Reproduction (2001) **121**, 51–75

Review

Maturation of human oocytes *in vitro* and their developmental competence

Alan Trounson, Cleola Anderiesz and Gayle Jones

Centre for Early Human Development, Monash Institute of Reproduction and Development, Monash University, Monash Medical Centre, Clayton, Victoria 3168, Australia

Complete maturation of oocytes is essential for the developmental competence of embryos. Any interventions in the growth phase of the oocyte and the follicle in the ovary will affect oocyte maturation, fertilization and subsequent embryo development. Oocyte size is associated with maturation and embryo development in most species examined and this may indicate that a certain size is necessary to initiate the molecular cascade of normal nuclear and cytoplasmic maturation. The minimum size of follicle required for developmental competence in humans is 5–7 mm in diameter. Maturation *in vitro* can be accomplished in humans, but is associated with a loss of developmental competence unless the oocyte is near completion of its preovulatory growth phase. This loss of developmental competence is associated with the absence of specific proteins in oocytes cultured to metaphase II *in vitro*. The composition of culture medium used successfully for maturation of human oocytes is surprisingly similar to that originally developed for maturation of oocytes in follicle culture *in vitro*. The presence of follicle support cells in culture is necessary for the gonadotrophin-mediated response required to mature oocytes *in vitro*. Gonadotrophin concentration and the sequence of FSH and FSH–LH exposure may be important for human oocytes, particularly those not exposed to the gonadotrophin surge *in vivo*. More research is needed to describe the molecular and cellular events, the presence of checkpoints and the role of gene expression, translation and protein uptake on completing oocyte maturation *in vitro* and *in vivo*. In the meantime, there are very clear applications for maturing oocytes in human reproductive medicine and the success rates achieved in some of these special applications are clinically valuable.

Oocyte maturation remains an enigmatic process that is generally understood to span the time from when messages initiate germinal vesicle breakdown (GVBD) to completion of the nuclear changes resulting in expulsion of the first polar body. The process of maturation encompasses a complex series of molecular and structural events, culminating in the arrest of the oocyte chromosomes on the metaphase II plate in anticipation of sperm penetration and activation for fertilization. There is no agreed assay for the completion of oocyte maturation, other than the full developmental competence of the fertilized oocyte to fully formed live born offspring. Completion of the nuclear changes to produce a metaphase II oocyte does not identify developmental competence and does not reflect the molecular and structural maturity of an oocyte, which is sometimes termed cytoplasmic maturation. It is well known that oocytes will progress spontaneously through the nuclear changes characteristic of oocyte maturation when they are liberated from the antral follicle and cultured *in vitro* (Pincus and Enzmann, 1935; Edwards, 1965). GVBD may be observed in oocytes in advanced stages of follicular atresia when follicular support cells have died. Hence progression of meiosis to metaphase I or II could be representative of either oocyte degeneration or oocyte maturation. Studies in the mid-1970s by Thibault *et al*. (1975) and Moor and Trounson (1977) showed that, in species such as rabbits and sheep that have an obligatory phase of protein synthesis during maturation, a complete follicle was necessary to mature oocytes fully. By maintaining the primary elements of follicular culture and retaining granulosa cell viability and function, Staigmiller and Moor (1984) were able to retain maturation and developmental competence of sheep oocyte–cumulus cell complexes in culture when they were removed from the follicular environment.

Email: christine.hi@med.monash.edu.au

1470-1626/2001

Follicular size, oocyte maturation and developmental competence

Follicular development in monovular species is characterized by recruitment of primordial follicles for an extended period of follicular growth, selection of a dominant follicle destined for ovulation, and atresia of the remaining cohort. Follicle development is a continuum that begins at birth and continues to the end of reproductive life. The human oocyte grows from a diameter of 35 μm to a final diameter of 120 μm over several months (Gosden and Bownes, 1995; Gougeon, 1996). During this period the oocyte synthesizes and stores mRNA and proteins essential for the completion of maturation and for the subsequent acquisition of embryo developmental competence and ongoing viability. In humans, during the late luteal phase of the preceding menstrual cycle, the largest non-atretic follicles range in size between 2 and 5 mm in diameter (Gougeon and Lefevre, 1983; McNatty *et al.*, 1983). In the early follicular phase, the largest follicle ranges in size from 5.5 to 8.2 mm and this is usually the follicle that achieves dominance and continues growth to ovulation (Gougeon and Lefevre, 1983). The preovulatory follicle develops to a diameter of 18.8 ± 0.5 mm by the late follicular phase (Gougeon and Lefevre, 1983) and expands rapidly after the mid-cycle LH surge to a diameter of ⩾ 25 mm.

Antrum formation in the rodent follicle occurs at about the time oocytes complete their growth and the oocyte has acquired the capacity to resume meiosis (Eppig and Schroeder, 1989). In mice, a polyovular species, oocytes from early antral follicles are competent to resume the first meiotic division (Sorensen and Wassarman, 1976) and when isolated from the follicle undergo spontaneous resumption of meiosis. However, competence of oocytes to complete maturation beyond the first meiotic division is not conferred in mice until the follicle is much larger (Eppig and Schroeder, 1989). Developmental competence, as shown by the ability to undergo fertilization and develop to the blastocyst stage, is dependent on both the size of the follicle and the size of the oocyte (Eppig *et al.*, 1992).

The capacity for meiotic competence in rabbits (Bae and Foote, 1975), cows (Sato *et al.*, 1977), pigs (Tsafiri and Channing, 1975), sheep (Moor and Trounson, 1977), goats (Martino *et al.*, 1994), horses (Goudet *et al.*, 1997) and rhesus monkeys (Schramm *et al.*, 1993) increases with follicle size and is not strictly correlated with antrum formation or maximum oocyte diameter, which occurs later. Marmoset monkeys are similar to mice in that meiotic competence occurs concomitantly with antrum formation (Adachi *et al.*, 1982; Gilchrist *et al.*, 1995). However, follicular antrum formation occurs at different stages of folliculogenesis in different species. Antrum formation occurs in human and bovine follicles at 2% of the final ovulatory diameter (Motlik and Fulka, 1986; Greenwald and Terranova, 1988). In rhesus monkeys, an antrum appears at 3% (Greenwald and Terranova, 1988), in pigs at 4.5% (Motlik and Fulka, 1986) and in marmoset monkeys at 15% of final ovulatory diameter (Gilchrist *et al.*, 1995). Consequently it is also more appropriate to consider meiotic competence in relation to follicular diameter as a proportion of ovulatory size. Marmoset monkey oocytes from follicles at 2% of final ovulatory diameter are meiotically incompetent (Gilchrist *et al.*, 1995).

In all non-rodent species studied, the ability of oocytes to resume meiosis is acquired when the diameter of the follicle is about 9–13% of the ovulatory diameter, a stage by which oocyte growth is completed (Gilchrist *et al.*, 1995). In oocytes, the ability to complete maturation to metaphase II and developmental competence is acquired progressively with increasing follicular diameter. Goat oocytes from follicles > 5 mm in diameter undergo fertilization and develop to blastocysts at rates comparable to ovulated oocytes (Crozet *et al.*, 1995). Cow oocytes from follicles > 6 mm have about twice the developmental potential of oocytes from 2–6 mm follicles (Tan and Lu, 1990; McCaffrey *et al.*, 1992; Lonergan *et al.*, 1994). Cow oocytes from follicles < 3 mm not only show a significantly lower rate of maturation and fertilization but also fail to cleave beyond the 8- to 16-cell stage of development (Pavlok *et al.*, 1992; Blondin and Sirard, 1995). Follicle diameter and oocyte diameter are significantly correlated in the cow for follicles in the range of < 1 mm to ⩾ 4 mm, and the proportion of oocytes completing maturation to metaphase II *in vitro* is associated with increasing oocyte diameter, at least up to 110 μm in diameter. Cow oocytes < 110 μm show a significant increase in the incorporation of tritiated uridine compared with larger oocytes, indicating that the smaller oocytes are in the growth phase and still actively synthesizing RNA that is essential to maturation and later development (Fair *et al.*, 1995). In cows, oocytes appear to acquire full meiotic competence at a diameter of 115 μm and attain the competence for preimplantation embryonic development to expanded and hatching blastocysts at a diameter of 120 μm (Otoi *et al.*, 1997).

Human and macaque oocytes resume spontaneous meiosis *in vitro* at a very low rate compared with other species (Edwards, 1965). The explanation for this observation could be that meiotic competence occurs relatively late in the growth phase or that maturation requires both stimulation and removal of inhibition (Lefevre *et al.*, 1987). The human oocyte has a size-dependent ability to resume meiosis and complete maturation *in vitro* and this increases significantly as the oocyte increases in diameter from 90 to 120 μm (Durinzi *et al.*, 1995). Furthermore, this size-dependent ability for meiotic competence may also depend on the size of the follicle and the stage of the menstrual cycle. Oocytes retrieved from the follicular phase of the menstrual cycle from follicles of 9–15 mm in diameter complete meiotic maturation to metaphase II at a higher rate than oocytes from follicles of 3–4 mm in diameter (Tsuji *et al.*, 1985; Whitacre *et al.*, 1998). Wynn *et al.* (1998) reported that 5 mm was the minimum follicle

diameter from which oocytes would mature *in vitro*. Oocytes retrieved from follicles in the luteal phase of the menstrual cycle completed maturation at the same rate irrespective of follicle size (Tsuji *et al.*, 1985).

Many studies have been undertaken to determine the effect of follicle size on the maturational status and subsequent fertilization, development and competence of the human oocytes from gonadotrophin-stimulated ovulatory cycles. The incidence of mature oocytes recovered after superovulation with human menopausal gonadotrophin (hMG) and human chorionic gonadotrophin (hCG) increases with increasing follicular size (Simonetti *et al.*, 1985). The proportion of mature oocytes from follicles ⩾ 15 mm in diameter remained constant. In follicles < 15 mm in diameter there was a significant reduction in mature oocytes; only 30% of oocytes mature in follicles of 12–14 mm and 9% in follicles < 11 mm in diameter (Scott *et al.*, 1989). There is widespread opinion that after gonadotrophin stimulation the fertilization rate is lower in oocytes obtained from follicles < 10 mm in diameter than in those obtained from larger follicles (Wittmaack *et al.*, 1994; Dubey *et al.*, 1995; Salha *et al.*, 1998a). This low fertilization rate is probably due to the increase in immature oocytes recovered from smaller follicles. Fertilization has been reported to increase progressively with an increase in follicle diameter from 10 mm (Dubey *et al.*, 1995), although others have reported that once a follicle has reached 10–12 mm in diameter the fertilization rate remains relatively constant with progressive follicle growth (Haines and Emes, 1991; Wittmaack *et al.*, 1994; Salha *et al.*, 1998a). Embryo cleavage rates were reported to be significantly decreased or not significantly different for oocytes obtained from follicles < 12 mm in diameter. Embryo quality (assessed on morphology of the blastomeres and degree of fragmentation), implantation, pregnancy and birth rates appear to be independent of follicle size in stimulated cycles (Wittmaack *et al.*, 1994; Salha *et al.*, 1998a).

In monovular species, one or two follicles become dominant in the final growth phase, leading to a marked increase in the ratio of oestrogen:androgen of the dominant follicle or follicles. The remaining cohort of antral follicles remain androgen dominated and ultimately undergo atresia. Prolonged exposure to androgens in sub-dominant follicles may have an adverse effect on oocyte viability and developmental competence (Anderiesz and Trounson, 1995). In humans, the dominant follicle can usually be recognized by ultrasonography when it has reached 10 mm in diameter (Fauser and van Heusden, 1997). In a large study of gonadotrophin-stimulated IVF cycles, Wittmaack *et al.* (1994) reported that the size of the leading follicle did not affect the fertilization and cleavage rates of cohort oocytes. In contrast, Russell (1998) reported a marked decrease in the rates of maturation, fertilization and transfer among cycles in which immature oocytes were retrieved when a dominant follicle of ⩾ 14 mm was present at the time of retrieval.

