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ARTICLE

Effect of incubation with different concentrations and durations of FSH for in-vitro maturation of murine oocytes

Yu-Hung Lin [a,b,c,d], Jiann-Loung Hwang [a,d,*], Kok-Min Seow [a,e], Lee-Wen Huang [a,b], Bih-Chwen Hsieh [a,b], Hen-Ju Chen [a,b], Chii-Ruey Tzeng [d], Chyi-Huey Bai [f]

[a] *Department of Obstetrics and Gynecology, Shin-Kong Wu Ho-Su Memorial Hospital, No. 95, Wen Chang Road, Shih Lin District, Taipei, Taiwan;* [b] *School of Medicine, Fu Jen Catholic University, Taipei, Taiwan;* [c] *Department of Obstetrics and Gynecology, National Taiwan University Hospital, Taipei, Taiwan;* [d] *Department of Obstetrics and Gynecology, Taipei Medical University, Taipei, Taiwan;* [e] *Department of Obstetrics and Gynecology, National Yang-Ming University, Taipei, Taiwan;* [f] *School of Public Health, Taipei Medical University, Taipei, Taiwan*

[*] Corresponding author. *E-mail address:* M002179@ms.skh.org.tw (J-L. Hwang).

Yu-Hung Lin graduated from the School of Medicine, National Taiwan University in 1991. After completing his residency training at the National Taiwan University Hospital, he served as an attending physician at the Shin Kong Wu Ho-Su Memorial Hospital. He is currently an associate professor of Fu Jen Catholic University and is also the chief of the Department of Obstetrics and Gynecology of Shin Kong Wu Ho-Su Memorial Hospital. His clinical interests include infertility treatment and minimally invasive surgeries. His research interest is assisted reproductive technologies, especially ovarian stimulation and in-vitro maturation of oocytes.

Abstract The purpose of the study was to evaluate whether a lower concentration of FSH or 2-h incubation with FSH would improve the outcome of in-vitro maturation of oocytes. The immature oocytes were obtained from FVB mice, and were allocated to four groups and incubated in the maturation media for 24 h. The maturation media were supplemented with 10 mIU/ml FSH for 24 h (group 1), 10 mIU/ml FSH for 2 h (group 2), 75 mIU/ml FSH for 24 h (group 3) or 75 mIU/ml FSH for 2 h (group 4). In each group, half of the in-vitro-matured oocytes were fertilized and cultured to blastocysts and the remaining matured oocytes were analysed for growth differentiation factor (GDF)-9 and bone morphogenetic protein (BMP)-15 mRNA to assess the oocyte quality. The maturation rates and oocyte BMP-15 mRNA concentrations were similar among the four groups. The GDF-9 mRNA concentrations were similar in group 2 and group 4. The fertilization and blastocyst rates were higher in groups 2 and 4 than in groups 1 and 3. It is concluded that 2-h incubation with FSH is better than 24-h incubation in terms of the fertilization rate and blastocyst development.

KEYWORDS: BMP-15, c-AMP, FSH, GDF-9, in-vitro maturation

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Introduction

Immature oocyte retrieval followed by in-vitro maturation (IVM) broadens the horizon for modern assisted reproductive technologies. Recent studies showed that IVM is a feasible alternative to IVF (Cha et al., 2005; Lin and Hwang, 2006). However, despite the recent progress, the implantation rate following IVM remains lower than that of traditional IVF. The poor outcome of human IVM is thought to be at least partly due to abnormalities in cytoplasmic maturation in in-vitro-matured oocytes.

During IVM, FSH is generally added to the maturation medium throughout the maturation period. The effect of FSH is achieved through secondary messengers such as cyclic adenosine 3′-5′-monophosphate (cAMP). FSH initially induces cumulus cells to produce cAMP, which is transferred to the oocyte via gap junctions; however, in the later stages, the cAMP concentrations decrease (Salustri et al., 1985). Andersen et al. showed that FSH induces maximal accumulation of cAMP after 30 min; thereafter, the cAMP concentrations decrease progressively, reaching the lowest concentrations at 120 min (Andersen et al., 2001). Further, cAMP plays dual roles in the meiotic maturation of oocytes. Although high cAMP concentrations inhibit oocyte maturation, the subsequent drop in the cAMP concentration triggers oocyte maturation (Salustri et al., 1985; Tornell and Hillensjo, 1993; Yoshimura et al., 1992). Byskov et al. (1997) showed that FSH induces cumulus cells within 30 min to produce, and after 2 h to secrete, a meiosis-activating substance to induce oocyte maturation in murine oocytes. These results suggest that short-term incubation with FSH can also induce oocyte maturation.

