Outlook

Strategies in human in-vitro maturation and their clinical outcome

Anne Lis Mikkelsen is a medical doctor who graduated in 1978 from University of Copenhagen, Denmark. In 1988 she became specialist with accreditation in obstetrics and gynaecology. After this Dr Mikkelsen worked for 2 years as senior registrar and 3.5 years as assistant consultant at different university hospitals in Copenhagen. During the last 6 years she has been a consultant at the Fertility Clinic Herlev University Hospital, which in 1998 became a part of a newly formed Institute for Human Reproduction. For 4 years she has been the head of a research group concerning in-vitro maturation of human oocytes. In order to optimize the clinical results, focus has been on improvement of the culture conditions during in-vitro maturation and improvement of the quality of the oocytes. Further scientific work includes endocrinology and fertility treatment.

Dr Anne Lis Mikkelsen
Anne Lis Mikkelsen
The Fertility Clinic, Herlev University Hospital, DK-2730 Herlev, Denmark
Correspondence: e-mail: anlimi@herlevhosp.kbhamt.dk

Abstract

The basis of in-vitro maturation (IVM) is the maturing in vitro of oocytes from the germinal vesicle (GV) stage of development to the metaphase II stage. Experience in handling immature oocytes has been obtained from two main groups. The first group is women suffering from polycystic ovarian syndrome, who are extremely sensitive to stimulation with exogenous gonadotrophins in assisted reproduction, and have a significant risk of developing ovarian hyperstimulation syndrome (OHSS). The second group is regular cycling women with normal ovaries referred for IVF due to severe male infertility. In both groups, aspiration of immature oocytes has been performed in unstimulated cycles and after priming with human chorionic gonadotrophin or FSH respectively. Clinical pregnancy rates of 24% per aspiration have been obtained. Children born after IVM appear to be healthy. These data, taken together, suggest that in future, immature oocyte retrieval combined with IVM could replace conventional IVF in selected patients.

Keywords: children, clinical application, in-vitro maturation

Introduction

In fully grown oocytes, resumption of meiosis in vivo is triggered by an LH surge. Removal of the oocyte from the follicle is the corresponding in-vitro signal.

The ability of immature oocytes to resume meiosis spontaneously when removed from the follicle was first demonstrated by Pincus and Enzman in 1935. This was later confirmed by Edwards (1965a,b). In 1969, Edwards et al. were able to demonstrate for the first time the fertilization of in-vitro matured human oocytes.

The end-point, whether in vivo or in vitro, is a metaphase II (MII) oocyte that can be fertilized and that can support normal embryonic development. The immature oocytes are retrieved by ultrasound-guided transvaginal aspiration from follicles of 2–10 mm in diameter. A high proportion of these in-vitro matured oocytes are able to resume meiosis and reach the MII stage in vitro. Their ability to be fertilized after 28–36 h of IVM is also high, and a similar proportion is able to undergo early cleavage (Cha et al., 2000; Mikkelsen and Lindenberg, 2001a,b; Mikkelsen et al., 2001a,b; Chian et al., 2004).

Oocyte growth and maturation

IVM of oocytes is not the activation of a quiescent gamete, but the culmination of a long series of preparatory processes occurring during oocyte growth.

Female germ cells enter meiosis early in fetal life, and a finite number of oocytes is determined before birth. The first meiotic germ cells appear around week 11, and shortly before birth there are in total 1–2 million oocytes arrested in prophase I at the first meiotic division. The oocytes are arrested in this phase during growth (Driancourt et al., 1993; Gougeon, 1996).

During a woman’s reproductive life, no more than 400 follicles (oocytes) reach ovulation. The great majority of follicles (99%) are destroyed through atresia. Primordial follicles enter the growth phase progressively, at a rate depending on the total number of units available in the intra-ovarian follicle pool.

When the follicles enter the growth phase, they actively grow.
by increasing the size of the oocyte from a diameter of 30 µm to a final diameter of 120 µm, and by granulosa cell proliferation and theca cell differentiation.

During the period of oocyte growth, there is a major increase in cytoplasmic organelles and the appearance of new structures, the cortical granules (Gougeon, 1996). During growth, both active transcription and translation occur in order to accumulate stores of proteins that are essential for later stages of oocyte maturation and are required to support the early embryonic cell divisions.

