In vitro maturation and fertilization of oocytes from an intact ovary of a surgically treated patient with endometrial carcinoma: Case report

A. Revel1, A. Safran1, A. Benshushan1, A. Shushan1, N. Laufer1 and A. Simon1

In vitro Fertilization Unit, Department of Obstetrics and Gynecology, Hadassah University Hospital, Ein Kerem, PO Box 12000, Jerusalem 91120, Israel

1To whom correspondence should be addressed. E-mail: revel@md.huji.ac.il

Polycystic ovary syndrome (PCOS) with prolonged anovulation had resulted in endometrial carcinoma in a 43-year-old woman. Since she and her husband did not share common biological children, they requested fertility preservation. Due to the woman’s age, high dose progesterone and postponing surgery were both considered inappropriate. We therefore proposed oocyte retrieval from the ovaries removed by staging laparotomy followed by in vitro maturation and ICSI. Surrogacy could then enable a future pregnancy. Fourteen of 17 (82%) retrieved oocytes matured in vitro. Following ICSI, eight embryos (two at the pronuclear stage and six cleaved) were cryopreserved. To the best of our knowledge, this is the first report of oocyte aspiration, maturation and fertilization from an ovary removed by laparotomy.

Key words: embryo cryopreservation/endometrial carcinoma/fertility preservation/in vitro maturation/polycystic ovary syndrome

Introduction

Patients with anovulatory polycystic ovary syndrome (PCOS) are at risk of developing endometrial carcinoma due to the unopposed and prolonged effect of estrogen on the endometrium (Salha et al., 1997). Young women diagnosed with well-differentiated endometrial carcinoma localized in the endometrium, who wish to preserve fertility, may be treated conservatively with progestagens (Kaku et al., 2001; Ogawa et al., 2001). Such treatment combined with assisted reproduction technologies does not seem to worsen the prognosis and provides the possibility of conceiving and carrying a normal pregnancy (Ogawa et al., 2001; Gotlieb et al., 2003). Ovulation induction for assisted reproduction technology, however, usually requires ovarian stimulation by gonadotrophins. The resulting rise in blood estradiol level is not recommended in patients with endometrial carcinoma (Schwarz, 1981). An alternative protocol to retrieve oocytes without the use of gonadotrophins would be more appropriate.

In vitro maturation (IVM) of human oocytes is an emerging assisted reproductive technology with great promise (Russell, 1998; Trounson et al., 1998; Tan and Child, 2002). This procedure was found to have the greatest success in PCOS patients. To be successful, this process must entail both nuclear and cytoplasmic maturation. Endogenous regulation of oocyte maturation is a complex sequence of events regulated by endocrine parameters, oocyte/ follicular cross-talk and intraoocyte kinase–phosphatase interactions (Tsafiri et al., 1983). Advances in immature oocyte isolation and oocyte and embryo culture conditions have increased the clinical feasibility of IVM (Cha et al., 2000). IVM for 2–3 days of cumulus-enclosed oocytes, retrieved transvaginally, is routinely performed in some IVF units and yields high quality embryos (Mikkelsen and Lindenberg, 2001).

Case report

We report a case of a 43-year-old patient who presented with amenorrhoea of 6 months duration and secondary infertility. The patient had had two spontaneous pregnancies and deliveries with her previous spouse. She currently is remarried and has no children with her present husband. Evaluation of this patient revealed hyperandrogenism, insulin resistance and anovulation. LH and FSH levels were 13 and 7 mIU/ml, respectively. Ultrasound revealed polycystic ovaries and suspicion of intrauterine polyps. Diagnostic hysteroscopy confirmed the presence of two intrauterine polyps. The patient was referred for operative hysteroscopy, prior to ovulation induction. Polypectomy and curettage produced a normal uterine cavity. The pathological report revealed well-differentiated endometrial carcinoma. Magnetic resonance imaging (MRI) to the abdomen and pelvis did not detect residual disease in utero, involvement of the cervix, or abdominal or pelvic spread. High dose progesterone at her age (43 years) and postponing surgery were both considered inappropriate. We suspected that this patient had a small chance of spontaneous pregnancy due to her PCOS and her age. In addition, ovulation...
induction was not considered to be appropriate with endome-
trial carcinoma. Total abdominal hysterectomy (TAH) and
bilateral salpingo-oophorectomy (BSO) with staging were
recommended. Since the patient and her husband were
interested in preserving fertility potential, we proposed
attempting the cryopreservation of embryos. The limitation
of a lower pregnancy rate at the age of 43 was discussed
thoroughly with the patient. Nevertheless, the large number of
antral follicles present in this patient’s ovary was a favourable
prognostic finding, perhaps overcoming the negative effect of
her age (Nahum et al., 2001).

Our plan was to aspirate oocytes from the ovaries removed
by laparotomy. In order to ensure available oocytes by IVM,
we administered one i.m. injection of HCG (10 000 IU, Teva,
Israel) 36 h prior to surgery. In accordance with the
IVM protocol followed (Mikkelsen et al., 1999), no priming
by FSH was used. TAH and BSO and staging were performed
under combined anaesthesia through a longitudinal incision.
No post-operative complications were observed. Neither
frozen sections nor paraffin-embedded slides detected any
myometrial invasion or metastatic disease. Accordingly, the
patient was staged as having IA disease. No adjuvant therapy
was required.