Intrafollicular concentrations of FSH are almost constant, at approximately 4–6 mIU/ml, in natural cycles (McNatty, 1978) and stimulated cycles (Filicori et al., 1996). The mean peak plasma concentration of FSH in natural cycles is 10 mIU/ml (Vermeiden et al., 1997), i.e. much lower than the 75 mIU/ml commonly used in the maturation medium. Some studies suggest that the correct concentration of FSH is required to promote oocyte growth. Andersen et al. (1997) found that serum FSH concentrations had a significant inverse correlation with the cleavage rate, suggesting that supraphysiological concentrations of FSH negatively affected oocyte maturation. Thomas et al. (2005) demonstrated in mice that the correct concentration of FSH was crucial to promote oocyte growth from preantral follicles and only a low FSH concentration (0.05 ng/ml) promoted oocyte growth. Exposure to high concentrations ($\geq$20 ng/ml) of FSH during IVM can even induce chromosome abnormalities (Roberts et al., 2005). Since the environment to which maturing oocytes are exposed can compromise subsequent embryo development (Rose and Bavister, 1992; van de Sandt et al., 1990), it was postulated for this study that continuous exposure to relatively high concentrations of FSH may result in inferior oocyte maturation.

Growth differentiation factor (GDF)-9 is localized exclusively in oocytes at all stages of follicular growth, except in primordial follicles (McGrath et al., 1995). GDF-9 promotes granulosa cell proliferation and is required for ovarian folliculogenesis (Elvin et al., 1999; Mazerbourg and Hsueh, 2003). Bone morphogenetic protein (BMP)-15 is also an oocyte-specific growth factor (Dube et al., 1998) and regulates granulosa cell proliferation and differentiation (Otsuka et al., 2000). Full complements of both GDF-9 and BMP-15 are essential for the development and function of oocytes (Su et al., 2004).

The present study used a murine model to observe whether a lower concentration of FSH or 2-h FSH incubation would enhance oocyte maturation and embryonic development. Besides the outcome of IVM, this study also analysed the mRNA expression of GDF-9 and BMP-15 to assess the oocyte quality.

Materials and methods

In-vitro maturation

Chemicals were obtained from Sigma Chemical Company (St Louis, MO, USA) unless stated otherwise. The mice used in the study were FVB/NJNarL mice (National Laboratory Animal Centre, Taipei, Taiwan). In each experiment, 10 female mice were used. Immature oocytes were obtained from 3- to 5-week-old female mice, 48 h after intra-peritoneal injection with 5 IU pregnant mare's serum gonadotrophin (PMSG). Immature oocytes with cumulus cells were collected by teasing the ovarian follicles. Only cumulus–oocyte-complexes (COC) that comprised oocytes with homogeneous cytoplasm and more than three layers of cumulus cells were used for the study.

The COC were pooled and evenly distributed into four groups. The basal maturation medium consisted of tissue culture medium 199, 10% fetal bovine serum, 4 mM hypoxanthine and 0.2 mM pyruvate, to which the following were added for the different treatment groups: (i) Group 1: 10 mIU/ml FSH (Gonal-F; Serono, Aubonne, Switzerland) and 0.5 IU/ml human chorionic gonadotrophin (HCG) for 24 h; (ii) Group 2: 10 mIU/ml FSH and 0.5 IU/ml HCG for 2 h, then 0.5 IU/ml HCG only for another 22 h; (iii) Group 3: 75 mIU/ml FSH and 0.5 IU/ml HCG for 24 h; and (iv) Group 4: 75 mIU/ml FSH and 0.5 IU/ml HCG for 2 h, then 0.5 IU/ml HCG only for another 22 h.