The oocyte secretes glycoproteins, which condense around it to form a translucent acellular layer called the zona pellucida. The zona separates the oocyte from the surrounding granulosa cells. However, contact with the oocyte is maintained via cytoplasmic processes, which penetrate the zona and form gap junctions at the oocyte surface. Gap junctions also provide the basis for an extensive network of intracellular communication among granulosa cells.

Transcription ceases at the time of ovulation, and therefore the oocyte and pre-embryo are dependent on the maternally inherited stores of ribonucleic acid (RNA) and protein until the embryonic genome is activated and RNA synthesis is initiated. The commencement of embryonic transcription varies between species; in humans, this is thought to occur at the 4–6 cell stage.

Oocyte maturation includes nuclear and cytoplasmic events. Nuclear maturation (meiotic resumption and progression from PI to MI) occurs spontaneously when the follicular inhibitory signal is suppressed.

Prior to meiotic maturation, the oocyte nucleus is characteristically large and pale. Following resumption of meiosis, the nuclear membrane dissolves (germinal vesicle breakdown; GVBD) and homologous chromosomes become separated with extrusion of the first polar body into the perivitelline space. After completion of the first meiotic division, the second meiotic division is initiated and the oocytes reach MIH prior to ovulation.

Beyond these nuclear aspects of oocyte maturation, cytoplasmic events occur and seem to be important for fertilization and developmental ability of the oocyte (Eppig and O’Brien, 1996). These aspects have been termed cytoplasmic maturation. Some ultrastructural and biochemical processes involved in the acquisition of cytoplasmic maturation have been described (Sathananthan, 1997), but events that determine the final quality of the mature oocyte remain to be identified.

Even though oocytes may be able to achieve complete nuclear maturation, they may still be deficient in cytoplasmic maturation (Eppig and O’Brien, 1996). Most deficiencies in oocytes during maturation are associated with cytoplasmic reprogramming rather than meiotic progression. The effects of cytoplasmic aberrations are seldom expressed at an early stage of development, but instead are more frequently associated with cleavage and peri-implantation stages (Eppig et al., 1994). Evaluation of the development of oocytes following fertilization is generally considered to be a valid criterion for developmental competence of the oocyte (Eppig et al., 1998).

Failing to satisfy any aspect of the maturation process results in pregnancy failure. The stage at which pregnancy terminates varies depending on the nature of the error induced during maturation. Gross abnormalities interrupt the meiotic cycle or block fertilization, while more subtle imperfections during maturation may be manifested during late cleavage or blastocyst stages, and survival to term has been a guide to controlling acquisition of full developmental competence during maturation (Eppig et al., 1998).

**IVM followed by IVF-embryo transfer**

Immature oocytes have been obtained from women who were undergoing routine superovulated IVF or intracytoplasmic sperm injection (ICSI) (rescue IVM). During ovarian stimulation, the oocyte population at the time of human chorionic gonadotrophin (HCG) administration may be heterogeneous, and this leads to retrieval of oocytes at different stages of maturation. About 15% of oocytes will remain in prophase I of meiosis. These oocytes can mature in vitro and develop into viable embryos. In 1983, Veeck and co-workers reported two pregnancies from embryo transfer of developed immature oocytes obtained from stimulated cycles in their IVF programme. Other groups have reported similar findings (Prins et al., 1987; Nagy et al., 1996, Liu et al., 1997). These oocytes, however, may represent an inferior population, as they failed to mature although the follicles were exposed to supra-physiological concentrations.

Experiences in IVM have been obtained from two main groups. The first group is women suffering from PCOS, as such women are extremely sensitive to stimulation with FSH in assisted reproduction, and they have a significant risk of developing OHSS. The second group is regular cycling women with normal ovaries referred for IVF (ICSI) due to severe male infertility.

**Immature oocytes obtained from women with PCOS**

Trounson et al. (1994) described the first pregnancy and delivery of a healthy baby after IVM of immature oocytes obtained in a patient with PCOS. In the following year, another pregnancy was reported in a patient with PCOS treated with IVM, combined with ICSI and assisted hatching (Barnes et al., 1995). Barnes et al. (1996) compared rates of maturation, fertilization and cleavage between untreated regularly ovulating and irregularly or anovulatory polycystic women. In almost all the parameters analysed, oocytes from regularly cycling patients performed better. The reason for this was not determined. Later, Cha et al. (2000) achieved a pregnancy rate of 27.1%. However, this high pregnancy rate was obtained after transfer of an average of 6.3 embryos per patient, since the implantation rate was still low (6.9%).