Studies in this laboratory indicate that oocytes retrieved from follicles in an untreated natural cycle, or from a cycle in which clomiphene citrate is used for minimal ovarian stimulation, are capable of complete development to the blastocyst stage after administration of exogenous hCG (5000 iu) to induce maturation *in vivo* as early as day 8 of the menstrual cycle with a leading follicle diameter of 12 mm (Table 1). Mature oocytes were recovered by transvaginal aspiration 36 h after hCG administration. Development of embryos in the cohort follicles in these cycles appeared to be independent of the diameter of the leading follicle at the time of hCG injection (Table 2). Development to the blastocyst stage was observed from the smallest follicle (6 mm) retrieved in this study (Fig. 1). A live birth resulted from the transfer of a single blastocyst from a follicle measuring only 11 mm at the time of hCG injection when the leading follicle was 19 mm in diameter. Sixty-one per cent of all the oocytes retrieved and fertilized developed to the blastocyst stage, a number comparable to that observed in gonadotrophin-stimulated cycles (hMG or FSH and hCG treated) using identical culture conditions (Jones *et al.*, 1998). In our studies, patients with regular menstrual cycles undergoing an *in vitro* maturation cycle have a leading follicle > 9 mm in diameter by days 8–13 of the menstrual cycle in 79% of cases, but anovulatory or irregularly ovulating patients with polycystic ovarian disease syndrome (PCOS) rarely have a leading follicle > 9 mm in diameter at the time of immature oocyte recovery. These data indicate that the developmental competence of the *in vitro* matured oocyte must be compromised by some inherent but unidentified problem in the maturation process *in vitro* rather than being directly related to reduced follicle size, at least for follicles > 6 mm in diameter.

Source of oocytes used for *in vitro* oocyte maturation

Oocytes of farm animals can be obtained from abattoirs after slaughter of the animals. Ovaries are recovered from the animals and kept in warm PBS for transport to the laboratory. Oocytes are then aspirated from the antral follicles visible on the surface of the ovary or identified under the dissecting microscope from minced ovarian tissue. Cows in different phases of the ovulatory cycle (follicular and luteal) or even pregnant at the time they are killed do not show any marked difference in the maturational competence of oocytes. Maturation *in vitro* of cow oocytes recovered by transvaginal ultrasonography show comparable developmental competence to oocytes matured in the ovaries of animals and grown to blastocysts in the donor (Bousquet *et al.*, 1999). In this large scale study, the donor cows were given 4 days of FSH treatment (total of 400 mg FSH) after removal of the dominant follicle and the oocytes were recovered 44–48 h after the end of FSH treatment. Oocyte developmental competence was not compromised by either maturation *in vitro*, IVF or embryo culture (Fig. 2). The medium used for maturation was tis-

Table 1. Diameter of the leading dominant follicle on the day of human chorionic gonadotrophin (hCG) injection and its relationship to human embryo development

Diameter of leading dominant follicle	Number of cycles	Oocytes recovered from leading dominant follicle	Oocytes fertilized from leading dominant follicle	Maximum stage of embryo development (%)			
				Cleavage only	Morula	Blastocyst	Expanded/hatched blastocyst
10	1	1	0				
11	0	0					
12	1	1	1			1	
13	3	2	2	1		1	
14	3	2	1				1
15	9	6	4	2			2
16	8	4	2				2
17	8	4	1			1	
18	6	2	2	1	1		
19	8	6	5	1	2	1	1
20	2	2	2	2			
21	4	2	2	1	1		
22	3	3	1		1		
26	1	1	1		1		
Total	57	36	24	8 (33%)	6 (25%)	4 (17%)	6 (25%)

Table 2. Influence of the diameter of the leading follicle on human embryo development in the cohort follicles

Diameter of leading dominant follicle	Number of cycles	Number of oocytes from cohort follicles	Number of zygotes from cohort follicles	Maximum stage of embryo development (%)			
				Cleavage only	Morula	Blastocyst	Expanded/hatched blastocyst
10	1	0					
11	0	0					
12	1	0					
13	3	2	1		1		
14	3	4	2	1		1	
15	9	11	5	4	1		
16	8	8	5	1		1	3
17	8	14	12	3		3	6
18	6	8	4		2		2
19	8	8	4	1	1	1	1
20	2	1	1				1
21	4	7	5			1	4
22	3	9	6	1	1	2	2
26	1	2	1		1		
Total	57	74	46	11 (24%)	7 (15%)	9 (20%)	19 (41%)

sue culture medium 199 (TCM 199) with 10% fetal bovine serum (FBS), 0.5 μg FSH ml^{-1}, 5 μg LH ml^{-1} and 1 μg oestradiol ml^{-1}, and is similar to that used by Moor and Trounson (1977) for the successful maturation of sheep oocytes in follicle culture. A detailed consideration of the gonadotrophin, steroid and growth factor benefits for bovine oocyte maturation (Bevers *et al.*, 1997) indicates that the major benefits are from components that are already present in the TCM 199 medium described above. This finding indicates that the factors governing oocyte maturation *in vitro* are primarily intrinsic to the follicle, oocyte–cumulus mass or the oocyte itself. Surprisingly, only relatively minor changes in developmental competence of animal and human oocytes have been recorded in numerous studies on culture media and conditions for oocyte maturation.

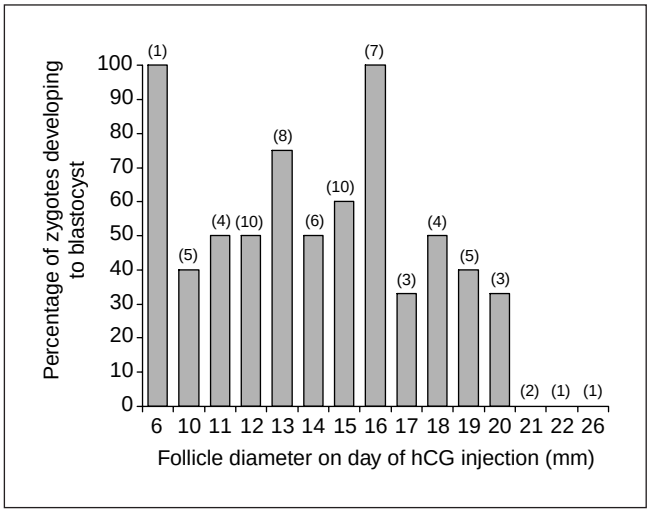

Fig. 1. Influence of the diameter of the follicle on the day of human chorionic gonadotrophin (hCG) injection on subsequent development to the blastocyst stage. The values in parentheses are number of embryos (*n*).

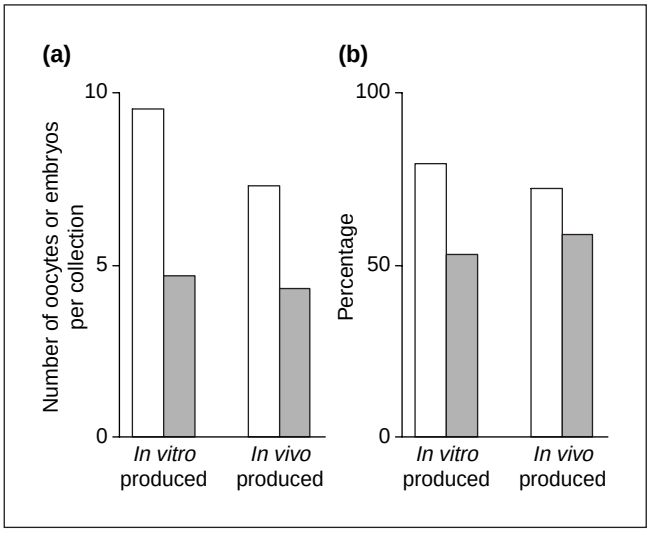

Fig. 2. Developmental competence of *in vitro* produced (matured, fertilized and cultured) bovine oocytes compared with that of oocytes produced *in vivo*. Data for 4145 *in vitro* matured oocytes and 1652 embryos recovered from superovulated donors (day 7 after oestrus). (a) Number of oocytes or embryos per collection (□); number of usable embryos (■). (b) Embryo cleavage (%, □); pregnancy at day 60 (%, ■). Data from Bousquet *et al*. (1999).

Immature oocytes from PCOS patients

Methods were developed by Trounson *et al*. (1994) for the recovery of oocytes from the ovaries of patients with PCOS in which the dominance of a particular follicle fails to occur and the cohort of growing follicles accumulates in the cortex. Most of these follicles are about 5 mm in diameter (range 3–8 mm) and remain under an androgen-dominated environment due to increased thecal cell secretion of androgens and a blockage of aromatization in the granulosa cell compartments (Almahbobi and Trounson, 1996). It is not uncommon for ⩾ 20 small to medium sized antral follicles to be present in each ovary of PCOS patients. When oocytes are recovered by transvaginal guided ultrasonography they can be readily identified and will undergo nuclear maturation in a number of different culture media, undergo fertilization after insemination or intracytoplasmic sperm injection (ICSI) and begin cleavage in culture (Trounson *et al*., 1994, 1996, 1998). Compared with oocytes recovered from regularly cyclic non-PCOS women, oocyte maturation, fertilization and embryo development in culture is significantly retarded (Barnes *et al*., 1996). However, babies have been born from the cleaving embryos and blastocysts produced as a result of *in vitro* oocyte maturation (Trounson *et al*., 1994; Barnes *et al*., 1995) although implantation rates are generally low (Trounson *et al*., 1998). Barnes *et al*. (1996) also suggested that oocytes from PCOS patients were at a disadvantage because of the abnormal endocrine environment and stasis of follicular growth. It is of interest to note that most of the follicles in these PCOS patients are not atretic (Almahbobi *et al*., 1996), nor does follicular atresia appear to reduce the developmental competence of human oocytes (Barnes *et al*., 1996). A marked improvement in viability of embryos produced from *in vitro* maturation of oocytes derived from PCOS patients was reported by Cha and Chian (1998). They recovered 910 oocytes from 72 PCOS cases (12.6 oocytes per case) and of 832 oocytes cultured, 499 (60%) matured to metaphase II. Eighty per cent of the metaphase II oocytes underwent fertilization and 306 embryos were transferred to 64 patients (4.7 embryos per patient) to yield 16 pregnancies (one twin pregnancy). The overall implantation rate was 5.6% per embryo transferred, but notably a mixture of pronuclear oocytes and early cleavage stage embryos was transferred in this study.

