of nuclear maturation of the oocytes was checked under an inverted microscope. The removed cumulus cells and granulosa cells from the maturation medium were collected to co-culture embryos. Semen analysis on the day of ICSI revealed 154×10⁶ spermatozoa/ml with 16% motility in 2 ml of ejaculate. ICSI was performed as described by Palermo et al. (1992). Injected oocytes were placed individually into cumulus and granulosa cell culture in 20 µl drops under oil. Fertilization was checked 18 h later by searching for two pronuclei and two polar bodies. Embryos were cultured for 2 more days before transfer and development was monitored daily. Embryo transfer was performed 5 days after oocyte retrieval.

Endometrial priming
From the day of oocyte retrieval onward, four tablets of oestradiol valerate (2 mg each) were given to the patient. 50 mg i.m. progesterone was injected daily starting 2 days after oocyte retrieval. Both medications were continued until a fetal heart beat was seen on ultrasound at 7 weeks.

Results
A total of 10 oocytes were obtained. All of them were enclosed by very dense layers of cumulus cells. There was no sign of cumulus expansion. All the oocyte–cumulus complexes showed a certain degree of expansion after 24 h and the cumulus cells were removed at 48 h. Nine had one polar body, and only one was at metaphase I stage. Among the nine oocytes injected with a spermatozoon intracytoplasmically, seven of them showed two distinct pronuclei and two polar bodies. One of the remaining two unfertilized oocytes was degenerate and the other was not fertilized. All the fertilized oocytes but one cleaved 48 h after ICSI. On the day of embryo transfer, the embryo quality was as follows; three 10-cell, one 6-cell with 10% fragmentation, one 6-cell with 30% fragmentation and one degenerate cleaved embryo. The four best quality embryos were transferred to the patient. Oestradiol concentrations on the day of cancellation, of oocyte retrieval and of embryo transfer were 14 000, 11 000 and 3300 pmol/l respectively. The progesterone concentration was 119 nmol/l on the day of embryo transfer. The endometrial thickness was 9 and 7 mm on the day of cancellation and embryo transfer respectively. Only one fetal heart beat was seen by ultrasound at 7 weeks gestation. Unfortunately, the pregnancy ended at 24 weeks shortly after premature rupture of membranes; a live healthy-looking girl was delivered who died 18 days postpartum.

Discussion
To our knowledge, this is the first report where a pregnancy resulted after in-vitro maturation and ICSI of an immature human oocyte retrieved after limited exposure to HMG without HCG administration. Follicular growth is stimulated by exogenous gonadotrophins to obtain sufficient oocytes for assisted reproduction. However, a certain percentage of these stimulated cycles have to be cancelled due to delayed response or to risk of hyperstimulation although a number of small follicles with immature oocytes are present. These patients must start a new cycle of stimulation with different dose of gonadotrophins and undergo the same procedure.

In-vitro maturation of mammalian oocytes has been available to the researcher since the pioneering studies of Pincus and Enzmann (1935) in rabbits. Live offspring in animals were obtained from in-vitro maturation and fertilization of immature oocytes (Fukuda et al., 1990; Yoshida et al., 1993). Human oocytes were also successfully matured (Edwards, 1965) and fertilized in vitro (Edwards et al., 1969). However, viability of these embryos was not confirmed until recently. Few babies have been born from in-vitro matured human prophase I oocytes (Cha et al., 1991; Trounson et al., 1994; Barnes et al., 1995; Russell et al., 1996). The procedure requires optimization from the recovery of oocytes to embryo development. There is little information on the best culture conditions for maturation and fertilization of immature human oocytes. The source of oocytes can affect the nuclear maturation rate. Oocytes from HMG-stimulated cycles showed a significantly higher maturation rate than those from unstimulated cycles, but no information has been provided on their ability to become fertilized (Gomez et al., 1993). Moreover, Barnes et al. (1996) have reported that oocytes from regularly cycling women have a better developmental capability than those from irregularly cycling and anovulatory women. The patient in our study received GnRHa and HMG and had a maximum-sized follicle of 13 mm until the signs of potential hyperstimulation were seen with elevated oestradiol concentrations. All the oocytes were enclosed with very dense cumulus cells with no sign of expansion and showing the typical characteristics of immature oocytes. BomseI-Helmreich et al. (1987) reported that, in patients who were stimulated with clomiphene citrate and HMG, all of the oocytes obtained from follicles <16 mm in diameter were at the germinal vesicle stage.

The oocytes obtained in this case were cultured with gonadotrophins and autologous granulosa cells and the embryos generated from these in-vitro matured oocytes were co-cultured with granulosa and cumulus cells from the same patient. Beneficial effects of gonadotrophins and co-culture with granulosa cells during maturation have been reported in oocytes obtained following HMG stimulation and HCG administration.

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(Prins et al., 1987; Dandekar et al., 1991; Zhang et al., 1993). In addition, early embryonic development has been shown to be enhanced by granulosa cell co-culture in regular IVF patients (Jaroudi and Carver-Ward, 1996). Human immature oocytes cultured in medium alone tend to have a higher rate of cleavage arrest than those co-cultured with granulosa cells (author’s unpublished observations).

ICSI was performed to avoid any male or oocyte factor fertilization failure. There have been concerns about zona hardening which has been shown to be inversely correlated with fertilizability during in-vitro maturation of mouse and rat oocytes under serum free conditions (Dodson et al., 1989; Zhang et al., 1991). There is no information on the zona hardening of human oocytes during in-vitro maturation. However, Schieve et al. (1995) reported that zona hardening did occur in human eggs and embryos discarded from IVF patients during in-vitro culture in medium containing human serum albumin. Moreover, Beckers et al. (1996) reported a very poor fertilization rate of in-vitro matured oocytes after regular insemination. Therefore, we decided to carry out ICSI to obtain an optimal fertilization rate and to minimize the possibility of fertilization failure.

One of the challenges of transferring embryos matured in vitro is the less than optimal preparation of the endometrium for implantation. Russell et al. (1996) showed that the commencement of endometrial priming was correlated with oocyte quality in unstimulated cycles; they reported that mid-follicular endometrial priming starting on cycle days 5–7 resulted in better oocyte maturation and embryo cleavage rates, and a better ongoing pregnancy rate when compared with early endometrial priming initiated on cycle day 3. In this case we had no choice but to start on the day of oocyte retrieval. Fortunately, the patient had a high oestradiol concentration on the day of oocyte retrieval and we assumed, based on our experience with frozen embryo replacement cycles, that the endometrium had been primed with this endogenous oestrogen before exogenous replacement was started.

This case report shows that the in-vitro maturation of human prophase I oocytes is feasible. They can be fertilized and undergo cleavage in vitro, and pregnancy can be achieved after this procedure. Patients who are at potential risk of hyperstimulation can be given an extra chance without HCG administration by an in-vitro ovocyte maturation protocol rather than cancelling that cycle. Furthermore, these cases may provide some information on the development of in-vitro maturation and embryo transfer protocols.


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Received on October 9, 1996; accepted on January 23, 1997