To compensate for this, endogenous priming with FSH (Suikkari et al., 2000; Mikkelsen and Lindenberg, 2001) or HCG (Chian et al., 2000) has been suggested before oocyte retrieval and IVM.
Suikkari et al. (2000) proposed low-dose (37.5 IU) recombinant FSH from the previous luteal phase until the leading follicle reached 10 mm. This resulted in maturation and fertilization rates in women with PCOS comparable with those in regularly cycling women; however, no pregnancies were achieved.

A beneficial effect of FSH priming has been found in a prospective randomized study (Mikkelsen and Lindenberg, 2000a,b). Oocytes obtained after priming with rFSH for 3 days followed by deprivation for 2–3 days before aspiration were compared with oocytes obtained in unstimulated PCOS patients. FSH priming improved the pregnancy (29 versus 0%) and implantation rate (21.6 versus 0%) rates compared with the non-primed group. Both FSH priming and subsequent FSH deprivation caused by withholding exogenous FSH should enhance the competence of the oocytes. It has been postulated that oocyte differentiation may be incomplete during follicular growth, and that oocytes from plateau phase follicles have increased competence (Barnes et al., 1996).

In 1999, Chian et al. reported that giving 10,000 IU HCG 36 h before oocyte retrieval improved maturation rate of immature oocytes from PCOS women (Chian et al., 1999). In a prospective randomized study, they later demonstrated that HCG priming not only improved the maturation rate, but also hastened the maturation process (Chian et al., 2000). Pregnancy rates of 30–35% have been obtained in a multicentre study, including 1000 cycles with HCG priming before immature oocyte retrieval (Chian, 2004). The implantation rate is, however, still low (10–15%). A similar pregnancy rate of 33.8% and implantation rate of 10.5% has been reported by Lin et al., using HCG priming. Additional FSH priming (75 IU per day for 6 days initiated on day 3) did not influence oocyte recovery, maturational and developmental potential, fertilization rate or pregnancy rate. The potential mechanism of the action of HCG on these small follicles is unclear.

Immature oocytes from regular cycling women with normal ovaries

Control of the menstrual cycle is a complex process involving both the hypothalamic–pituitary axis and local (paracrine and endocrine) factors (Baker and Spears, 1999). Those follicles destined to ovulate will be selected from a cohort of follicles that enter the follicular phase of the menstrual cycle with a diameter of 2–6 mm. The selected follicle will grow to a diameter of 20–25 mm at the time of ovulation. Circulating concentrations of FSH and LH regulate follicular growth and development. The rise in serum FSH concentrations during the early follicular phase causes a cohort of follicles responsive to FSH stimulation to grow. The dominant follicle can be distinguished from other cohort follicles by size (>10 mm diameter) (Pache et al., 1990). Synthesis of oestradiol is closely linked to development of the pre-ovulatory follicle and the concentrations of oestradiol in the follicle and serum are significantly related to the size of the follicle. The increase in the concentration of oestradiol is the principal factor for establishment of dominance. Oestradiol has a negative feedback effect on the hypothalamic axis, with subsequent decrease in the concentration of FSH. The dominant follicle withstands this decline, while subordinate follicles are susceptible to a decline in gonadotrophins and undergo atresia. The subordinate follicles, however, can be rescued and thereby avoid atresia by stimulatory treatment with FSH (Macklon and Fauser, 2000), or by retrieval of immature oocyte and IVM.

The first birth from IVM of immature oocytes from unstimulated cycles used oocytes that had been retrieved at different times during the menstrual cycle (Barnes et al., 1996; Russell et al., 1997; Thornton et al., 1998; Cobo et al., 1999).

Mikkelsen et al. (2000) timed oocyte collection to coincide with selection of the dominant follicle. Oocytes were aspirated after a leading follicle of 10 mm and an endometrial thickness of at least 5 mm were observed at ultrasound, and in 87 cycles a pregnancy rate of 18% per transfer was obtained. Oocytes originating from the ipsilateral ovary did not have impaired competence to mature and cleave compared with oocytes originating from the contralateral ovary (Mikkelsen et al., 2001b).

Some studies have examined the effect of priming with FSH before aspiration of immature oocytes in regularly menstruating women. (Trounson et al., 1998; Wynn et al., 1998; Mikkelsen et al., 1999, 2002; Suikkari et al., 2000). The series is small, a variety of stimulation regimens have been used, and limited conclusions can be drawn.

