Immature oocyte retrieval combined with in-vitro oocyte maturation

J.B. Russell

The Center for Human Reproduction, 4745 Ogletown Stanton Road, Suite 111, Newark, DE 19713, USA

Immature oocyte retrieval combined with in-vitro oocyte maturation has considerable potential to increase knowledge on the microenvironment and nuclear and cytoplasmic maturation, and may be an alternative or even replacement for routine in-vitro fertilization as practised today. Understanding the critical steps to accomplish this task demands ongoing research to produce a comparable microenvironment to that of the developing follicle. This will allow cytoplasmic maturation to occur, and clarify knowledge on the selection of the dominant follicle, and utilize novel aspects fertilization and embryo culture in vitro. A second aspect of the procedure concerns its clinical aspects. These include a better understanding of the number of antral follicles that can be retrieved transvaginally and the nature of the endometrial window and its advancement in order to provide a window of opportunity for implantation to occur.

Key words: embryo transfer/endometrial priming/immature oocytes/in-vitro fertilization/in-vitro maturation

Introduction

Since the momentous birth of Louise Brown in 1978, the focus of assisted reproductive technology (ART) has been on securing numerous mature oocytes prior to their removal from the ovary (Laufer et al., 1983; Pellicer et al., 1989). This procedure allows optimal in-vivo oocyte maturation prior to their retrieval, challenging the laboratory to accomplish fertilization and early embryonic division prior to their transfer and hopefully implantation to the endometrial cavity. The past decade of refinements have evolved with the oocyte retrieval process moving toward a more simplified, minimally invasive transvaginal ultrasound-guided aspiration combined with extremely technological advanced fertilization procedures, such as intracytoplasmic sperm injection (ICSI) (Russell et al., 1986; Palermo et al., 1992).

Laboratory culture techniques have examined a variety of conditions including different media preparations with or without serum supplementation, different media enhancing additives, and the controversial use of co-culture techniques.
Clinically, ovulation induction protocols have focused on enhancing recruitment of the cohort of follicles and synchronizing their development by down-regulation of the central axis using gonadotrophin-releasing hormone (GnRH) agonists (Porter et al., 1984; Meldrum et al., 1989). All of these factors have added to our ability to offer in-vitro fertilization (IVF) to numerous patients with multiple diagnoses with gradually improving clinical pregnancy rates.

A process introduced several years ago in animal studies utilized in-vitro oocyte maturation as a supply of mature oocytes. This practice was then introduced into human clinical trials during the early days of IVF (Edwards et al., 1965; Tsuji et al., 1985.) This procedure has been successful in producing live born offspring in animals (Goto et al., 1988; Keskintepe et al., 1994), and in humans (Cha et al., 1991; Trounson et al., 1994; Barnes et al., 1995; Russell et al., 1997). The challenge with human in-vitro oocyte maturation is to provide optimal conditions for in-vitro maturation by mimicking the microendocrine environment of the developing follicle to enable the immature oocyte to achieve nuclear and cytoplasmic maturation. Once oocyte maturation in vitro has been completed, fertilization and culture conditions must also be optimal prior to the transfer of embryos to the uterus for implantation to take place.

Initial studies leading up to the first birth from an unstimulated immature oocyte retrieval used surgically-removed ovarian specimens (Cha et al., 1991). The ovary was then dissected for the identification of antral follicles in the laboratory prior to the oocytes being matured and fertilized. The fertilized oocytes were then frozen, thawed, and transferred to a host uterus in which a triplet gestation was obtained and delivered.

Trounson et al. (1994) reported their clinical experience with performing in-vitro oocyte maturation after transvaginal ultrasound guidance in anovulatory and ovulatory patients with polycystic ovarian (PCO) disease. PCO patients are excellent candidates for transvaginal immature oocyte retrieval due to their ovarian size and numerous arrested early antral follicles in the range 5–8 mm. In addition, oocyte maturation offers them an alternative to the associated complications encountered by PCO patients during controlled ovarian hyperstimulation. Problems with overall oocyte quality associated with polycystic ovarian disease patients include a compromised quality of oocytes and a toxic luteinizing hormone (LH) secretion due to the elevated androgen microenvironment in the patients (Anderiesz et al., 1995).

A significant part of Trounson’s clinical achievement with human in-vitro oocyte maturation was the ability to retrieve immature oocytes transvaginally in polycystic ovarian disease patients, mature them in vitro, fertilize them with direct insemination and transfer the embryo to the uterus in the cycle in which the oocytes were retrieved. This innovative technique ended with the conception and delivery of a child born in 1994 (Trounson et al., 1994). The clinical features of this successful procedure were the transvaginal retrieval of oocytes from the ovary, and replacement of the embryos into a uterus which had to be prepared...
In-vitro oocyte maturation in a very short period of time. This was accomplished by the administration of exogenous oestrogen and progesterone after oocyte retrieval.

