CHAPTER 18

Combination of FSH priming and hCG priming in IVM cycles

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INTRODUCTION

After birth, human oocytes remain at prophase I of meiosis until they are stimulated by gonadotropin to resume meiosis before ovulation. Throughout a woman's lifetime, only a few hundred oocytes will complete meiosis and maturation and be ovulated, while the majority of oocytes will undergo apoptosis. In conventional assisted reproductive technologies, ovarian stimulation is usually utilized to increase the number of available oocytes and embryos, and therefore the pregnancy rate. However, the use of stimulation drugs increases the patient's cost and suffering, and is associated with side-effects such as nausea, abdominal pain, mood swings, menopausal symptoms, ovarian hyperstimulation syndrome (OHSS), and a potential cancer risk. The recovery of immature oocytes followed by in-vitro maturation (IVM) and fertilization is an attractive alternative because it reduces the patient's cost and suffering and avoids the side-effects associated with ovarian stimulation.

Another application of IVM is preservation of women's fertility, especially for those who are going to undergo cancer treatment. Cryopreservation of immature oocytes or ovarian tissue, coupled with IVM, is a potential way to preserve fertility, although the successful cases are limited[1]. Immature oocytes can also be a new source of oocyte donation. Pregnancies resulting from immature oocyte donation have been reported from oophorectomy specimens[2], during cesarean section[3], and from woman with polycystic ovaries[4].

OOCYTE MATURATION

Oocyte maturation is a complex process that comprises nuclear maturation and cytoplasmic maturation. Nuclear maturation refers to the resumption of meiosis and progression from germinal vesicle breakdown (GVBD) to metaphase II (MII). Cytoplasmic maturation refers to the preparation of oocyte cytoplasm for fertilization and embryonic development[5]. GVBD is initiated by the preovulatory surge of LH. LH probably induces GVBD by an indirect action mediated by cumulus cells. The oocyte and the cumulus cells are coupled by gap junctions. Inhibitory factors are transported from the cumulus cells to the oocytes to maintain meiotic arrest of the oocytes. Some evidence suggests cyclic adenosine monophosphate (cAMP) as a potential inhibitor of meiotic resumption[6,7]. It was speculated that LH causes dissociation of the cumulus cells and the oocyte, and thus terminates the flow of the

meiosis-inhibiting substances into the oocyte[5]. It is also possible that LH induces the production of a GVBD-inducing signal in the cumulus cells that is subsequently transferred to the oocyte through the gap junctions[8]. Cytoplasmic maturation involves complicated processes that prepare the oocyte for activation, fertilization, and development. During this process, RNA molecules, proteins, and imprinted genes are accumulated in the cytoplasm to regulate oocyte meiosis and development[9]. Insufficient cytoplasmic maturation will fail to promote male pronucleus formation and will increase chromosomal abnormalities after fertilization[10].

HISTORY OF HUMAN IVM

In 1935, Pincus and Enzmann found that rabbit oocytes, when liberated from Graafian follicles, would undergo spontaneous meiotic maturation in vitro[11]. Edwards demonstrated in 1965 that human oocytes removed from follicles could mature in medium supplemented with serum[12]. The first human birth resulting from IVM was reported by Cha et al. in 1991[2]. They obtained immature oocytes from oophorectomy specimens. After maturation in vitro, the oocytes were donated to a woman of premature menopause, and a set of triplets was born. In 1994, Trounson et al.[13] reported the first birth from IVM in PCOS women, and he developed a special aspiration needle for immature oocyte retrieval. However, the maturation rate of human IVM was about 30–50%[2,14,15], which was much lower than that of other species, and the pregnancies resulting from IVM were limited. In addition, Trounson et al.[16] demonstrated that the in-vitro matured human oocytes had reduced developmental competence. The poor outcome of human IVM was thought to be at least partly due to abnormalities of cytoplasmic maturation in in-vitro matured oocytes. Several treatment modalities have been proposed to improve the outcome of human IVM.

FSH PRIMING

The early studies on human IVM did not use gonadotropin before oocyte retrieval. Since FSH acts on cumulus cells and promotes steroid production, oocyte RNA, and protein synthesis[17], it has been postulated that pretreatment with FSH might increase either the number of immature oocytes recovered or the maturation potential and developmental competence of the oocytes.