In two studies (Wynn et al., 1998; Suikkari et al., 2000) an improvement in the harvest of oocytes has been reported, and perhaps oocyte quality can be increased by mild ovarian stimulation with FSH prior to oocyte collection. However, Wynn et al. (1998) did not perform fertilization of the oocytes and no conclusion concerning developmental capacity can be drawn from that experiment. No beneficial effect of FSH priming was found in a prospective randomized study (Mikkelsen et al., 1999). In one group, oocytes were aspirated after priming with rFSH for 3 days, followed by deprivation for 2–3 days. In the other group, oocytes were obtained in unstimulated cycles and the day of aspiration was fixed in the same way (after a follicle of 10 mm was demonstrated). FSH priming did not increase the number of oocytes recovered, and no benefit on FSH priming compared with natural cycle on maturation rate, fertilization rate, cleavage rate or pregnancy rate could be demonstrated.

Oocyte aspiration technique

Ultrasound guided transvaginal aspiration of immature oocytes was described by Trounson and co-workers (1994). They introduced two major modifications compared with conventional IVF ultrasound guided oocyte retrieval; firstly, a new more rigid aspiration needle with a shorter bevel at the tip (Cook Australia Ltd, Brisbane, Australia) and secondly, a reduced vacuum of 80–100 mmHg. The reduced vacuum seemed to produce the most significant change. The adapted Cook needles and standard double lumen needle may be used with no difference in recovery rate (Wynn et al., 1998). Most reports have described the use of a single lumen needle under ultrasound guidance, and follicles of 2–10 mm may be aspirated without flushing (Barnes et al., 1996; Russell et al., 1997; Wynn et al., 1998; Cobo et al., 1999; Cha et al., 2000; Chian et al., 2000).
In previous studies, transvaginal oocyte aspiration has been performed under general anaesthesia (Wynn et al., 1998) or spinal anaesthesia (Chian et al., 2000). Mikkelsen et al. (1999) described the use of paracervical block.

Culture medium

Very few reports based on human data are available on the composition of culture media for human oocyte maturation. Furthermore, often too few GV oocytes have been available to make meaningful comparisons (Salha et al., 1998; Smitt and Cortwintd, 1999; Van den Hurk et al., 2000; Trounson et al., 2001).

It is generally accepted that the presence of granulosa cells is beneficial for human oocyte maturation (Cha and Chiang, 1998; Goud et al., 1998; Hwang et al., 2000). However, it is largely unknown how granulosa cells support cytoplasmatic maturation in vivo or in vitro.

FSH is important for the development of preovulatory follicles in vivo (Macklon and Fauser, 1988) and for induction of LH receptors (Gougeon, 1996), and is normally added to the culture medium. Studies in humans provide support for the responsiveness of human oocytes to gonadotrophins during IVM. Improvements in human oocyte maturation and embryo cleaving in the presence of FSH and LH have been reported (Prins et al., 1987; Durenzi et al., 1997; Andereiz et al., 2000). HCG and LH are equally effective in promoting oocyte maturation in vitro (Hreinsson et al., 2003). However, there is still a need to consider variations in relative concentrations for optimizing developmental capacity (Andereiz et al., 2000).

Culture medium for human IVM is usually supplemented with serum. The most commonly used protein sources in human IVM are fetal cord serum (Cha et al., 1991; Hwu et al., 1998) and fetal bovine serum (Trounson et al., 1994; Barnes et al., 1996; Cha et al., 2000; Chian et al., 2000; Suikkari et al., 2000). Due to potential sources of infectious agents, it is advisable not to supplement with serum sources from other patients or from animals, and therefore the patient’s serum has been used (Mikkelsen et al., 2000; Smith et al., 2000). Some clinics have used HSA (Wynn et al., 1998; Cobo et al., 1999) or synthetic serum substitute (Russell et al., 1997; Whitacre et al., 1998) as protein supplementation in IVM.