The media used to mature human oocytes by Cha and Chian (1998) was TCM 199 with 20% FBS and contained 10 iu equine chorionic gonadotrophin (eCG) ml^{-1} and 10 iu hCG ml^{-1}. eCG has approximately 50:50 FSH and LH bioactivity. This medium is again very much like the original follicle culture medium of Moor and Trounson (1977) and, like the successful bovine oocyte maturation medium, contains a large quantity of FBS. FBS is considered crucial for bovine oocyte maturation and may also contain factors essential for human oocyte maturation (Barnes, 1999). A medium designed to meet the nutritional and maturational needs of human oocytes based on a detailed review of oocyte metabolism and developmental competence (human oocyte maturation (HOM) medium; Table 3) has been tested for maturation, fertilization and embryo development of oocytes recovered from PCOS patients without any apparent benefit for these parameters compared with a commonly used commercial amniocyte culture medium that contains bovine serum (Chang's medium; Trounson *et al*., 1998). There is a need to explore more extensively the role of culture medium and additives

Table 3. Composition of human oocyte maturation (HOM) medium

Component	Concentration
NaCl	105 mmol l^{-1}
KCl	5.5 mmol l^{-1}
$NaH_2PO_4{\cdot}2H_20$	0.5 mmol l^{-1}
$CaCl_2{\cdot}2H_20$	1.8 mmol l^{-1}
$MgSO_4{\cdot}7H_20$	1.0 mmol l^{-1}
$NaHCO_3$	25.0 mmol l^{-1}
Sodium lactate	3.0 mmol l^{-1}
Sodium pyruvate	0.3 mmol l^{-1}
Glucose	5.5 mmol l^{-1}
Glutamine	1.0 mmol l^{-1}
Taurine	0.1 mmol l^{-1}
Cysteine	0.1 mmol l^{-1}
Cysteamine	0.5 mmol l^{-1}
Essential amino acids (EAA)	1 ×
Non-essential amino acids (NEAA)	2 ×
Penicillin	0.06 g l^{-1}
Streptomycin	0.05 g l^{-1}
EDTA	0.1 mmol l^{-1}
Vitamins	0.1 ×
Human serum albumin	2 mg ml^{-1}
Recombinant FSH	0.1 iu ml^{-1}
Recombinant LH	0.5 iu ml^{-1}
Epidermal growth factor (EGF)	10 ng ml^{-1}
Insulin transferrin sodium selenite medium supplement (ITS)	10 μl per 10 ml
Oestradiol	1 μg ml^{-1}

EAA: ICN (catalogue no. 1681149) 50 × solution; use 200 μl per 10 ml to give 1 × concentration.
NEAA: ICN (catalogue no. 1681049) 100 × solution; use 200 μl per 10 ml to give 2 × concentration.
Vitamins: ICN (catalogue no. 1601449) 100 × solution; use 10 μl per 10 ml to give 0.1 × concentration.
EGF: Sigma (catalogue no. E1264) 100 μg per vial; prepare 100 μg per 10 ml stock solution in HOM salts + 0.3% (3 mg ml^{-1}) HSA. Add 10 μl per 10 ml to give 10 ng ml^{-1}.
ITS: Sigma (catalogue no. I1884) prepare 50 ml stock from one bottle and store stock frozen in working aliquots, dilute stock 1:1000 in final solution = 10 μl per 10 ml.

for maturation, particularly the importance and replacement of the FBS component with an appropriate combination of growth factors and other components that are present in the follicular milieu. FBS continues to be used with some success despite the concerns about potential transmission of infectious agents. From this point of view, HOM medium may be preferable for the clinical application of oocyte maturation *in vitro*.

A rather unusual variant of treatment for PCOS patients with irregular menstrual cycles was reported by Chian *et al.* (1999a,b) and involves oocyte maturation *in vitro*. The patients were given intravaginal progesterone (300 mg once a day) for 10 days to induce withdrawal bleeding and 10–14 days later were given 10 000 iu hCG and the oocytes that were visible by transvaginal ultrasonography recovered from antral follicles. The oocytes were matured *in vitro* for 24–48 h in TCM 199 containing the patient's own serum (20%), 25 mmol pyruvic acid l^{-1} and 75 miu hMG. Of 25 patient treatment cycles, ten resulted in pregnancy (40%) when an average of 2.9 embryos were transferred. The authors claimed that priming with hCG before oocyte recovery increased the developmental competence of the immature oocytes recovered. However, no data were provided to show that the oocytes were immature after hCG injection. Our observations reported earlier in this review show that the oocytes recovered are mature 36 h after hCG, even from follicles as small as 6 mm in diameter at the time of hCG injection. In a further study (Chian *et al.*, 2000), the authors showed that 46% of oocytes were already maturing in hCG primed patients and the oocytes completed meiosis 12–24 h ahead of oocytes from unprimed (no hCG administration) patients. The final outcomes of fertilization, embryo development and pregnancy rates for hCG primed and unprimed patients were not significantly different.

Oocyte maturation after superovulation of IVF patients with gonadotrophins (hMG, FSH and hCG)

Normal methods for superovulation in human IVF entail the control of endogenous gonadotrophins, usually by suppression of their secretion and release from the pituitary by gonadotrophin releasing hormone (GnRH) agonists or antagonists. Multiple follicular growth is induced by administration of exogenous FSH and the final phase of oocyte maturation is induced by hCG (Eldar-Geva *et al.*, 1999). However, about 5–7% of oocytes recovered after superovulation for IVF are immature at the germinal vesicle (GV) stage and require further maturation *in vitro* (see Table 4). Some oocytes will complete maturation spontaneously in culture *in vitro* over 4–6 h before insemination (Osborn, 1993) and after being denuded of cumulus cells in preparation for fertilization by ICSI (De Vos *et al.*, 1999). Fertilization may be lower with these oocytes and their cleavage and capacity to develop to term may be reduced (De Vos *et al.*, 1999). The maturation of these oocytes may be considered to be delayed, and any reduced developmental competence compared with mature metaphase II oocytes may be related to the inability of the follicles to respond synchronously with other large follicles to hCG administration.

The time course for GVBD differs for GV stage oocytes recovered from superovulated IVF patients and untreated women (Cha and Chian, 1998). After 12 h of culture 80% of GV oocytes have undergone GVBD, whereas all the oocytes recovered from untreated patients still had intact GVs (Fig. 3). This leads to a disparity in the completion of nuclear maturation in the oocytes from both sources (Cha and Chian, 1998). About 75% of oocytes from superovulated patients reach metaphase II by 30 h of culture, whereas 75% of oocytes from untreated patients have extruded the first polar body by 42–45 h (Fig. 3). The difference in the onset of GVBD may be due to the priming of follicles by FSH and hCG before recovery of the

oocytes. However, follicles that have immature oocytes after administration of large doses of hCG (5000–10 000 iu hCG) must lack sufficient blood supply to receive the ovulatory stimulus or have insufficient LH receptors to induce the oocyte maturation response *in vivo* (reviewed by Salha *et al.*, 1998b). Since many of the GV oocytes also have unexpanded cumuli, it is likely that insufficient FSH and LH is available to these follicles *in vivo* to induce oocyte maturation.

Oocytes considered to be immature by cumulus and corona cell expansion after superovulation of IVF patients can be matured in culture without gonadotrophin or steroid supplements (Veeck *et al.*, 1983; Dandekar *et al.*, 1991) but the culture medium usually contains the patient's own serum and occasionally granulosa cells recovered from the same follicle or from other follicles that contained a mature oocyte. There are numerous case reports of development to term after maturation of oocytes recovered from superovulated patients without any special additives to culture medium (Nagy *et al.*, 1996; Edirisinghe *et al.*, 1997; Tucker *et al.*, 1998). Pregnancies have also been reported for IVF patients who were not given the final ovulating dose of hCG either by mistake (Liu *et al.*, 1997) or to reduce the probability of ovarian hyperstimulation syndrome (OHSS) (Jaroudi *et al.*, 1997; Coskun *et al.*, 1998). In the former case, 50 iu FSH ml^{-1} and 50 iu hCG ml^{-1} in B2 Ménézo medium was used for oocyte maturation. In the latter case, hMG and hCG were also added to the culture medium for oocyte maturation (75 miu hMG ml^{-1} and 500 miu hCG ml^{-1} in human tubal fluid or Ham's F10 culture medium supplemented with 10% synthetic serum substitute; Irvine Scientific, Santa Ana, CA). Jaroudi *et al.* (1999) used this procedure and reported a pregnancy rate of 9.5% and an implantation rate of 4.5% per transferred embryo.

It is interesting that oocytes appear to mature spontaneously or in the presence of gonadotrophins when patients are treated as if to recover mature oocytes for conventional IVF. However, there is very limited application for maturation *in vitro* in association with superovulation except to use the few immature oocytes that are recovered, or as a precaution to prevent OHSS, although in overstimulated IVF patients other strategies are available for treating this potential problem.

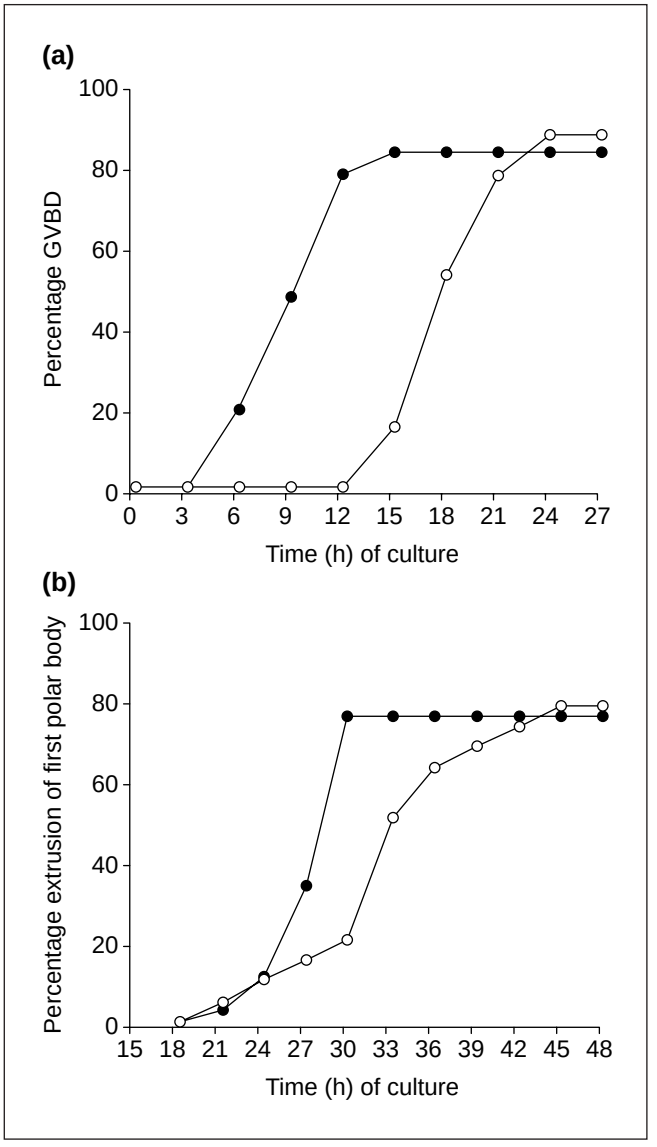

Fig. 3. Time course of (a) germinal vesicle breakdown (GVBD) and (b) the completion of maturation to metaphase II (extrusion of first polar body) in immature oocytes obtained from superovulated (stimulated, ●) and untreated patients (unstimulated, ○). (a) Stimulated 83.3% = 30/36; unstimulated 87.5% = 35/40. (b) Stimulated 75.0% = 27/36; unstimulated 77.5% = 31/40. Reproduced with permission from Cha and Chian (1998).

Pre-treatment of women with hMG or FSH for oocyte maturation

Preliminary studies of the treatment of women for 1 or 3 days with recombinant human FSH (rhFSH) early in the follicular phase showed no difference in the recovery rate of oocytes, or oocyte maturation, fertilization or development in culture (Trounson *et al.*, 1998). This finding was confirmed by Mikkelsen *et al.* (1999) who also treated women for 3 days with rhFSH (150 iu once a day, days 3–5 of the cycle) and found no improvement in any parameter of oocyte recovery, maturation or developmental competence. Oocytes were matured in TCM 199 with 75 miu rhFSH ml^{-1}, 500 miu hCG ml^{-1} and 10% patient's own serum from the day of oocyte recovery. Mikkelsen *et al.* (1999) claimed a benefit of reduced culture period for maturation (36 versus 48 h) on implantation rates of embryos produced. However, this aspect was not tested in the same experiment. Implantation rate was 15% for oocytes matured for 36 h and 7% for oocytes matured for 48 h, for embryos transferred in the two experiments. No benefit of an abbreviated culture interval for maturing

Table 4. Maturation, fertilization, embryo cleavage and pregnancy rates from *in vitro* matured human oocytes from cycles in which oocytes had been exposed to exogenous human chorionic gonadotrophin (hCG) *in vivo*

Number of cycles	Number of immature oocytes	Resumption of meiosis	Maturation (metaphase II)	Fertilization	Embryo cleavage	Pregnancy from transfer of *in vitro* matured embryos only	Study
44	74	97%	86%	85%	90%	Singleton	Veeck *et al.*, 1983
40	65	NR	36–74% dependent on supplementation with hMG	36–64%	NR	NR	Prins *et al.*, 1987
85	132	NR	NR	20–54% dependent on co-culture with granulosa cells	NR	NR	Dandekar *et al.*, 1991
66	254	NR	75–83%	57–58%	79–90% 3–12% blastocysts	NR	Toth *et al.*, 1994[a]
56	145	82–84%	38–82% dependent on culture conditions	44%	100%	NR	Janssenswillen *et al.*, 1995
1	14	100%	64%	78%	71%	Singleton	Nagy *et al.*, 1996
1	5	100%	100%	80%	NR	Singleton	Edirisinghe *et al.*, 1997[a]
26	80	50–100%	10–80% dependent on ± cumulus intact and ± sperm co-culture	50–87%	NR	NR	Fahri *et al.*, 1997
92	315	75–90%	34–64% dependent on cumulus denuded ± EGF 80–82% dependent on cumulus intact ± EGF	59–66% ± cumulus 46–72% + cumulus ± EGF	48–89% ± cumulus	NR	Goud *et al.*, 1998
59	101	NR	30–32% ± follicular fluid	62–77% ± follicular fluid	NR	Singleton Singleton[a]	Thornton *et al.*, 1998[a]
1	13	NR	67%	100%	100%	Singleton	Tucker *et al.*, 1998[a]
2804	4716	Only GVBD oocytes studied	27% by 4 h	53% (less than sibling metaphase II oocytes)	83% (comparable to sibling metaphase II oocytes)	Singleton	De Vos *et al.*, 1999
2	14	NR	79%	100%	NR	Singleton Triplet	Chian *et al.*, 1999a
25	249	NR	84%	87%	95%	Singleton Singleton Twins Five ongoing Two miscarriages	Chian *et al.*, 1999b
13	102	NR	84%	91%	95%	Five clinical	Chian *et al.*, 2000

[a]Includes cryopreservation of immature oocytes.
EGF: endothelial growth factor; GVBD: germinal vesicle breakdown; hMG: human menopausal gonadotrophin.
NR: not reported or not carried out.