For IVM, the COC were incubated in the maturation media for a total of 24 h. Only oocytes that displayed a distinct first polar body were classified as metaphase II (MII). In each group, half of the MII oocytes went through fertilization and culture and the other half of MII oocytes were analysed for mRNA expression.

Fertilization

The spermatozoa were retrieved from the cauda epididymis of 5- to 7-week-old male mice. The spermatozoa were dispersed in human tubal fluid medium (Irvine Scientific, Santa Ana, CA, USA) plus 5 mg/ml fetal bovine serum and diluted to a concentration of 1×10^6/ml. The MII oocytes were incubated with the spermatozoa for 4 h. The oocytes were then washed to eliminate excess spermatozoa and then cultured overnight in a drop of mKSOM medium (Speciality Media, Phillipsburg, NJ, USA) covered with mineral oil. The next morning, the number of 2-cell embryos was counted and the embryos were transferred to fresh droplets of mKSOM medium under mineral oil and cultured to blastocysts.

Fertilization was considered to be the percentage of 2-cell embryos 24 h after insemination. The maturation rates, fertilization rates and embryo development were recorded.

RNA isolation and reverse transcription

In the second part of the experiment, the other half of the in-vitro-matured oocytes in each group were analysed for GDF-9 and BMP-15 mRNA after stripping of the cumulus cells with 0.03% hyaluronidase and mechanical pipetting. The experiment was repeated six times.

Total RNA was extracted from approximately 15 in-vitro-matured oocytes per treatment per experiment by using the TRIzol RNA Purification Kit (Invitrogen, USA) according to the manufacturer's instructions. Briefly, pools of oocytes were mixed with 500 μl of the TRIzol reagent and were lysed by repetitive pipetting, followed by addition of 100 μl chloroform and vigorous shaking. After centrifugation at 12,000*g* for 15 min, the RNA in the upper aqueous phase was recovered, precipitated and washed with ethanol and dried. After extraction, the RNA pellets were resuspended in 40 μl of water and stored at −80°C. The RNA concentration was determined by UV absorbance at 260 nm (GeneQuant RNA/DNA calculator; Pharmacia LKB Biochrom, Cambridge, UK)

The reverse transcription (RT) reaction was performed, using Invitrogen reagents, in a 20 μl mixture, containing extracted RNA, oligo(dT), reverse transcriptase buffer, dNTP mixure, recombinant ribonuclease inhibitor and 200 IU SuperScript III reverse transcriptase. The total RNA, oligo(dT) and dNTP mixture were incubated at 65°C for 5 min and then chilled on ice. The contents were collected by brief centrifugation at 12,000*g* for 5 s, and first-strand buffer, DTT, RNaseOU recombinant RNase inhibitor and 200 IU SuperScript III reverse transcriptase were added. The reaction was carried out at 50°C for 60 min and heat inactivated at 70°C for 15 min. The resultant cDNA mixtures were stored at −20°C.

Real-time quantification PCR

Oligonucleotide primers for PCR were designed to amplify GDF-9 and BMP-15 and custom ordered from Invitrogen. The sense and antisense primer sequences were as follows: GDF-9 (5′-CAACGACACAAGCACC-3′ and 5′-CAACGACACAAGCACC-3′); BMP-15 (5′-AAATGCCGGACCAAGC-3′ and 5′-CGGACACTCCGCATGA-3′). In order to quantify gene expression, the expression levels of specific mRNA were calculated relative to the housekeeping gene β-actin.

PCR amplification of cDNA was performed by using the LightCycler FastStart DNA Master^{PLUS} SYBR Green I Kit (Roche, Mannheim, Germany). The procedure was performed with 2 μl cDNA in a final volume of 20 μl containing the reaction mix (FastStart Taq DNA polymerase, reaction buffer, $MgCl_2$, SYBR Green I dye and dNTP mix) and the sense and antisense primers. Real-time PCR was carried out in a thermal cycler (LightCycler 1.5; Roche). The reaction conditions were as follows: pre-denaturation at 95°C for 5 min and 35 cycles consisting of denaturation at 95°C for 10 s, annealing at 58°C for 10 s and extension at 72°C for 25 s.