Serum concentrations have varied between 7.5 and 20% and the concentration of HSA and synthetic serum protein has varied between 0.1–0.4 and 10% respectively (Trounson et al., 2001). Significantly increased rates of maturation, pregnancy and implantation have been obtained from oocytes matured in culture medium with serum supplementation compared with oocytes matured in medium supplemented with HSA (Mikkelsen et al., 2001a). The reasons for the higher performance of serum-supplemented media in the IVM system remain to be elucidated. Serum may contain growth factors such as epidermal growth factor or insulin-like growth factor-I, which are thought to be important for cytoplasmic maturation (Gomez et al., 1993). Other factors such as inhibins and activins may also improve nuclear maturation and subsequent fertilization of immature oocytes (Alak et al., 1996).

Time interval of maturation

Previous studies have shown that 80% of immature human oocytes show nuclear maturation (extrusion of a polar body) and will be at MII by 48–54 h of culture (Trounson et al., 1994; Russell et al., 1997). Significant asynchrony of maturation has been observed and a number of MII oocytes can be obtained already after 24 h of maturation. If these oocytes are inseminated after 48 h, they have been at MII arrest for 20–30 h, which places them well past the optimal fertilization time and may compromise their developmental competence.

Adverse consequences of inseminating aged oocytes that were already at metaphase II by 23–25 h have been observed. Barnes et al. reported that oocytes reaching metaphase II first seemed to be the most competent to develop into blastocysts.

To ensure that early-matured oocytes did not remain in metaphase arrest for a prolonged period, Smith et al. (2000) evaluated the effects of shortening the duration of oocyte maturation from 36 to 28 h. No significant difference in rates of maturation, fertilization, or pregnancy, was observed when oocytes were matured for 28 h compared with 36 h. The optimal time of insemination has not yet been established. The 28-h IVM period had a significant benefit in that it allowed insemination to be performed during working hours; it had to be performed at night when the 36-h IVM schedule was used.

Fertilization, embryo culture and priming of the endometrium

Compared with conventional insemination techniques, ICSI has resulted in higher fertilization rates in human oocytes matured in vitro (Barnes et al., 1995; Hwang et al., 2000). A fertilization rate of 45% was reported when oocytes were inseminated conventionally, compared with 70–75% when ICSI was performed (Trounson et al., 1994; Russell et al., 1997; Hwang et al., 2000). Furthermore, by removal of granulosa cells, it was possible to identify extrusion of the first polar body, i.e. MII ova. However, recently conventionally IVF have been used successfully in cases with normal sperm parameters (A-M Suikkari, personal communication).

Puncturing follicles before they have reached maturity may result in an endometrium that is inadequately primed for implantation, due to lack of adequate endogenous oestriadiol and progesterone produced by granulosa cells. This may explain the low implantation rate reported in a previous study (Trounson et al., 1994). Exogenous priming with oestriadiol and progesterone is needed, and the window of implantation must be synchronized with embryo development. It is well known from hormone replacement in recipients of donor oocytes that 2-day-old embryos are best transferred into the endometrial cavity on days 3 or 4 of progesterone exposure (Rosenwaks et al., 1987; Younis et al., 1996). Imitating normal priming as closely as possible by initiating oestriadiol on the day of aspiration, and supplementation with progesterone 2 days later, has been suggested (Mikkelsen et al., 1999).
**Children born after IVM**

Data are available on approximately 300 babies delivered following IVM (Cha et al., 2000; Chian et al., 2004; A-M Suikkari, personal communication).

All IVM deliveries obtained from the beginning of the IVM programme to August 2004 have been followed \((n = 46)\). The mean age of the mothers was 31 years (range 26–38 years) and 30 were primiparae.

In the group of 47 children, 45 were singletons and two were twins; 26 boys and 21 girls, including one case of stillbirth.

Pros and cons of prenatal diagnosis were discussed in detail with the parents at 5–8 weeks of gestation. A nuchal translucency (NT) screening at 10–14 weeks of gestation and a second trimester ultrasound screening at 18–22 weeks of gestation was offered to all couples. All ultrasound examinations were performed by a medical doctor experienced in first and second trimester scanning.

In view of possible risk factors due to the new technique of IVM, couples were also offered a prenatal test by chorionic villous sampling (CVS) or amniocentesis.

Nuchal translucency (NT) measurement was possible in all fetuses, and in one case the measured NT was ≥3 mm. Second trimester ultrasound was performed in 35 pregnancies, and they were all normal.

The fetus with NT ≥3 mm had chromosomal analyses performed by CVS, resulting in normal karyotyping. The pregnancy was continued. NT normalized, and no structural defects were observed, either by fetal echography or at second trimester ultrasound. The mother delivered at term a girl with a soft cleft palate, who was otherwise healthy. Except for this soft cleft palate, no further malformations were observed.