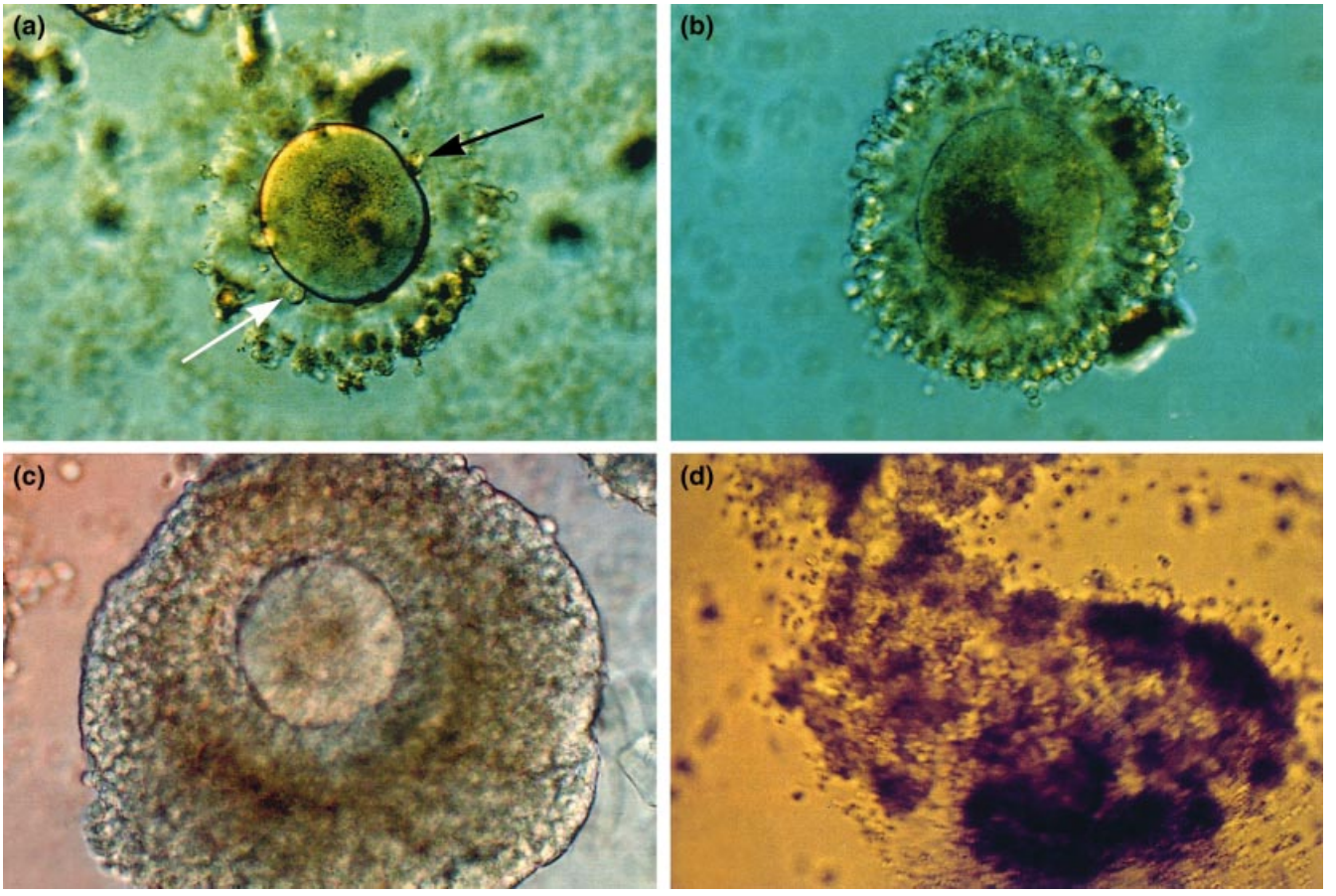

Fig. 4. Human *in vitro* matured oocytes. (a) *In vitro* matured oocyte with the first polar body visible (black arrow) and remnants of cumulus cell cytoplasmic processes (white arrow). Note expansion of the cumulus cells. (b) Germinal vesicle (GV) stage oocyte surrounded by cumulus cells. The oocyte cytoplasm is darker towards the pole opposite the GV. (c) Healthy GV stage oocyte deeply embedded in follicular granulosa cells. (d) GV stage oocyte embedded in dark atretic cumulus cells.

oocytes of untreated patients (24, 36 or 48 h) could be detected by Trounson *et al.* (1994, 1996).

Mikkelsen *et al.* (1999) did not observe any benefit of extending the rhFSH pre-treatment from 3 to 6 days to produce follicles > 10 mm in diameter. However, Wynn *et al.* (1998) administered 600 iu rhFSH to women over 5 days (300 iu on day 2, 150 iu on day 4 and 150 iu on day 6) for recovery of immature oocytes (day 7). A mean of 7.5 oocytes was recovered after rhFSH treatment compared with 5.2 oocytes from untreated women. Maturation to metaphase II was higher in rhFSH treated women (71%) compared with untreated women (44%) and hence more metaphase II oocytes resulted after rhFSH treatment (mean of 4.8 versus 2.1 for untreated women). The medium used was Eagles' minimum essential medium (EMEM) with a number of additives including 10 miu rhFSH ml^{-1} and 100 miu hCG ml^{-1}.

The failure to achieve a substantial improvement in the number and developmental competence of oocytes by pre-treatment with FSH indicates that maturation *in vitro* is not limited by the growth phase of follicles in the ovaries. Normally one or two follicles become dominant in the ovulatory cycle and the other antral follicles cease growth and will enter atresia as the follicular phase progresses. There seems little detectable difference in the maturational and developmental competence of oocytes recovered from the dominant follicle or those from atretic follicles, a surprisingly common observation that was first made by Moor and Trounson (1977) in sheep follicle culture. There would be no clinical application of pre-treatment of women with FSH to collect immature oocytes, unless a very substantial benefit could be demonstrated for maturation and development to term. Such a benefit appears to be unlikely from the present data. It would also seem unlikely that pre-treatment with FSH after removal of the dominant follicle, a protocol demonstrated to maximize bovine embryo production, would be similarly beneficial when applied to women (Bousquet *et al.*, 1999; Fig. 2).

Maturation of oocytes from untreated women

The human ovary contains numerous antral follicles during the follicular and luteal phase of the ovulating cycle and during pregnancy. The harvest of immature

Table 5. Maturation and embryo culture conditions in human *in vitro* maturation (IVM) cycles in which oocytes had not been exposed to exogenous human chorionic gonadotrophin (hCG) *in vivo*

Source of oocytes	IVM culture medium	IVM culture conditions	Maturation time (h)	Embryo culture medium	Embryo culture time (h)	Authors
Ovarian resection Oophorectomy	Ham's F10 + glutamine + 15% FBS + penicillin	5% CO_2 in air	43	NR	NR	Tsuji *et al.*, 1985
Ovarian resection Oophorectomy	B_2	5% CO_2 in air	48	NR	NR	Lefevre *et al.*, 1987
Ovarian biopsy Oophorectomy	Ham's F10 + 20% FCS/50% FF	NR	32–48	Ham's F10 + 20% FCS	24–48	Cha *et al.*, 1991
Aspiration, laparotomy Ovarian resection Oophorectomy	B_2 ± EGF ± IGF-I	NR	24–48	NR	NR	Gomez *et al.*, 1993
Aspiration, vaginal ultrasonography Polycystic ovaries	EMEM + Earle's salts + glutamine/ TCM 199 + 10% FBS + 75 miu hMG ml^{-1} + 1 ml oestradiol ± 500 miu hCG ml^{-1} ± granulosa cell co-culture	Microdrops under paraffin oil 1 ml in culture tubes 1 ml in co-culture dishes 5% CO_2 in air/5% CO_2, 5% O_2, 90% N_2	21–54	HTF + 10% patient serum	58–68	Trounson *et al.*, 1994
Aspiration, vaginal ultrasonography Polycystic ovaries	TCM 199 + 10% FBS + 75 miu rhFSH ml^{-1} + 500 miu hCG ml^{-1} + pyruvate + penicillin + streptomycin	Under silicon oil 5% CO_2 in air	36–46	HTF +10% patient serum or G1/G2 + BSA	68–110	Barnes *et al.*, 1995[a]
Aspiration, vaginal ultrasonography Polycystic ovaries and normal ovaries	TCM 199 + 10% FBS + 75 miu rhFSH ml^{-1} + 500 miu hCG ml^{-1} + pyruvate + penicillin + streptomycin	Under silicon oil 5% CO_2 in air	NR	HTF + 10% patient serum	NR	Barnes *et al.*, 1996
Oophorectomy	Ham's F10 + 7.5% FBS ± 75 miu urofollitropin ml^{-1}	5% CO_2 in air	24–72	NR	NR	Durinzi *et al.*, 1997
Aspiration, vaginal ultrasonography	HTF + 10% SSS + 75 miu hMG ml^{-1} + 500 miu hCG ml^{-1} + granulosa cells	Under mineral oil	48	HTF + 10% SSS + cumulus + granulosa cells	≈ 72	Jaroudi *et al.*, 1997[a]
Aspiration, vaginal ultrasonography Gonadotrophin stimulated ovaries	B_2 + 50 iu FSH ml^{-1} + 50 iu hCG ml^{-1}	Under paraffin oil 5% CO_2 in air	48	B_2	48 frozen	Liu *et al.*, 1997[a]
Aspiration, vaginal ultrasonography Oestradiol primed ovaries	EMEM/TCM 199 + 1 ml oestradiol + 75 miu FSH ml^{-1} + 500 miu hCG ml^{-1} + 3% SSS	NR	52	HTF + 6% SSS	72	Russell *et al.*, 1997

Continued.