In a study performed on rhesus monkeys, FSH priming for 6–7 days enhanced nuclear and cytoplasmic maturation of oocytes in vitro[18]. In comparison to the non-stimulated monkeys, greater percentages of oocytes completed meiotic maturation (74% vs. 41%), were fertilized (85% vs. 61%), and cleaved to the two–four-cell stage embryos (79% vs. 38%) in the FSH-primed monkeys.

The effects of FSH priming on human IVM were contradictory. Gómez et al.[19], in a small series, found that only 16.7% of immature oocytes obtained from non-stimulated ovaries reached MII after 48 h. The percentages of immature oocytes from stimulated cycles (with HMG) that reached MII were 50% at 24 hours and 87.5% at 48 hours, which were comparable with animal studies. They speculated that the intrafollicular environment induced by FSH may be able to generate the protein synthesis involved in oocyte maturation.

Wynn et al. gave a truncated course of 600 IU FSH (300 IU on day 2, 150 IU on days 4 and 6) to normal women; after which, a significantly greater percentage of oocytes completed meiotic maturation in vitro (71.1% vs. 43.5%) and higher serum estradiol (E_2) concentrations on the day of oocyte retrieval were found after FSH treatment (1049 ± 241 pg/ml vs. 154 ± 17 pg/ml). Immature oocyte numbers and endometrial thickness were not significantly different[20].

Cha and Chian[5] demonstrated that the time courses of germinal vesicle breakdown (GVBD) and oocyte maturation were faster in the oocytes

retrieved from stimulated ovaries, although the final percentages of GVBD and MII oocytes were not different between stimulated and unstimulated ovaries. Mikkelsen and Lindenberg pretreated PCOS women with 150 IU FSH per day for 3 days from day 3, and they found that the maturation rate was significantly higher in the FSH-primed group (59%) compared with the non-primed group (44%). There were no significant differences in the rates of fertilization and embryo cleavage between the two groups[21]. However, in another similar study on normal cyclic women, FSH priming did not increase the number of oocytes obtained and did not improve the maturation and cleavage rates or embryo development[22]. Similarly, Trounson et al. found no significant differences in the number of oocytes recovered, maturation rate, fertilization rate, and embryo development in patients pretreated with 1 day or 3 days of 150 IU recombinant FSH compared to patients without treatment[16].

Jaroudi et al.[23] reported 21 IVM cycles in women who were at risk of OHSS (with too many follicles or high serum E_2 levels). The women were stimulated with gonadotropin-releasing hormone agonist (GnRHa) on long or short protocols. Oocyte retrieval was performed, without hCG injection, when leading follicles were <15 mm. No MII oocytes were obtained at oocyte retrieval. The maturation rate was 70.8%, and the fertilization rate was 58.7% with ICSI. However, only two pregnancies (9.5%) were obtained.

In a bovine study it was found that, although superovulation with FSH increased follicular size and decreased atresia, the oocytes were developmentally less competent[24]. It was speculated that superovulation forced the follicles into an accelerated growing phase, leaving the oocytes with insufficient time to acquire competence. In humans, it has also been found that HMG stimulation results in follicular asynchrony[25]. Therefore the role of FSH priming in IVM seems inconclusive and contradictory. Whether normal women and PCOS women respond differently to FSH priming is also unknown.

hCG PRIMING

In the final stages of follicular maturation, the LH surge initiates the continuation of meiosis in the oocyte and luteinization of the granulosa cells. Because of their structural similarity, hCG has been used in assisted reproduction to mimic the endogenous LH surge. Since the LH surge or hCG injection induces final oocyte maturation, it is tempting to give hCG before oocyte retrieval in IVM cycles.

In 1999, Chian et al.[26] reported a series of 25 IVM cycles in PCOS women. By giving 10 000 IU of hCG 36 h before oocyte retrieval, they obtained an impressive pregnancy rate of 40%. In their other randomized study to compare the outcome of IVM with and without hCG priming, the maturation process was faster in the hCG-primed group[27]. At the time of oocyte retrieval, GVBD had occurred in 46.2% of oocytes in the hCG-primed group, but in none in the non-hCG-primed group. The percentage of MII oocytes after 48 h of culture was higher in the hCG-primed group (84.3%) than in the non-hCG-primed group (69.1%), although the rates of fertilization and cleavage and embryo quality were similar between the two groups.