Chromosomal analyses were performed by CVS or amniocentesis in 12 more fetuses, and in one case the measured NT was ≥3 mm. Second trimester ultrasound was performed in 35 pregnancies, and they were all normal.

The fetus with NT ≥3 mm had chromosomal analyses performed by CVS, resulting in normal karyotyping. The pregnancy was continued. NT normalized, and no structural defects were observed, either by fetal echography or at second trimester ultrasound. The mother delivered at term a girl with a soft cleft palate, who was otherwise healthy. Except for this soft cleft palate, no further malformations were observed.

Two preterm deliveries were observed. One boy was delivered at gestational week 32 + 4 by Caesarean section due to severe pre-eclampsia. The birth weight was 1745 g. One set of twins (boys) was delivered vaginally at 34 gestational weeks, with birth weights of 2240 and 2060 g.

The remaining babies were born at term with median gestational age 40 weeks (range 37–42 weeks) and median weight 3720 g (range 2900–5290 g). In total, 31 babies were delivered vaginally and 15 by Caesarean section.

One pregnancy resulted in the delivery of a dead female child at gestational age 42 weeks + 2 days. According to the consultant on the delivery ward, the death of the baby had no relation to the method by which the patient became pregnant. The pregnancy was uneventful and the fetus was in good condition at follow-up attendance at 39 gestational weeks + 6 days. At 14 days past the estimated date of delivery, a cardiotocograph (CTG) test was not reassuring and it was decided to induce labour. The attempt to induce labour was unsuccessful, the CTG returned to normal and the patient went home for the night. On the next morning, ultrasound examination showed fetal death. The patient delivered vaginally a dead female child, weighing 3720 g, with no observable abnormalities. An autopsy showed signs of asphyxia, but otherwise no pathological findings. The remaining children are all healthy, the oldest child being 6 years old.

**Future perspectives**

The IVM protocol is relative simple, with a shorter period of treatment. In addition, the side effects of stimulation, in particular OHSS, are eliminated and costs are reduced compared with conventional IVF. In future, immature oocyte retrieval combined with IVM could possibly replace standard stimulated IVF in selected patients, e.g. regular cycling women referred for IVF/ICSI due to impaired sperm quality of the man, and women with PCOS.

In the future, in-vitro follicle culture in combination with IVM may become possible. Progress is being made towards complete development of follicular oocytes in vitro (Cortvriendt and Smitz, 2001). Complete in-vitro follicle growth from primordial follicle up to Graafian stage has been achieved so far only in mice (Eppig and O’Brien, 1996). Methods for long-term in-vitro follicle growth of human primordial follicles are at present under development (Salha et al., 1998; Smitz and Cortvriendt, 1999; Van den Hurk et al., 2000), but a successful culture system has not yet been reported. Human primordial follicles have been found to proliferate up to secondary follicles even after freeze–storage (Hovatta et al., 1997).

Research is continuing in optimizing methods for the freezing of isolated immature oocytes and of a complete human ovary. This may have advantages in assisted reproductive technologies and may help to restore fertility in the treatment of cancer in children and young women.

**References**


Outlook - Human in-vitro maturation - AL Mikkelsen


Cha KY, Han SY, Chung HM 2000 Pregnanacies and deliveries after in vitro maturation culture followed by in vitro fertilization and embryo transfer without stimulation in women with polycystic ovary syndrome. Fertility and Sterility 73, 978–983.

Chian RC 2004 In-vitro maturation of immature oocytes for infertile women with PCOS. Reproductive BioMedicine Online 8, 547–552.


Mikkelsen AL, Smith SD, Lindenberg S 1999 In vitro maturation of human oocytes from regular menstruating women may be successful without FSH priming. Human Reproduction 14, 1847–1851.


Smith SD, Mikkelsen AL, Lindenberg S 2000 Development of human oocytes matured in vitro for 28 or 36 h. Fertility and Sterility 73, 541–554.


Reproduction 121, 51–75.
Whitacre KS, Seifer DB, Friedman CI et al. 1998 Effects of ovarian source, patients’ age, and menstrual cycle phase on in vitro maturation of immature human oocytes. Fertility and Sterility 70, 1015–1021.


Received 16 December 2004; refereed 5 January 2005; accepted 16 February 2005.