Statistical analysis

Statistical analysis was carried out using Statistical Package for Social Sciences version 13.0 (SPSS, Chicago, IL, USA). The outcome of IVM, including maturation rate, fertilization rate, embryo cleavage and blastocyst development among each group was compared by the chi-squared test. Bivariate logistic regression was performed to analyse the effects of incubation duration and concentration of FSH on IVM. Mean mRNA concentrations were compared by ANOVA, followed by Tukey post-hoc multiple comparisons. A *P*-value <0.05 was considered significant.

Results

The results of IVM are shown in **Table 1**. The maturation rates were similar between the four groups. The maturation rate was slightly higher, but not significantly, in group 2. However, the fertilization and blastocyst rates were higher in groups 2 and 4 than in groups 1 and 3. The fertilization and blastocyst rates between groups 2 and 4 were similar. The results of logistic regression analysis are shown in **Table 2**. Incubation in 10 IU/ml and 75 mIU/ml FSH produced similar IVM outcomes. Compared with 24-h incubation, 2-h incubation with FSH slightly improved the maturation rate (odds ratio (OR) 1.35) although not statistically significantly. Two-hour incubation with FSH significantly improved the fertilization rate (OR 2.16, $P = 0.0002$) and, even more, blastocyst development (OR 6.93, $P = 0.0021$). GDF-9 and BMP-15 mRNA were expressed in all four groups of oocytes. The oocyte BMP-15 mRNA concentrations were similar in all four groups. The GDF-9 mRNA concentrations were similar in group 2 and group 4, but the GDF-9 mRNA concentration in group 3 was slightly higher than that in group 1 ($P = 0.045$, **Figure 1**).

Discussion

This study is the first to show for IVM that 2-h incubation with FSH is better than 24-h incubation in terms of the fertilization rate and embryonic development. Two-hour incubation with 10 mIU/ml and 75 mIU/ml FSH produced similar results and both concentrations produced higher fertilization rates and blastocyst rates than 24-h exposure. Continuous 24-h incubation with FSH at 10 mIU/ml (group 1) and 75 mIU/ml (group 3) produced similar IVM outcomes. Expression of BMP-15 mRNA was similar in group 1 and group 3, and GDF-9 mRNA concentration in group 3 was slightly higher than that in group 1 ($P = 0.045$).

Although different centres have used different media for human IVM (Chian et al., 2000; Child et al., 2001; Cha et al., 2005; Lin et al., 2003; Mikkelsen et al., 1999; Trounson et al., 1994), all media contain FSH. The effects of FSH on oocyte maturation are mixed. Durinzi et al. (1997) showed that addition of urofollitropin did not significantly increase the ability of oocytes to achieve MII. On the contrary, FSH supplementation has been shown to increase fertilization (Schroeder et al., 1988) and embryonic development (Jinno et al., 1989; Morgan et al., 1991). It is generally believed that FSH supplementation in the maturation medium is

Table 1 Outcome of in-vitro maturation of oocytes in different media.

	Group 1	*Group 2*	*P-value*	*Group 3*	*Group 4*	*P-value*
Oocytes (*n*)	190	190	—	190	190	—
Maturation rate	66.8	74.2	NS	62.1	67.9	NS
Fertilization rate	53.8	78.0	0.004	65.0	80.3	0.038
Morula	20.0	42.1	0.04	25.6	34.0	NS
Blastocyst	2.9	17.5	0.047	5.1	17.0	0.05

Values are % unless otherwise stated.
Group 1 = 10 mIU/ml FSH for 24 h; group 2 = 10 mIU/ml FSH for 2 h; group 3 = 75 mIU/ml FSH for 24 h; group 4 = 75 mIU/ml FSH for 2 h; NS = not significant.

Table 2 Logistic regression analysis of the effects of incubation duration and concentration of FSH on in-vitro maturation.