The mechanism of hCG priming in improving the outcome of IVM is not clear. It was hypothesized that follicles might possess hCG receptors to respond to hCG priming[28]. During ovarian stimulation, hCG is used to substitute for LH to induce final oocyte maturation. Besides inducing GVBD, LH also causes cumulus expansion by secreting a hyaluronic acid-rich proteoglycan matrix[29]. Indeed, we found that many cumulus–oocyte complexes (COC) obtained after hCG priming were class 3 (slight expansion in outer layers of cumulus) in Hazeleger's classification of bovine COC[30], which was shown to have the highest developmental rate[31]. This is in contrast

to our previous experience of IVM without hCG priming, in which most of the COC were cumulus compact. This implies that hCG can initiate the oocyte maturation process in small follicles.

Trounson et al.[32] questioned whether the oocytes obtained after hCG priming were really 'immature' since they have demonstrated that mature oocytes could be obtained even from 6-mm follicles. However, no oocytes obtained at oocyte retrieval were MII in Chian's studies and in our experience. Barnes et al. found that the maturation, fertilization, and cleavage rates were higher in women with regular cycles than in PCOS women. They thought the reason may be related to elevated androgen levels in follicular fluid in PCOS women[33]. It has also been shown with murine oocytes that testosterone significantly reduces their ability to mature and undergo normal embryonic development[34]. However, in the study of Child et al.[35] using hCG priming before oocyte retrieval, although the maturation rate at 24 h was higher in women with normal ovaries than in PCOS women, by 48 h the rates of maturation were similar. The fertilization and cleavage rates were also similar between the two groups. It seems therefore that hCG plays a more important role in oocyte maturation than FSH priming, and can overcome the deleterious effect of androgens.

COMBINATION OF FSH AND hCG PRIMING

Since Chian et al.[26,27] proposed hCG priming for IVM in PCOS patients, we followed their protocol and we obtained several pregnancies. However, if IVM is to be applied to regularly cycling women, a major limitation is the small number of antral follicles available. It has been shown that the pregnancy rate of IVM is correlated with the number of immature oocytes retrieved, with the highest in those with >10 immature oocytes[36]. Although Child et al.[35] obtained an average of 5.1 (± 3.7) immature oocytes in normal, cyclic women, we rarely obtained more than 2 oocytes in these women. Futhermore, a thin endometrium (<7 mm) found in some PCOS women, which may be associated with a reduced pregnancy rate. In order to see if gonadotropin would stimulate follicular growth or enhance the growth of endometrium, we gave small doses of rFSH (Gonal-F, 75 IU per day, for 3 to 6 days) to 10 PCOS women whose endometrium was <7 mm on day 9. The endometrium thicknesses before and after rFSH stimulation were 5.2 mm and 7.9 mm, respectively. Two out 10 women became pregnant (20.0%). We then performed a randomized study on PCOS women in whom 35 cycles were pretreated with 75 IU of rFSH for 6 days and 33 cycles were not[37]; 10 000 IU of hCG was given 36 hours before oocyte retrieval. The overall maturation rate, fertilization rate, and pregnancy rate were 74.2%, 72.8%, and 33.8%, respectively. As shown in Table 18.1, serum E_2 level on the day of hCG injection was higher in the FSH-primed group, but the maturation rates, fertilization rates, and pregnancy rates were similar between the two groups. The numbers of oocytes obtained and endometrial thicknesses were also similar. It was concluded that, with hCG priming, FSH priming had no additional beneficial effect on IVM[37]. It should be noted, however, that this study was conducted on PCOS women. Whether the combination of FSH priming and hCG priming will improve the outcome of IVM in normal women is not known.

It has been shown in cows that withdrawing FSH stimulation before oocyte pick-up creates a 'coasting' period that provides a favorable follicular microenvironment for the oocyte to complete final maturation[38,39]. An interesting experiment was performed by Blondin et al.[40] in cows, in which FSH stimulation and different 'coasting' periods were compared. With four injections of FSH (200 IU in total) and 33 h of coasting, administration of LH 6 h before oocyte retrieval increased the percentage of blastocysts and the embryo production rate on days 7 and 8. However, if a 48-h coasting period was used,

Table 18.1 Clinical variables and outcome of FSH-priming and non-FSH-priming groups[37]