The purpose of this paper is to assess the clinical ability to retrieve, mature, and fertilize immature oocytes retrieved from patients who are ART candidates with different diagnoses.

**Methods and materials**

Patients with different diagnoses who had failed a previous IVF cycle were enrolled in the immature oocyte retrieval programme. Patients signed an informed consent before the procedure. The patient was assessed on cycle day 2 or 3 using baseline levels of follicle stimulating hormone (FSH), oestradiol and progesterone. A baseline ultrasound was also performed to assess and quantify early follicular development, and rule out any residual pathology. Follicles were measured on either day 6, 7, or 8 by ultrasound and compared to the number of immature oocytes retrieved on days 9–12. The retrieval was scheduled between day 9 and day 12. If a dominant follicle (≥1.3 cm) was visualized by ultrasound by day 8 or at the time of the retrieval, the procedure was cancelled and rescheduled earlier in the next month.

**Oocyte retrieval**

Oocyte retrieval was performed transvaginally with ultrasound guidance. The retrieval of immature oocytes is very similar to the retrieval of mature oocytes from stimulated follicles. The vaginal vault was cleansed with antibacterial soap and sterile water. A paracervical block was administered with 1% lidocaine and the vaginal vault was then filled with culture media. The sterile biopsy guide and a 5 MHz transvaginal probe was inserted into the lateral fornix. An ultrasound evaluation was performed and all follicles identified as 6–12mm were punctured using 80–100 mm Hg pressure until all follicular contents including fluid and cells were curetted from the follicle. The fluid was collected without flushing the follicle into a test tube with 3 ml of culture media maintained at 37°C. All patients undergoing oocyte retrieval received i.v. sedation with Fentanyl (Elkins-Sinn Inc, A.H.Robbins), Versed (Roche Laboratories), and Propofol (Zeneca). All of the follicles sized ≥6 mm were aspirated. The needle was a 17 gauge, 30 cm long Cook aspiration needle with a needle with a short bevel.

An Emcon embryo filter unit (75 μm, Veterinary Concepts, Spring Valley, WI, USA) was primed with at least 10 ml of heparinized modified human tubal fluid (M-HTF) (Irvine Scientific, Irvine, CA, USA) prior to the first aspirate. When the follicular fluid was obtained, it was filtered by gravity and rinsed with additional M-HTF to remove any red blood cells. The fluid remaining after filtration was decanted into Petri dishes which were scanned under a stereomicroscope for the identification of immature oocytes. These immature oocytes were graded and placed into Falcon tubes with M-HTF and 3% synthetic serum.
supplement (SSS) (Irvine Scientific). The immature oocytes, once graded, were placed in maturation media. The base maturation media consisted of tissue culture media (TCM 199) (Sigma Chemical, St Louis, MO, USA) with fetal calf serum (10%), urinary human FSH (0.075 IU) (Serono, Randolph, MA, USA) 17β-oestradiol (1 μg/ml) (Sigma) and human chorionic gonadotrophin (0.5 IU) (Steris, Phoenix, AZ, USA).

Immature oocytes were allowed to mature over the next 48 h. They were examined every 12 h and those with extruded a polar body at the end of this 48 h period were prepared for insemination. ICSI was performed to eliminate male factor bias on oocytes that matured without significant retraction, vacuolization or fragmentation.

Normally fertilized oocytes were then transferred to culture media with Vero cells for co-culture. Embryo transfer was performed 2–3 days after insemination.

*Morphology of retrieved oocytes*

Three types of oocytes were retrieved from the ovary during the retrieval of immature oocytes. These categories were compact, bald, and atretic. Compact immature oocytes possessed 1–2 layers and occasionally 4–5 layers of corona cells overlying the oocyte. Bald oocytes had very few corona cells covering the zona pellucida and were void of any continuity with these cells.

*Endometrial priming*

Endometrial priming consisted of a model typical of mid- to late follicle priming. Exogenous hormones were used for this purpose. Oestradiol valerate (i.m.) or micronized 17β-oestradiol (PO) was started 1–3 days prior to oocyte aspiration. Progesterone (50–100 mg i.m.) was initiated 1–2 days after oocyte retrieval.