Table 5. (*Continued*)

Source of oocytes	IVM culture medium	IVM culture conditions	Maturation time (h)	Embryo culture medium	Embryo culture time (h)	Authors
Aspiration, vaginal ultrasonography Polycystic ovaries	TCM 199 + 20% FBS + 10 iu PMSG ml^{-1} + 10 iu hCG ml^{-1}	NR	NR	NR	≈ 48	Cha and Chian, 1998
Aspiration, vaginal ultrasonography Gonadotrophin stimulated ovaries	HTF/Ham's F10 + 10% SSS + 75 miu hMG ml^{-1} + 500 miu hCG ml^{-1} + granulosa cells	Under paraffin oil	44	NR	NR	Coskun *et al.*, 1998
Aspiration, Caesarean section	HTF + 150 miu hMG ml^{-1} + 1 μg oestradiol ml^{-1} + 10% FCS	Under mineral oil 5% CO_2 in air	36–48	HTF + 10% FCS ± human ampullary epithelial cell monolayer	≈ 168	Hwu *et al.*, 1998
Aspiration, laparoscopy/ laparotomy Oophorectomy	HTF + 10% SSS	Under mineral oil 5% CO_2 in air	24–48	NR	NR	Whitacre *et al.*, 1998
Aspiration, vaginal ultrasonography ± gonadotrophin 'primed' ovaries	EMEM + Earle's salts + 0.1% HSA + pyruvate + penicillin + streptomycin + insulin + transferrin + selenium + glutamine + 10 miu rhFSH ml^{-1} + 100 miu hCG ml^{-1}	Under mineral oil 5% CO_2 in air	24–52	NR	NR	Wynn *et al.*, 1998
Aspiration, vaginal ultrasonography Gonadotrophin stimulated ovaries	HTF + 10% SSS + 75 miu hMG ml^{-1} + 500 miu hCG ml^{-1}	Under mineral oil	44	HTF + 10% SSS + cumulus/ granulosa cells	≈ 72	Jaroudi *et al.*, 1999
Aspiration, vaginal ultrasonography	TCM 199 + 0.4% HSA + pyruvate +75 miu rhFSH ml^{-1} + 500 miu hCG ml^{-1} + 2 ng EGF ml^{-1} + 1 μg oestradiol ml^{-1}	5% CO_2 in air	24–36	SIS IVF50: S2 + human endometrial epithelial cells	⩾ 110	Cobo *et al.*, 1999
Aspiration, vaginal ultrasonography ± gonadotrophin 'primed' ovaries	TCM 199 + pyruvate + penicillin + streptomycin + 1 μg oestradiol ml^{-1} + 75 miu rhFSH ml^{-1} + 500 miu hCG ml^{-1} + 10% patient serum	Under paraffin oil 5% CO_2 in air	36–48	Medicult IVF	≈ 48–72	Mikkelsen *et al.*, 1999
Aspiration, vaginal ultrasonography ± hCG primed ovaries	TCM 199 + pyruvate + 75 miu FSH ml^{-1} + LH + 20% FBS	5% CO_2 in air	48	Medicult IVF	≈ 48–72	Chian *et al.*, 2000

[a]Case report.

B_2: Menezo's medium; BSA: bovine serum albumin; EGF: epidermal growth factor; EMEM: Eagle's minimal essential medium; FBS: fetal bovine serum; FCS: human fetal cord serum; FF: follicular fluid; hCG: human chorionic gonadotrophin; hMG: human menopausal gonadotrophin; HTF: human tubal fluid medium; HSA: human serum albumin; IGF-I: insulin-like growth factor I; SIS: Scandinavian IVF Sciences AB; SSS: synthetic serum supplement (Irvine Scientific, Santa Ana, CA); TCM 199: tissue culture medium 199.

'Primed' indicates a truncated course of treatment compared with routine IVF stimulation cycles.

NR: not reported or not carried out.

Table 6. Maturation, fertilization, embryo cleavage and pregnancy rates from *in vitro* matured human oocytes from cycles in which oocytes had not been exposed to exogenous human chorionic gonadotrophin (hCG) *in vivo*

Number of cycles	Number of immature oocytes	Resumption of meiosis	Maturation (metaphase II)	Fertilization	Embryo cleavage	Pregnancy	Study
90	96	35–69% dependent on size of follicle and cycle stage	9–35% dependent on size of follicle and stage of cycle	NR	NR	NR	Tsuji *et al.*, 1985
11	24	31–73% dependent on state of follicle atresia	NR	NR	NR	NR	Lefevre *et al.*, 1987
23	157	NR	36–56% dependent on culture medium supplement	32–81% IVF	78.7% normal embryos	Triplets	Cha *et al.*, 1991
40	125	33–75% at 24 and 48 h 82–100% at 24 and 48 h with EGF 88–100% at 24 and 48 h with IGF-I	0–16% at 24 and 48 h 20–55% at 24 and 48 h with EGF 24–38% at 24 and 48 h with IGF-I	NR	NR	NR	Gomez *et al.*, 1993
42	159	81–100% at 21–22 and 48–54 h	18–81% at 23–25 and 48–54 h	41–45% IVF	Retarded	Singleton	Trounson *et al.*, 1994
1	13	NR	47–77%	0–80% IVF/ICSI	75–100% (68 h) 17% (110 h)	Singleton	Barnes *et al.*, 1995
20	234	NR	60–100% dependent on menstrual cycle regularity and cumulus cover of oocytes	20–67% IVF	10–60% Retarded only in irregular cycles	NR	Barnes *et al.*, 1996
11	58	79–90% dependent on urofollitropin	38–59% dependent on urofollitropin	NR	NR	NR	Durinzi *et al.*, 1997
1	10	100%	90%	78% ICSI	100%	Singleton (death after premature delivery)	Jaroudi *et al.*, 1997
1	5	100%	100%	20% ICSI	100%	Singleton	Liu *et al.*, 1997
14	161	NR	40–62% dependent on time of oestradiol follicular priming	75% ICSI	64–92%	Singleton	Russell *et al.*, 1997
72	832	NR	60%	80% ICSI	90%	Twins 15 singletons	Cha and Chian, 1998
20	162	78%	66%	NR	NR	NR	Coskun *et al.*, 1998

Continued.

Table 6. (*Continued*)

Number of cycles	Number of immature oocytes	Resumption of meiosis	Maturation (metaphase II)	Fertilization	Embryo cleavage	Pregnancy	Study
51	268	NR	67%	70% IVF	87–89% Comparable development to IVF for initial 48 h 30% blastocysts in co-culture	NR	Hwu *et al.*, 1998
88	289	46–59% at 24 and 48 h 48–60% dependent on source of oocytes 54–61% dependent on patient age 44–63% dependent on stage of menstrual cycle	9 and 36% at 24 and 48 h 29–32% dependent on source of oocytes 30–35% dependent on patient age 31–33% dependent on menstrual cycle stage	NR	NR	NR	Whitacre *et al.*, 1998
26	160	72–84% dependent on 'priming' 59–61% at 20 and 54 h	44–71% dependent on 'priming' 8–47% at 20 and 54 h	NR	NR	NR	Wynn *et al.*, 1998
21	171	NR	71%	59% ICSI	75% Poorer quality than IVF cycles	Singleton Singleton (death after premature delivery at 24 weeks)	Jaroudi *et al.*, 1999
19	112	NR	37–52% dependent on presence of dominant follicle	66–88% ICSI	36–57% Development to blastocyst	NR	Cobo *et al.*, 1999
32	115	NR	71–81% dependent on duration of 'priming'	61–79% ICSI	53–72%	Two singletons delivered Three singletons ongoing One singleton miscarried	Mikkelsen *et al.*, 1999
11	81	NR	69%	84%	96%	Three clinical	Chian *et al.*, 2000

NR: not reported or not carried out.
EGF: epithelial growth factor; ICSI: intracytoplasmic sperm injection; IGF-I: insulin-like growth factor I.

oocytes from antral follicles visible in the ovarian cortex provides the opportunity to study the maturation process *in vitro* and to avoid the administration of gonadotrophins to women for collection of multiple oocytes. Reports to date of maturation *in vitro* of human oocytes from untreated women are summarized (Tables 5 and 6).

It was originally shown by Cha *et al.* (1991) that oocytes recovered from ovaries removed from women for various gynaecological conditions can be matured in culture in Ham's F10 medium with 20% fetal cord serum (FCS) or 50% follicular fluid from preovulatory follicles that contained mature metaphase II oocytes. Maturation in

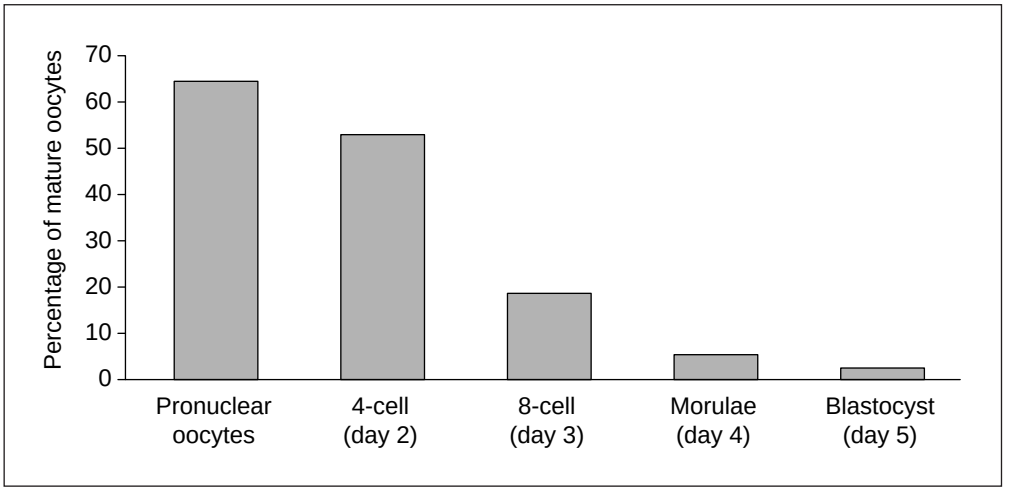

Fig. 5. Proportion of *in vitro* matured oocytes that underwent fertilization and developed to cleavage stage embryos, morulae and blastocysts *in vitro*. Oocytes were matured in TCM 199 + gonadotrophins or Chang's medium for 48 h, fertilized by intracytoplasmic sperm injection (ICSI) and cultured in Earle's medium with 10% patient's serum. Reproduced with permission from Trounson *et al*. (1998).

medium with follicular fluid resulted in 56% metaphase II oocytes after 32–48 h and 81% of the mature oocytes underwent fertilization. Lower results were obtained with FCS (36% maturation to metaphase II and 32% fertilization). When five embryos were transferred to a patient, a triplet pregnancy resulted. No difference in oocyte maturational or developmental competence could be identified between oocytes recovered in the follicular or luteal phase of the ovulatory cycle.

The introduction of ultrasound guided oocyte retrieval of immature oocytes by Trounson *et al*. (1994) allowed greater access to growing follicles in the ovaries and the examination of an alternative to administration of high doses of gonadotrophins to women for superovulation. In EMEM with 10% FBS, 75 miu hMG ml^{-1}, 500 miu hCG ml^{-1} and 1 μg oestradiol ml^{-1}, 81% of oocytes matured to metaphase II by 48–54 h of culture. The replacement of EMEM with TCM 199 produced the same outcomes for maturation, fertilization and embryo development, including births from maturation in both media (Trounson *et al*., 1994; Barnes *et al*., 1995, 1996). These studies again confirmed that oocyte–cumulus health (tight cumulus–corona cover, loose or partly absent corona cover, denuded oocytes, obvious atresia in cumulus–corona cover; Fig. 4) had no obvious effect on maturational or developmental competence (Barnes *et al*., 1996). It was apparent that oocytes from women with regular ovulatory cycles had a better cleavage rate than those who were anovulatory or had irregular menstrual cycles (Barnes *et al*., 1996). Russell *et al*. (1997) claimed that administration of oestradiol to patients in the early to mid-follicular phase before recovery of immature oocytes, increases maturation, fertilization and their developmental competence. However, in controlled studies reported by Trounson *et al*. (1998) there was no benefit of oestrogen treatment of patients in any of the parameters examined. It is possible that prolonged oestrogen treatment may increase uterine receptivity for embryo implantation, although this remains to be proven.

In an interesting variation of natural cycle IVF, Thornton *et al*. (1998) recovered immature GV oocytes from follicles secondary to the one dominant follicle after hCG injection (10 000 iu). These follicles were ⩽ 12 mm and were matured in Ham's F10 + 50% follicular fluid as described by Cha *et al*. (1991) or in standard culture medium. There was no difference in maturation rates *in vitro* (30% and 32%, respectively) or fertilization rates (77% and 62%, respectively). A birth resulted from the transfer of an embryo in the same cycle as the immature egg retrieval. An additional birth resulted from one of six transfers of cryopreserved embryos derived from oocytes matured *in vitro*. A similar approach was adopted by Chian *et al*. (1999a,b, 2000) for PCOS patients with irregular menstrual cycles, and good pregnancy rates were achieved using maturation medium with gonadotrophins and serum additives.