	FSH priming	*Non-FSH priming*	*p*
No of cycles	35	33	
Age (years)	30.1 ± 2.8	31.3 ± 4.1	NS
Day 3 FSH (mIU/ml)	5.10 ± 1.43	5.54 ± 1.55	NS
Day 3 LH (mIU/ml)	12.46 ± 7.37	11.63 ± 6.61	NS
E_2 on day of hCG (pg/ml)	102.78 ± 98.58	39.17 ± 14.52	0.001
Endometrial thickness on day of hCG (mm)	8.09 ± 1.49	7.77 ± 1.03	NS
No of immature oocytes per patient	21.9 ± 9.4	23.1 ± 11.0	NS
Mean MII oocytes	16.7 ± 7.6	16.6 ± 5.8	NS
Maturation rate at 24 h	43.2%	39.2%	NS
Maturation rate at 48 h	76.5%	71.9%	NS
2PN oocytes per patient	12.7 ± 6.2	11.6 ± 4.6	NS
Fertilization rate	75.8%	69.5%	NS
Cleavage rate	89.4%	88.1%	NS
No of transferred embryos	3.8 ± 1.0	3.8 ± 0.9	NS
Pregnancy rate	31.4%	36.4%	NS
Implantation rate	9.7%	11.3%	NS

LH injection did not affect the rates of blastocyst or embryo production on days 7 and 8. The best results were obtained when the cows received six doses of FSH (300 IU in total) with 48-h coasting, and the administration of LH did not affect the rates of blastocyst production. The results suggested that with four injections of FSH and 33-h coasting, follicles were still in the growing phase, so extending the coasting period to 48 h and LH administration allowed oocytes to acquire developmental competence in vivo. A standard FSH stimulation protocol and 48-h coasting, in association with LH administration, creates an optimal follicular environment for oocyte maturation. These results seem to contradict our study. In our study, rFSH was given from day 3 to day 8, and oocyte retrieval was performed after day 10, so there were at least 48 h of coasting. But we found that the combination of FSH priming and hCG priming is no better than hCG priming alone. It is not known if extended stimulation with FSH would be more helpful, but prolonged ovarian stimulation will offset the major benefit of IVM – namely avoiding the use of gonadotropins.

In our center, we use the traditional double-lumen aspiration needle (K-OPSD-1735-ET; Cook, Australia) for oocyte retrieval instead of the special aspiration needle for immature oocytes (K-OPS-1235-Wood; Cook, Australia) designed by Trounson et al.[13]. Because the COCs are tenacious and detachment from the follicle wall may be difficult, we always flush every follicle until a COC is found, or three times at most. Table 18.2 shows the mean numbers of COCs obtained in different studies. There is a trend to obtain more

COCs in PCOS women than in regularly cycling women. We obtained more COCs than other studies, probably because of follicular flushing. As shown in Tables 18.1 and 18.2, FSH priming does not increase the number of oocytes recovered. Mikkelsen et al. also found that extending FSH stimulation (150 IU/day) from 3 days to 6 days did not increase the number of oocytes obtained[22].

IVM IN STIMULATED CYCLES

IVM with FSH priming and hCG priming is similar to 'rescue' IVM in conventional IVF cycles, in which gonadotropin and hCG are used before oocyte recovery. After ovarian stimulation with gonadotropin and hCG, about 15% of oocytes are found in GV or MI stage at the time of oocyte retrieval. Veeck et al.[41] reported two pregnancies out of 15 cases resulting from transfer of in-vitro matured oocytes from stimulated cycles, but one of them ended up as a miscarriage. Nagy et al.[42] reported a birth resulting from in-vitro matured GV oocytes from a woman in whom hCG was injected when the leading follicles were only 16 mm. Jaroudi et al.[43] reported a pregnancy resulting from IVM to prevent OHSS. In that case, 10 immature oocytes were obtained, without hCG injection, when the leading follicles were 13 mm. Unfortunately, the pregnancy was lost at 24 weeks. The outcome of rescue IVM in stimulated cycles, however, is very poor, and only limited cases have been reported[23,41–43].