The ovaries were immediately transferred in phosphate-
buffered saline (PBS) at 37°C to the IVF laboratory. Oocyte
aspiration was performed using a 17 gauge needle (Figure 1).
Seventeen oocytes were retrieved and immediately transferred
to P1 medium (Irvine Scientific, Santa Ana, CA) containing
10% synthetic serum supplement (SSS) (Irvine Scientific). P1
medium supplemented with 0.075 IU/ml Gonal F (Serono,
Herzliya, Israel), 0.5 IU/ml HCG (Teva) and 10% maternal
serum was used for IVM.

Although sperm analysis was in the normal range for
conventional IVF, we opted for the use of ICSI treatment to
maximize fertilization, acknowledging the effect of the
patient’s age and IVM on the physical properties of the zona
dellucida. ICSI has been suggested as being preferable to IVF
in cases of reduced oocyte quality due to patient age (Saito
et al., 2000)

Table I describes the maturation of oocytes within 42 h of
retrieval. Of the 17 oocytes collected, two were at the M2
(mature oocyte) stage and ICSI was performed, but no
fertilization was observed. The remaining 15 oocytes were at
the germinal vesicle (GV) stage and therefore underwent IVM.
The following morning, three of them had matured to the M2
stage and, following ICSI with fresh sperm, we observed a 2PN
and a 1PN zygote.

These zygotes were cryopreserved using a standard protocol
(Testart et al., 1997). On day 2, 10 of the GV oocytes
developed further, seven to M2 and three to the germinal
vesicle breakdown (GVBD) stage. This enabled another ICSI
attempt which was followed by the cryopreservation of an
additional six cleavage stage embryos (Table I).

Discussion
Oophorectomy in reproductive age patients who still have a
desire for fertility can be performed for malignant disease or
for the purpose of fertility preservation prior to ovary-toxic
chemotherapy (Revel and Schenker, 2004). We previously
have described how aspiration of ovaries removed for
cryopreservation could enable cryopreservation of oocytes
and embryos (Revel et al., 2003). Indications for oopher-
ectomy in benign disease include ovarian torsion, severe

Figure 1. Oocyte retrieval from a polycystic ovary removed by laparotomy due to endometrial carcinoma.
endometriosis or to treat critical ovarian hyperstimulation syndrome (OHSS) (Amarin, 2003).

We report here the possibility of aspirating oocytes from ovaries that were removed by laparotomy and TAH and BSO. Furthermore, we were able to achieve IVM in 14 of 17 (82%) oocytes and to obtain eight (47%) embryos (two at the pronuclear and six at the cleavage stage) from the 17 oocytes retrieved. A high number of retrieved oocytes has indeed been reported in PCOS patients (Child et al., 2001).

To the best of our knowledge, the possibility of obtaining normal embryo development from oocytes retrieved from an unstimulated surgically removed ovary has not been reported previously. Nevertheless, many factors affect the chances of obtaining a pregnancy in this case. Most noteworthy is this patient’s age. However, the large number of follicles observed may be a better predictor than patient age (Nahum et al., 2001).

IVM studies in cows have indicated that morphological characteristics such as the appearance of cumulus cells, oocyte size and the time of polar body extrusion are related to the ability of oocytes to fertilize and develop to viable embryos (Younis et al., 1989). Both estradiol (Eroglu, 1993) and FSH (Singh et al., 1993) have been shown to delay GVBD. We therefore perform micromanipulation of GVBD oocytes and obtain pregnancies following this approach, showing that extraction of the second polar body could occur after ICSI.

The number of blastomeres of each embryo was noted. Since day 3 embryos of $\geq 4$ cells can achieve pregnancies, we froze cleaving embryos containing 3–10 cells, although only one was an 8-cell embryo.

Embryos were graded before freezing according to the blastomere symmetry, the presence of anucleate fragments and the extent to which they filled the embryos’ content (Simon et al., 1998). We obtained embryos scored B (irregularity and asymmetry of the blastomeres but no fragmentation) and C (regular or irregular blastomeres with <20% of their surface filled with anucleate fragments).

Two of the eight embryos were observed to contain one pronucleus (1PN) at fertilization. 1PN embryos have been shown to be the result of normal fertilization in 70–75% of cases (Staessen and Van Steirteghem, 1997), the remainder resulting from parthenogenetic activation. Moreover, 1PN embryos can develop to blastocysts (14.3%) (Plachot and Crozet, 1992) and enable pregnancy and birth of healthy children (Gras and Trounson, 1999). We thus decided to cryopreserve 1PN embryos. The quality and limitation of such embryos were discussed with the patient. This patient required no adjuvant chemotherapy and recovered well from surgery. She and her husband are currently coordinating embryo transfer to a surrogate gestational carrier. We believe that she has a fair chance of obtaining a successful pregnancy from the cryopreserved embryos.

While acknowledging the limitations of this approach in this 43-year-old patient, we offer this presentation to submit the possibility that IVF units interested in fertility preservation should obtain experience in IVM in order to be able to offer fertility preservation in patients in need of oophorectomy.

References


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