It has also been reported that oocytes recovered at the end of pregnancy (Caesarean section) can be matured in human tubal fluid (HTF) medium with 150 miu hMG ml^{-1}, 10% FCS and 1 μg oestradiol ml^{-1} (Hwu *et al*., 1998). Embryos failed to cleave beyond two to six cells in HTF medium with 10% FCS, but 26% of the pronuclear zygotes developed to blastocysts in human ampullary epithelial cell co-culture. This finding again demonstrates the flexibility of the origins of oocytes for maturation and underlines the importance of factors intrinsic to oocyte nuclear and cytoplasmic health for developmental competence. Providing culture medium and culture conditions are appropriate and suitable for maintaining the essential metabolism, growth and molecular expression patterns, a

viable embryo can be obtained from a wide range of physiological and endocrine states. The challenge is to recognize the key elements intrinsic to oocyte maturational competence and to ensure that these are catered for in the interventions required for oocyte maturation *in vitro*. A recent examination of oocytes recovered after superovulation in humans, or after ovulation in mice, for molecular markers of apoptosis does not support the hypothesis that apoptosis is responsible for fertilization and developmental abnormalities (Van Blerkom and Davis, 1998). Hence the cytoplasmic and chromosomal defects that occur in human oocytes are unlikely to be preprogrammed at least by apoptotic mechanisms. The occurrence of these defects may be due to interventions that interrupt the completion of essential cellular processes and interfere with messages from support cells required for molecular and cytoplasmic maturation events.

Developmental competence of *in vitro* matured oocytes

Nuclear maturation, the resumption of meiosis and completion of the first meiotic division, occurs *in vitro* for all species studied to date. Significant numbers of immature oocytes can be matured to metaphase II. Subsequent fertilization, cleavage and development to viable offspring vary according to the species studied. However, aberrations in cytoplasmic maturation are more likely to be apparent as failure in later stages of development (Moor *et al*., 1998). It is of interest to note the improved rates of fertilization and embryo development and viability after administering a priming dose of hCG to patients, and further research is needed to determine the physiological mechanisms involved in the apparent improvement in cytoplasmic maturation of human oocytes *in vitro*.

Recently, Anderiesz *et al*. (2000a) showed that exposure of cattle and human oocytes to a 1:10 ratio of FSH:LH (1 iu rhFSH ml^{-1} and 10 iu rhLH ml^{-1}) resulted in significantly higher developmental competence evident by increased development to the blastocyst stage *in vitro* compared with FSH alone (1 iu rhFSH ml^{-1}) or no gonadotrophins. In the human maturation system, FSH (1 iu rhFSH ml^{-1} in TCM 199 + 2 mg human serum albumin ml^{-1}) was used for the first 24 h of culture and both 1 iu FSH ml^{-1} and 10 iu LH ml^{-1} were included for the remaining 24 h of culture to complete maturation. The biphasic addition of gonadotrophins may enable the maturation of LH receptors on cumulus and granulosa cells and improve their responsiveness to LH in the second culture phase. In these experiments, 24% of the fertilized oocytes developed to blastocysts *in vitro* and no blastocysts were obtained in maturation medium containing FSH alone or no gonadotrophins. Further experiments are needed to confirm the benefit of biphasic exposure of human oocytes to gonadotrophins and to determine the mechanism responsible for improved development of embryos. Development of bovine blastocysts was highest (24%) in medium with an FSH:LH ratio of 1:10, but since these blastocysts mature within 24 h, biphasic exposure to gonadotrophins was not examined.

It is important that evaluation of oocyte maturation in humans includes an adequate assessment of developmental competence *in vivo* or *in vitro*. Very few studies of *in vitro* matured human oocytes have included an assessment of development to the blastocyst stage at which stage it can be revealed that preimplantation development is seriously compromised (see for example Fig. 5). Barnes *et al*. (1995) were the first to report successful development to the blastocyst stage in sequential culture medium designed specifically to optimize blastocyst development. A pregnancy resulted from the transfer of a single blastocyst after assisted hatching. However, only one of six embryos produced in this case study was competent to develop to the blastocyst stage. Hwu *et al*. (1998) demonstrated that embryos derived from *in vitro* matured oocytes when cultured in HTF culture medium alone arrest at the 2- to 16-cell stage of development. However, when embryos are co-cultured with human ampullary cells, 30% of zygotes are capable of developing to the blastocyst stage. Cobo *et al*. (1999) similarly reported high rates of development of embryos derived from *in vitro* matured oocytes to the blastocyst stage (49%) when embryos were co-cultured with endometrial epithelial cells. These recent reports compare favourably with the proportion of zygotes developing to the blastocyst stage in the absence of co-cultured cells from *in vivo* matured oocytes harvested from stimulated cycles.

Molecular checkpoint controls in oocyte maturation

The meiotic cell cycle is a dynamic system that functions via an elaborate sequence of highly ordered and interrelated events. Mammalian oocytes are arrested at the G2-phase of the cell cycle, specifically at the dictyate stage of prophase in the first meiotic division. The resumption of oocyte maturation releases oocytes from this developmental arrest and re-initiates meiosis. The meiotic cell cycle then proceeds through two consecutive M-phases, which result in a reduction in the maternal genome. The passage through the cell cycle is regulated by checkpoints and molecular controls.

Checkpoint controls

Cell cycle checkpoints are genetically encoded controls and biochemical pathways that represent an important mechanism in cell cycle progression as they impose delays in the cell cycle to ensure the systematic completion of each cell cycle phase before the initiation of the next phase (Hartwell and Weinert, 1989). In somatic cells, there are three major checkpoints, which are present at G1, G2 and M-phase and specifically monitor cell size, DNA replication, DNA damage and spindle abnormalities.

Table 7. Checkpoints responsive to DNA replication

Checkpoints	Species	Gene/gene product	Function	Study
Checkpoints responsive to DNA replication	*Drosophila*	GRP	Delays mitosis in response to inhibition of DNA replication	Fogarty *et al.*, 1997
Checkpoints responsive to DNA replication	Human	p53	Prevents G1 to S-phase transition after aberrant mitosis	Casenghi *et al.*, 1999
			Prevents DNA re-replication	Di Leonardo *et al.*, 1997
Genes homologous to yeast	Mouse	HUS1	Function in checkpoint control to be determined	Weiss *et al.*, 1999
DNA replication checkpoint genes		RAD1	Biological function as checkpoint remains to be determined	Parker *et al.*, 1998a
Genes homologous to yeast	Mouse	RAD17	Shares sequence homology with *S. pombe*	Bluyssen *et al.*, 1999
DNA replication checkpoint genes			Rad 17 protein: function in cell cycle checkpoints to be determined	
Genes homologous to yeast	Human	RAD17	Shares sequence homology with *S. pombe*	Parker *et al.*, 1998b
DNA replication checkpoint genes			Rad 17 protein: function in cell cycle checkpoints to be determined	Bluyssen *et al.*, 1999
		RAD1	Shares sequence homology with *S. pombe* rad1+ checkpoint	Parker *et al.*, 1998a Marathi *et al.*, 1998
		RAD9	Shares sequence homology with *S. pombe*	
			Functions as a checkpoint for a block in replication	Lieberman *et al.*, 1996

Table 8. Checkpoints responsive to DNA damage

Checkpoints	Species	Gene/gene product	Function	Study
Checkpoints responsive to DNA damage	*Drosophila*	GRP	Involved in checkpoint pathway for DNA damage	Fogarty *et al.*, 1997
Checkpoints responsive to DNA damage	Human	RAD9 RAD1 HUS1	Form a protein complex that functions as checkpoint for DNA damage	Volkmer and Karnitz, 1999
Checkpoints responsive to DNA damage	Human	p53	Arrests cell cycle at both the G1 and G2/M-phase	Agarwal *et al.*, 1995
Checkpoints responsive to DNA damage	Human	RAD1	Involved in checkpoint control after DNA damage	Freire *et al.*, 1998 Parker *et al.*, 1998b
Genes homologous to yeast DNA damage checkpoints	Mouse	HUS1	Function in checkpoint control to be determined	Weiss *et al.*, 1999
Genes homologous to yeast DNA damage checkpoints	Mouse	RAD17	Share homology with *S. pombe* rad 17+ checkpoint gene	Bluyssen *et al.*, 1999
Genes homologous to yeast DNA damage checkpoints	Human	RAD17	Share homology with *S. pombe* rad 17+ checkpoint gene	Parker *et al.*, 1998b Bluyssen *et al.*, 1999

Checkpoints responsive to cell size

Human oocytes must reach a critical size before they gain the ability to progress from the G2 to the M-phase. Whether the inability of small oocytes to progress through the G2-phase to M-phase is due to checkpoints responsive to cell size is unclear. Studies by Canipari *et al.* (1984) indicate that it is cell maturity rather than cell size that may govern meiotic progression. Alternatively, the inhibition of meiotic maturation in small oocytes may be due to a deficiency in products required for meiotic progression.

Checkpoints responsive to DNA replication

Genes have been identified that function as checkpoints for DNA damage and arrest, or delay cell cycle progression until DNA replication is complete (Table 7). Fulka *et al.* (1994, 1995a) demonstrated that the G2 to M-phase transition in murine oocytes is not influenced by the presence of unreplicated or replicating DNA. Although the genes associated with the checkpoint monitoring DNA replication have not been investigated directly in mammalian oocytes, current data indicate that the replication

Table 9. Spindle associated checkpoints

Checkpoints	Species	Gene/gene product	Function	Study
Spindle sensitive checkpoint	*Drosophila*	BUB3	Associates with BUB1 to form a multi-protein spindle assembly checkpoint	Basu *et al.*, 1998
Spindle sensitive checkpoint	*Drosophila*	BUB1	Demonstrates that BUB1 is an important component of spindle checkpoint in higher eukaryotes	Basu *et al.*, 1999
Spindle sensitive checkpoint	Mouse	BUB1	Checkpoint response to spindle damage	Taylor and McKeon, 1997
	Mouse	p53	Checkpoint ensures the maintenance of diploidy	Cross *et al.*, 1995
			Monitors disruption of the mitotic spindle	Lanni and Jacks, 1998
Spindle sensitive checkpoint	Human	MAD2	Monitors spindle–kinetochore attachment	Li and Benezra, 1996
	Human	hBUB1 and hBUBR1 kinases	Component of spindle checkpoint pathway	Ouyang *et al.*, 1998
			Monitor kinetochore–microtubule interactions	Jablonski *et al.*, 1998
			Function in checkpoint that monitors chromosome alignment	

responsive checkpoints that function during mitosis do not function during oocyte meiosis (Fulka *et al.*, 1994). On the basis of these findings it is possible that human oocytes also lack the checkpoints responsive to DNA replication.

Checkpoints responsive to DNA damage

Mammalian homologues to the yeast genes involved in the DNA damage checkpoints have been identified (Table 8) and are thought to participate in the same checkpoint functions as their yeast counterparts. Human oocytes have not been examined directly for the presence and function of these highly conserved DNA damage checkpoints. However, Fulka *et al*. (1997) demonstrated that the presence of damaged chromatin did not prevent the progression of meiosis in fused mouse oocytes. Therefore, it appears that DNA damage checkpoints may be either functionally inactive or absent during mammalian oocyte meiosis (Fulka *et al.*, 1994, 1997).

Spindle sensitive checkpoints

Gene homologues to the spindle-sensitive checkpoints of yeast have been isolated in somatic cells of higher species (Table 9). However, relatively few studies have examined the presence of spindle associated checkpoints in the meiotic cell cycle of oocytes (Fulka *et al.*, 1994, 1995a; LeMarie-Adkins *et al.*, 1997; Cross and Smythe, 1998). *Xenopus* oocytes were shown to contain a spindle assembly checkpoint, part of which was p42 MAP kinase (Cross and Smythe, 1998). Fulka *et al*. (1994, 1995b) demonstrated the presence of checkpoints in murine M-phase oocytes that can delay or accelerate meiotic progression, but LeMarie-Adkins *et al*. (1997) found that the checkpoint mechanism that functions at the metaphase to anaphase transition was absent in mouse oocytes.