The major difference of IVM and 'rescue IVM' is the timing of oocyte retrieval. In conventional IVF, hCG is given when the leading follicles reach 18 mm and the serum E_2 level is adequate. On the contrary, in the IVM program, we always retrieve oocytes before the leading follicles reach 12 mm. Cobo et al.[44] demonstrated that if follicles were aspirated when a dominant follicle was >10 mm, there was a significant decrease in the rate of oocyte retrieval (50.5%, compared to 70.8% when the follicle was <10 mm). Maturation rates and fertilization rates were similar between the

Table 18.2 Mean numbers of COCs obtained in various studies

Authors	*Patients*	*Priming*	*Mean no of COCs*
Trounson et al.[13] (1994)	PCOS	—	15.3
Wynn et al.[20] (1998)	Normal	FSH	8.9
Mikkelsen et al.[22] (1999)	Normal	—	3.7
Mikkelsen et al.[22] (1999)	Normal	FSH	4.0
Chian et al.[27] (2000)	PCOS	hCG	7.8
Cha et al.[57] (2000)	PCOS	—	13.6
Smith et al.[58] (2000)	Normal	—	5.6
Child et al.[35] (2001)	PCOS	hCG	11.3
Child et al.[35] (2001)	Normal	hCG	5.1
Mikkelsen and Lindenberg[21] (2001)	PCOS	FSH	7.5
Du et al.[54] (2004)	PCOS	hCG	11.4

COC, cumulus–oocyte complex

two groups. However, development to blastocyst stage was also lower in the group in which the follicle was >10 mm. Russell found a dramatic decrease in the rates of maturation, fertilization, and transfer of embryos among cycles in which immature oocytes were retrieved when a dominant follicle (>14 mm) was present at the time of oocyte retrieval[45].

It is generally thought that after dominant follicles have formed, the secondary follicles will become atretic. However, little is known about how dominant follicles affect the developmental potential of oocytes in the smaller follicles, and several recent studies refute this notion. Smith et al. showed that in cattle the developmental competence of oocytes from small antral follicles is not adversely affected by the presence of a dominant follicle[46]. Chian et al.[47] demonstrated with bovine ovaries that, although the number of oocytes obtained in the early follicular phase (before dominant follicles have formed) was higher than those from the late follicular and luteal phases, the rates of maturation and fertilization and embryo cleavage were not significantly different.

In humans, Thornton et al.[48] reported a series of IVM in natural-cycle IVF. Ovulation was triggered with 10 000 IU of hCG when follicle maturity was achieved. After 24 h, 32% GV oocytes matured in standard culture medium, and 30% GV oocytes matured in 50% follicular fluid. The fertilization rates were 62% and 77%, respectively, and two pregnancies resulted from the transfer of embryos derived from immature oocytes. Chian et al.[49] reported three pregnancies resulting from natural-cycle IVF combined with IVM. At the time of oocyte retrieval, the largest follicles were 14–19 mm, but the immature oocytes obtained could still be matured in vitro and produce embryos. These reports also suggest that the maturational and developmental competence of immature oocytes may not be affected by the presence of dominant follicles.

The GV oocytes obtained from superovulated ovaries are different from the GV oocytes in IVM cycles. Nogueira et al.[50] found that the in-vitro matured oocytes from stimulated cycles had a 21% incidence of non-cleavage after fertilization, and chromosomal anomalies were found in 78.5% of embryos analyzed. However, the incidence of aneuploidy or chromosome aberration in in-vitro matured oocytes from unstimulated cycles was about 20%[51,52], which was similar to that reported for in-vivo matured oocytes after gonadotropic stimulation in IVF cycles[51,53]. Besides, the approximate 200 babies after IVM did not show increased anomalies[28,37,54,55].

We think the GV oocytes from stimulated cycles behave differently from the GV oocytes in IVM cycles (either with or without FSH and hCG priming). The GV oocytes obtained from stimulated ovaries had a lower fertilization rate even with ICSI[56], and the resulting embryos had a high incidence of cleavage arrest and chromosome anomalies[50]. The problem does not lie in the dominant follicles, since there is no solid evidence to show that the formation of dominant follicles adversely affects the GV oocytes from smaller follicles. The oocytes remaining at the GV stage in spite of ovarian stimulation may be of inferior quality or there may be an intrinsic defect in the oocytes or follicles.

CONCLUSIONS

Human chorionic gonadotropin priming initiates oocyte maturation in vivo and produces a favorable outcome in IVM. The role of FSH priming is controversial. The combination of FSH priming and hCG priming does not produce additional benefit over hCG priming alone.

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