*Results*

Patients (n = 37) who were candidates for ART underwent the immature oocyte retrieval process. Their mean age was 34.7 ± 4.6 years, range 27–41. Their diagnoses included tubal disease (n = 9), endometriosis (n = 7), anovulation (n = 11), male factor (n = 6), and unexplained (n = 4). FSH concentrations on cycle day 2 or 3 were 7.4 ± 3.9 mIU/ml. LH concentrations were 9.1 ± 5.2 mIU/ml, and baseline oestradiol 43 ± 15.5 pg/ml. Oocytes were retrieved from all patients who underwent immature oocyte retrieval, and a total of 431 immature eggs were collected (11.6/patient). The correlation between age and the number of oocytes is shown in Figure 1. A total of 28 patients underwent ultrasound evaluation on cycle days 6, 7, or 8 when all follicles ≥6 mm were measured; the number of follicles was compared with the number of oocytes retrieved. The retrieval efficiency was 86.4% from 302 follicles sized ≥6–12 mm and 261
immature oocytes retrieved. A classification of the morphology of the first 431 aspirated oocytes is shown in Figure 2.

The ratio of bald:compact:atretic oocytes was (156/431) 36%: (198/431) 46%: (77/431) 18%. The overall maturation rate was 63% (223/354) with no significant difference between bald and compact oocytes (P > 0.05). The fertilization rate was similar between bald and compact oocytes, being overall 72% (160/223). The proportion of oocytes with two pronuclei (2PN), as a percentage of all those injected, was 67%. Abnormal fertilization or eggs with $>3$PN, were observed in 6% (10/160) of the oocytes and 5% were damaged, i.e. eight of the 160 oocytes injected. Comparisons between compact and bald oocytes revealed a very similar rate of maturation and fertilization, although the cleavage rate appeared to favour oocyte that were morphologically graded as compact at the time of their retrieval.
Our present retrieval time averages ~36 min. In all 81% (30/37) of the patients had an embryo transfer.

Discussion

Our initial study revealed a significant decrease in rates of oocyte maturation and cleavage rates of embryos exposed to early exogenous oestrogen prior to retrieval (Russell, 1997). Although the uterus was primed sufficiently for implantation to take place, oocyte quality and cleavage dramatically decreased when the oocytes were exposed to exogenous oestrogen early in the cycle.

Further refinements are clearly needed to advance the endometrial environment from a day 9 or 10 follicular-phase endometrium to a day 19 or 20 endometrium within a 5 day timetable. Navot et al. (1989) showed how patients with a short exposure to exogenous oestrogens had a higher spontaneous abortion rate. In our study, immature oocytes must undergo a competent cytoplasmic maturation, and embryos must be able to implant at an acceptable rate. These processes must be synchronized if implantation is to occur. The luteal phase must be supported with enough exogenous oestrogen and progesterone support to maintain a secretory endometrium until the pregnancy test is performed. If a pregnancy is established, the exogenous hormonal administration is continued until the placenta functions independently.

Endometrial priming is a significant aspect of this procedure. It will impact on the success and ability to retrieve immature oocytes, and must be combined with in-vitro oocyte maturation to become a clinical therapy for those couples attempting to conceive using assisted conception. The challenge is to retrieve immature oocytes from the ovary in the mid- to late follicular phase and then return them to the implantation cavity 4–5 days later. The endometrium presumably has an endometrial ‘window’ which opens at day 18–19 after an incomplete follicular phase exposure to oestrogen and a very short exposure to progesterone.

During the final selection of the dominant follicle, there appears to be a critical point where the selection process has a negative effect on the maturation of existing follicles containing immature oocytes. This point apparently involves an increased rate of atresia among those follicles not selected to ovulate.

Our early experience has shown a dramatic decrease in the rates of maturation, fertilization and transfer among cycles in which immature eggs were retrieved when a dominant follicle (≥1.4 cm) was present at the time of retrieval. The key to timing the retrieval of immature oocytes is to allow endogenous gonadotrophins to enhance an early recruitment and to initiate early cytoplasmic and possibly nuclear maturation, which can be completed in vitro.

The future of in-vitro oocyte maturation as a therapy for couples attempting to conceive by means of assisted conception has enormous potential. It can alleviate the medication required to recruit the cohort of follicles in the month of aspiration, eliminate medications, reduce the risks of hyperstimulation and the long-term theoretical implications of ovarian cancer. The economic impact is
also profound, since there is a sharp reduction in the numbers of repeated laboratory studies and ultrasound analyses required for monitoring. It is also beneficial in relation to the patients’ time required to perform these studies.

In addition, an initial diagnostic laparoscopic procedure permits the retrieval of immature eggs for diagnostic purposes and an assessment of oocyte quality. It also allows the patient the potential of a conception in the early stages of their workup. The retrieval of immature oocytes combined with in-vitro oocyte maturation, for women in an oocyte donation programme, will appeal to individuals concerned about the administration of gonadotrophins, and of any potential short- and long-term side effects. Understanding cytoplasmic and nuclear maturation may provide information on the spontaneous chromosomal abnormalities frequently associated with recurrent pregnancy loss.

References


J.B. Russell