Molecular control of oocyte maturation

Maturation promoting factor/M-phase promoting factor (MPF)

In addition to checkpoint controls, cell cycle progression is also regulated by endogenous products. The most common and widely studied factor in the area of cell cycle progression is maturation promoting factor (MPF). The original research by Masui and Markert (1971) led to the discovery that an activity in the cytoplasm of mature amphibian oocytes could induce meiosis when injected into G2 arrested frog oocytes. This activity was found to be MPF and it is now widely accepted that MPF is the primary molecule involved in meiotic cell cycle progression and the factor responsible for M-phase induction in all eukaryotic cells.

MPF is a serine–threonine kinase protein heterodimer composed of a catalytic subunit, $p34^{cdc2}$, and a regulatory subunit, cyclin B (Labbe *et al.*, 1989; Pines and Hunter, 1989; Gautier *et al.*, 1990). During the cell cycle, MPF activity is regulated by the phosphorylation–dephosphorylation of $p34^{cdc2}$ and its association with cyclin B (Clarke and Karsenti, 1991). Specifically, MPF activation involves the association of cyclin B and $p34^{cdc2}$ and selective dephosphorylation of $p34^{cdc2}$ on two residues, tyrosine 15 (Tyr15) and threonine 14 (Thr14) (Gautier *et al.*, 1988, 1990; Dunphy and Newport, 1989). This dephosphorylation is mediated by the *cdc25* and *nim1* genes (Russell and Nurse, 1986; Gautier *et al.*, 1991). In oocytes, MPF activation occurs initially at the G2 to M-phase transition. However, before the induction of M-phase, $p34^{cdc2}$ and cyclin B are present as pre-MPF, a bound and $p34^{cdc2}$ phosphorylated protein heterodimer, and are present in this inactivated state because of interaction with the *WEE1* gene (Parker *et al.*, 1991) (Fig. 6).

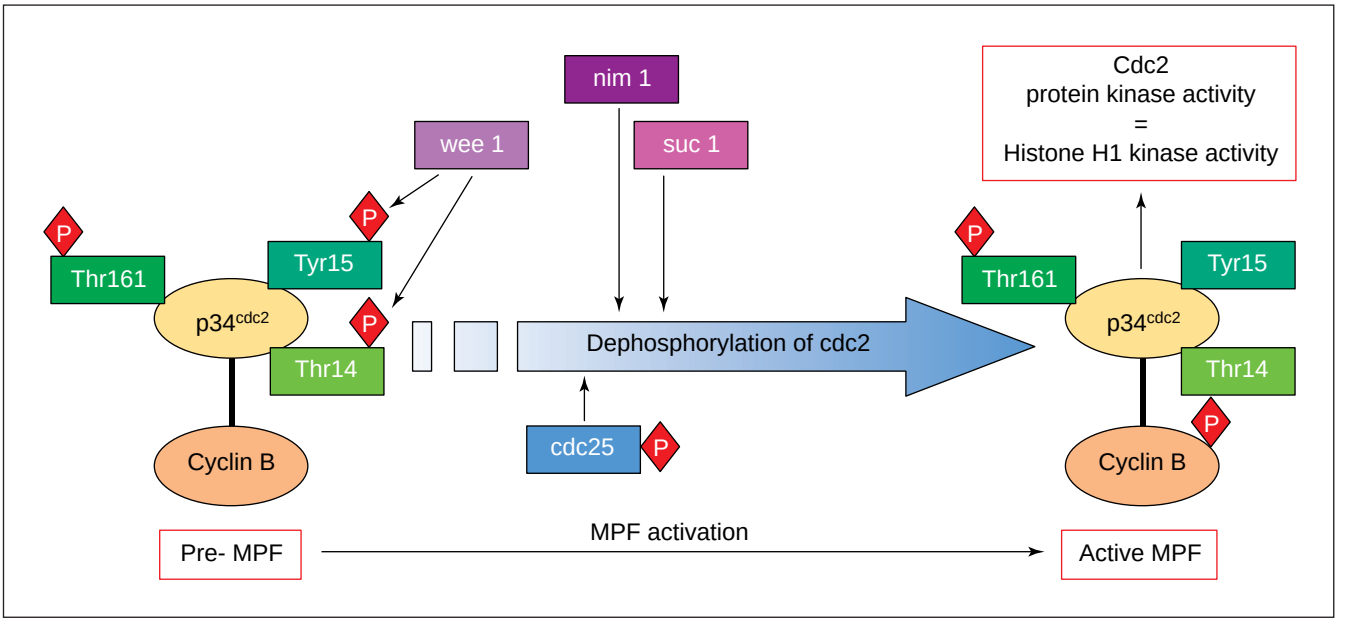

Fig. 6. Diagrammatic representation of the molecular structure and activation of maturation promoting factor (MPF). MPF is shown as a heterodimer composed of $p34^{cdc2}$ and cyclin B. In its pre-MPF state, the MPF heterodimer is phosphorylated on tyrosine 15 (Tyr15) and threonine 14 (Thr14). Threonine 14 and tyrosine 15 phosphorylation is due to the action of hypo-phosphorylated wee1. Then activation of MPF is primarily due to the interplay between cdc25 and nim1 and wee1. The phosphorylated form of cdc25 is responsible for dephosphorylating the cdc2 protein on Tyr15 and Thr14, thus activating MPF. Hypo-phosphorylated wee1 prevents MPF activation by maintaining the Tyr15 and Thr14 residues of the cdc2 protein in a phosphorylated form. Nim1 maintains wee1 in a hyper-phosphorylated form that is unable to phosphorylate the Tyr15 and Thr14 residues of the cdc2 protein and thereby prevents wee1 from holding MPF in a phosphorylated and inactive form. The active MPF molecule is dephosphorylated on Tyr15 and Thr14 of the cdc2 protein and the cyclin protein is phosphorylated. The active form of MPF displays cdc2 kinase activity, known as histone H1 kinase activity.

Cyclic MPF activity is a characteristic feature of oocytes and is attributable to the periodic activation and inactivation of the MPF heterodimer. MPF activity is detected just before, or concomitantly with, GVBD. MPF activity increases until metaphase I and decreases during the anaphase to telophase transition. The activity increases again until the oocyte reaches metaphase II, and is maintained at a high level by the interaction of cytostatic factor (CSF) and the viral oncogene c-mos until fertilization. Inactivation of MPF results in exit from metaphase, and during oocyte maturation MPF inactivation occurs at the transition of metaphase I to anaphase I and again at fertilization. $P34^{cdc2}$ phosphorylation (Dorée et al., 1989) and cyclin degradation (Murray et al., 1989) are both required for MPF inactivation. However, despite the fact that both events are required for the cessation of M-phase, the proteolytic destruction of cyclin B is seen as the principal event associated with the inactivation of MPF activity (Glotzer et al., 1991).

Cyclin destruction requires the presence of intact microtubules and occurs via ubiquitin-mediated proteolysis, a process that involves the attachment of ubiquitin molecules to the cyclin and is mediated in oocytes by three enzymes, E1, E2-C and E3 (Hershko *et al.*, 1994). The ubiquinated cyclin is presented to the proteasome for degradation. The factors that activate the cyclin proteolytic pathway have not yet been fully elucidated. However, it has been demonstrated that cyclin degradation can be triggered by cdc2 (Felix *et al.*, 1990; Hershko *et al.*, 1994). Alternatively, the calcium, calmodulin, CaM kinase II pathway presents itself as an alternative candidate for initiating cyclin degradation because the calcium ionophore has been demonstrated to activate the proteasome (Kawahara and Yokosawa, 1994) and CaM kinase II activates cyclin destruction and mos and CSF degradation (Whitaker, 1996). The calcium, calmodulin, CaM kinase II pathway probably represents the mechanism by which MPF is inactivated at fertilization.

MPF is activated *in vivo* at the onset of oocyte maturation in response to an endogenous LH surge. Mammalian oocytes resume meiotic maturation spontaneously *in vitro* upon liberation from the ovarian follicle. Our studies show that *in vitro* maturation of human oocytes activates MPF activity, as evidenced by the increase in histone H1 kinase activity at metaphase II (Fig. 7). This finding demonstrates that MPF is activated *in vitro* in a manner similar to that for oocytes *in vivo*.

c-mos

c-mos is a proto-oncogene originally identified as the transforming gene of the Moloney murine sarcoma virus

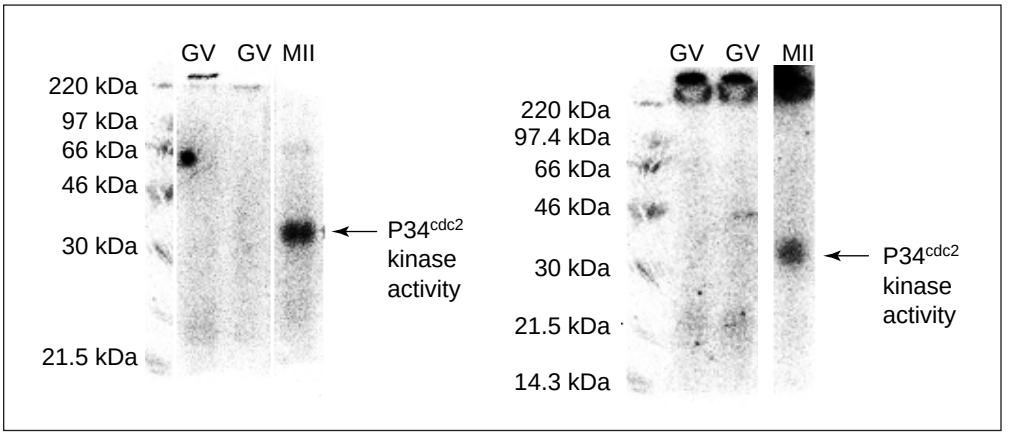

Fig. 7. Maturation promoting factor (MPF) activity in single germinal vesicle stage (GV) and metaphase II stage (MII) human oocytes. The oocytes were retrieved from women who had not received exogenous gonadotrophin treatment and the metaphase II oocytes were matured *in vitro*.

(Frankel and Fischinger, 1976). The c-*mos* proto-oncogene encodes a serine–threonine protein kinase (mos), which is expressed in oocytes. In the oocyte, mos is involved in several aspects of oocyte maturation. Mos has been demonstrated to phosphorylate cyclin B (Roy *et al.*, 1990) and thereby affects cyclin stability (O'Keefe *et al.*, 1991; Xu *et al.*, 1992) and MPF activity. As part of the CSF complex, mos is involved in the maintenance of metaphase II arrest in mammalian oocytes and similarly, in *Xenopus* oocytes, mos has been implicated in the pathway that is involved in metaphase arrest (Kosako *et al.*, 1994). In *Xenopus*, murine and bovine oocytes, mos activates mitogen activated protein (MAP) kinase (Nebreda and Hunt, 1993; Fissore *et al.*, 1996; Verlhac *et al.*, 1996). In addition, mos initiates and regulates meiotic maturation in *Xenopus* oocytes (Sagata *et al.*, 1988; Roy *et al.*, 1996) and has been shown to participate in, but is not essential for, murine oocyte maturation (O'Keefe *et al.*, 1989; Araki *et al.*, 1996). However, it is important for murine oocyte spindle and chromosome morphology, assembly and function (Zhao *et al.*, 1991; Verlhac *et al.*, 1996).

Mos has been detected in human oocytes (Pal *et al.*, 1994; Heikinheimo *et al.*, 1995, 1996) and its expression is restricted to the oocyte, as both the mos protein and mRNA are degraded during embryonic development (Heikinheimo *et al.*, 1995). Although the action of mos during human oocyte maturation *in vitro* has not been investigated directly, it is likely to play a role in the regulation of meiotic maturation by interacting with cyclin B and CSF to stabilize MPF and maintain meiotic arrest, as well as participate in the activation of MAP kinase.

Mitogen activated protein (MAP) kinase

MAP kinase, which is alternatively known as extracellular regulated kinase (ERK), is a serine–threonine kinase that is activated via a protein kinase cascade at the onset of oocyte maturation in *Xenopus* (Haccard *et al.*, 1990), mouse (Verlhac *et al.*, 1993) and pig (Inoue *et al.*, 1995) oocytes. MAP kinase activity (ERK) is associated with the induction of meiosis in *Xenopus* oocytes (Haccard *et al.*, 1995) and although activated at the onset of oocyte maturation in mice, MAP kinase is not necessarily required for GVBD in mouse oocytes (Sun *et al.*, 1999a). However, MAP kinase activity is associated with a plethora of cytoplasmic events including the regulation of microtubule dynamics, spindle assembly and chromosome condensation (Verlhac *et al.*, 1993, 1994; Dedieu *et al.*, 1996). In most oocytes (clam, starfish and ascidian oocytes are the notable exceptions), high concentrations of MAP kinase are maintained during meiotic progression to metaphase II and concentrations decrease after fertilization (Ferrell *et al.*, 1991; Dedieu *et al.*, 1996).

In human oocytes, p42ERK2 is the main form of MAP kinase (Sun *et al.*, 1999b). The activation pattern of MAP kinase in human oocytes is reminiscent of MAP kinase activity in other mammalian species such as pigs and mice. In humans, MAP kinase is inactive in immature oocytes, active in mature oocytes and the activity decreases after pronuclei formation after fertilization (Sun *et al.*, 1999b). Therefore, although not widely studied in human oocytes, it appears that the MAP kinase pattern of activation in the cell cycle is similar to that of other mammalian species and, therefore, MAP kinase may serve a similar function during human oocyte maturation.

Transcription and translation

During the period of oocyte growth a large number of genes are transcribed and translated (Schultz, 1986). Protein translation actively continues during oocyte maturation and the accumulated transcripts and macromolecules are subsequently used for meiotic and early embryonic developmental events (Wickramasinghe and Albertini, 1993;

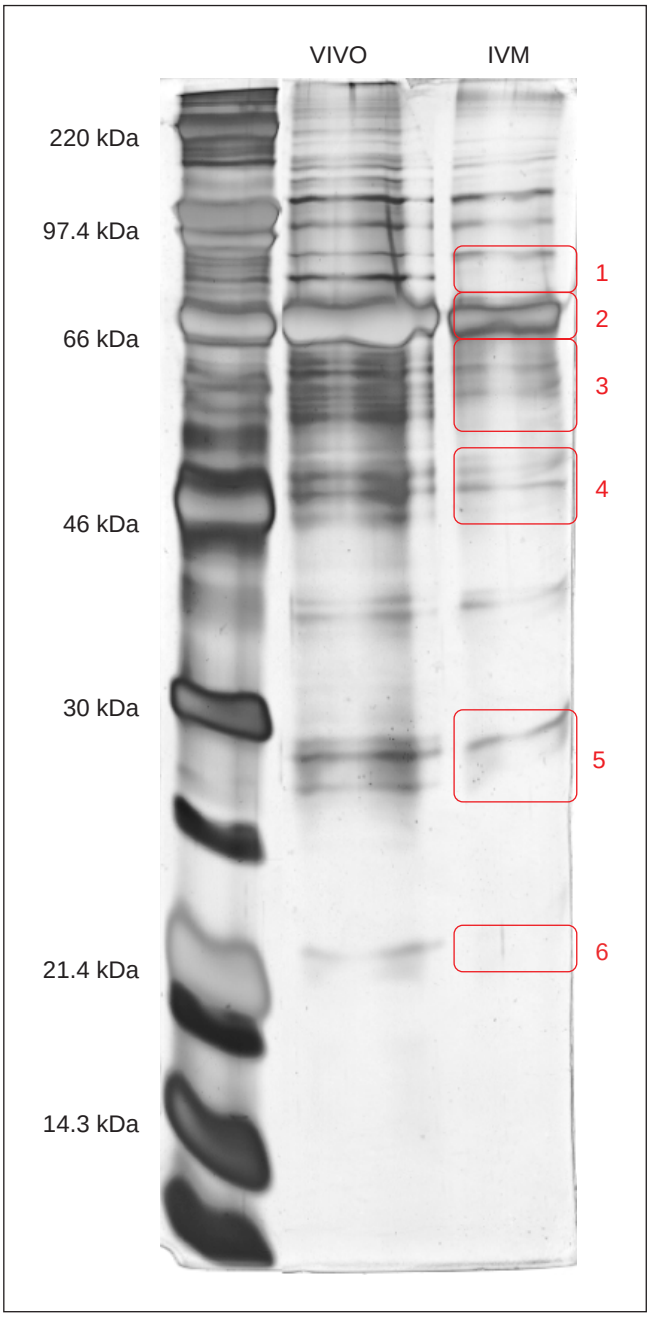

Fig. 8. One-dimensional 12% SDS-polyacrylamide gel of human oocyte proteins stained with silver nitrate. Each lane contains a single denuded *in vitro* matured (IVM) or *in vivo* matured (VIVO) human oocyte. Areas 1, 3, 4, 5 and 6 represent regions in which there appears to be an absence of particular proteins compared with the VIVO group.

Fair *et al.*, 1995). Thus, it is commonly accepted that transcription and translation play an integral role in meiotic progression and subsequent embryo developmental competence.

Although it is clear that immature human oocytes retrieved from unstimulated ovaries can mature from the GV stage to metaphase II *in vitro*, their subsequent embryonic development appears to be severely compromised (Trounson *et al.*, 1994, 1996, 1998; Barnes *et al.*, 1996; Moor *et al.*, 1998). It has been suggested that the reduced developmental potential in human oocytes matured *in vitro* may be attributable to sub-optimal culture conditions, incomplete oocyte growth or abnormal cytoplasmic maturation (Moor *et al.*, 1998).

Studies on protein content have revealed that *in vitro* matured metaphase II human oocytes, derived from ovaries of untreated women (IVM group), display a reduced protein content compared with *in vivo* matured metaphase II oocytes retrieved from ovaries of gonadotrophin treated patients (VIVO group). The one-dimensional protein profiles of single human metaphase II stage oocytes from the IVM and VIVO groups following silver staining are presented (Fig. 8). The human IVM oocytes have a reduced protein content compared with the VIVO group. Areas 1, 3, 4, 5 and 6 represent regions in which there appears to be an absence of particular proteins. Isoelectric focusing and SDS-PAGE were used in combination to isolate and identify further variations in particular oocyte proteins. When the protein profiles of IVM and VIVO human oocytes were examined by two-dimensional SDS-PAGE there were nine specific human oocyte proteins expressed in the VIVO group that were not detected in the IVM group. A number of proteins cannot be detected in human oocytes matured *in vitro* and these are likely to include molecules essential for cell cycle regulation and normal embryo development. A reduction in oocyte protein content after *in vitro* maturation has also been reported in bovine oocytes (Kastrop *et al.*, 1991). Whether these protein deficiencies are attributable to transcriptional inadequacies or translational defects is currently unknown.

Measurements of protein synthesis (Schultz *et al.*, 1979; Salustri and Matrinozzi, 1983) reveal that oocytes cannot synthesize all the proteins they require during the growth phase (Schultz, 1986). Part of the protein content of the oocyte may be obtained from proteins taken up from serum (Glass, 1971). Alternatively, the follicular cells surrounding the oocyte could contribute to the protein content via a gap junction mediated transfer of molecules between the granulosa cells and the oocyte. Premature liberation of oocytes from small growing follicles may prevent the accumulation of follicular fluid proteins or cumulus cell transfer of proteins essential for developmental competence.

The available data indicate that *in vitro* matured human oocytes have a reduced protein content and the isolation and identification of the specific proteins that are either absent or at lower than required concentrations are needed.

Control of progression of maturation in vitro and developmental competence

Human oocytes appear to be unsynchronized in their response to maturational signals *in vitro*. This proposal is

best demonstrated by the data reported by Trounson *et al.* (1994, 1998). A small proportion of oocytes (15–20%) will extrude the first polar body by 24 h of culture, and by 43–47 h, 60% have matured to metaphase II. Similar data have been reported by Cha and Chian (1998) for oocytes obtained from untreated PCOS patients (Fig. 3). As discussed earlier, the factor responsible for the resumption of meiotic maturation is MPF and its associated $p34^{cdc2}$ kinase activity is responsible for the cascade of events associated with meiotic maturation. The serine–threonine protein kinase inhibitor 6-dimethylaminopurine (DMAP) blocks GVBD and cell cycle progression in immature oocytes by inhibiting the post-translational dephosphorylation of $p34^{cdc2}$ that triggers MPF activity (Jessus *et al.*, 1991), but does not interfere with protein synthesis (Rime *et al.*, 1989; Fulka *et al.*, 1991).

Studies by Anderiesz *et al.* (2000b) have shown that DMAP in culture medium will reversibly block GVBD of mouse and human oocytes for 7 and 24 h, respectively. Removal of DMAP and subsequent culture in medium without DMAP results in the same timing of formation of metaphase II oocytes as the untreated controls (Anderiesz *et al.*, 2000b). It was hypothesized that DMAP treatment may synchronize nuclear maturational events in oocytes but there was no evidence of this effect in either mouse or human oocytes. DMAP treatment of human oocytes had no effect on fertilization or development to the blastocyst stage but decreased the developmental capacity of mouse embryos.

Prospects for the future application of human oocyte maturation in vitro

The recovery and maturation of oocytes from PCOS patients remains a very suitable alternative to surgery or diathermy of ovaries, for the establishment of pregnancy in these infertile women. It is apparent that large numbers of oocytes can be recovered by ultrasound-guided follicular aspiration and a reasonable pregnancy rate can be achieved when large numbers of unselected pronuclear or early cleavage stage embryos are transferred to the patients (Cha and Chian, 1998). The selection of the more developmentally competent embryos by extended culture (day 3 to day 6) and preimplantation genetic diagnosis to remove embryo aneuploidies would reduce the number of embryos transferred but still retain an acceptable pregnancy rate. Clearly, improvements can be made to the maturation conditions for retention of the potential developmental competence of immature oocytes and this aim should continue to be a priority for research on oocyte maturation. The benefits for PCOS patients is to avoid the need to administer fertility drugs to induce superovulation for mature oocyte collection and IVF. PCOS patients are susceptible to OHSS (Danninger and Feichtinger, 1997) and recovery of immature oocytes and their maturation *in vitro* remains a very attractive treatment strategy for pregnancy.

The recovery of immature oocytes from patients who have been treated with high doses of gonadotrophins for IVF and in whom administration of hCG has been purposefully withheld is attractive because LH and hCG are potent inducers of vascular endothelial growth factor (VEGF), which is closely related to the initiation and exacerbation of OHSS. Follicular aspiration and recovery of immature oocytes has already been successful in such a case (Jaroudi *et al.*, 1997). This option could be adopted in clinical IVF to prevent the occurrence of OHSS.

It is also beneficial to recover oocytes from small non-dominant follicles in natural ovulatory cycles, in patients that have had minimal ovarian stimulation with clomiphene citrate (Thornton *et al.*, 1998; this review) or in PCOS patients with irregular menstrual cycles given progesterone for withdrawal bleeding, hCG at 10–14 days after progesterone and from which oocytes are recovered 36 h later (Chian *et al.*, 1999a,b, 2000). Oocytes from these small follicles (down to 7 mm diameter) appear to be developmentally competent and may contribute to successful pregnancy and development to term. This will be a new application for *in vitro* maturation and is obviously clinically useful for IVF patients wishing to avoid treatment with large doses of FSH, or for patients for whom superovulation treatment poses an unacceptable financial burden.

In the cases of recovering immature oocytes from follicles of patients at the normal time of ovulation, uterine receptivity for implantation and development will be similar to that for normal IVF patients or naturally cyclic women. The conditions required for uterine receptivity in anovulatory PCOS patients is not known and the use of progesterone for withdrawal bleeding may enhance implantation rates after embryo transfer.

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