Model	*10 IU versus 75 IU (ref.)*		*2 h versus 24 h (ref.)*	
	OR	*P-value*	*OR*	*P-value*
Maturation	1.29	NS	1.35	NS
Fertilization	1.00	NS	2.16	0.0002
Morula	1.13	NS	2.89	0.0006
Blastocyst	1.00	NS	6.93	0.0021

NS = not statistically significant; OR = odds ratio.

beneficial to oocyte maturation. However, most of the studies have used supraphysiological concentrations of FSH. The concentration of FSH is crucial for oocyte development. Repeated ovarian stimulation in mice has been shown to reduce in-vitro meiotic competence (Combelles and Albertini, 2003). Similarly, in human IVF, supraphysiological concentrations of FSH have been shown to negatively affect oocyte maturation (Andersen et al., 1997). On the other hand, some studies have shown that lower concentrations of FSH are enough for IVM. Byskov et al. (1997) showed that FSH at 8 IU/ml is sufficient to induce the resumption of meiosis in murine oocytes. Similarly, Andersen et al. (1999) demonstrated that 50% of murine oocytes resumed meiosis at FSH concentrations of 6–12 mIU/ml. The present study showed that 10 mIU/ml was sufficient to enhance oocyte maturation *in vitro*.

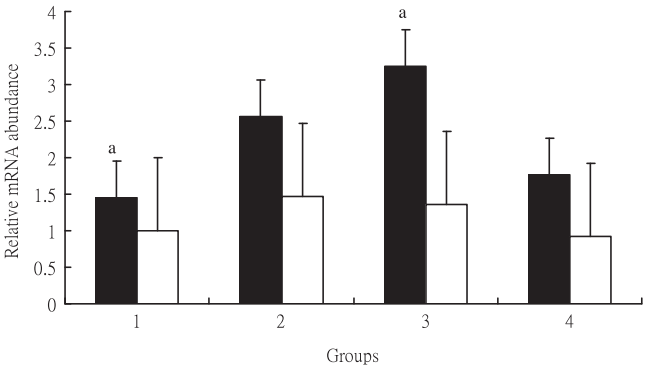

Figure 1 Real-time PCR of GDF-9 (black bars) and BMP-15 (open bars) mRNA in different treatment groups. Group 1 = 10 mIU/ml FSH for 24 h; group 2 = 10 mIU/ml FSH for 2 h; group 3 = 75 mIU/ml FSH for 24 h; group 4 = 75 mIU/ml FSH for 2 h. [a]*P*-value is 0.045.

The present study showed that 2-h incubation with FSH produced higher fertilization and blastocyst rates than 24-h incubation. These results are compatible with those of the study by Byskov et al. (1997), in which 2-h priming with FSH induced oocyte maturation in murine oocytes. Similarly, Xia et al. (2000) used pig oocytes to demonstrate that priming with FSH for 30 min to 4 h stimulated germinal vesicle breakdown and a plateau was reached at 2–4 h of priming. Lu et al. (2000) showed that the presence of 50 mIU/ml FSH for 1 h was sufficient to induce meiotic resumption, which reached a plateau after 2 h. These studies, however, did not compare the outcomes of IVM between short-term priming and long-term incubation with FSH.

The explanation for the beneficial effects of short-term FSH priming may be two-fold. First, FSH causes an increase in oocyte cAMP concentration via the stimulation of cumulus cells (Webb et al., 2002). The initial high concentrations of cAMP maintain the oocytes in meiotic arrest. However, FSH withdrawal results in a fall in the cAMP concentration, leading to initiation of meiosis resumption (Eppig, 1989; Grondahl et al., 2003). Second, continuous FSH incubation results in the desensitization of FSH receptors. FSH has been shown to induce a dose-dependent and almost complete down-regulation of FSH receptor mRNA in Sertoli cells after 4 h of culture (Themmen et al., 1991). Lambert et al. (2000) showed that the granulosa cells obtained from oocyte retrieval after ovarian stimulation were refractory to immediate FSH stimulation, and at least 1 day of culture was required for the granulosa cells to obtain their responsiveness to FSH. Moreover, short-term incubation with FSH

may be more physiological, since the pituitary release of FSH is pulsatile (Padmanabhan et al., 1997).

FSH and LH play important roles in the regulation of oocyte growth and maturation. The pre-ovulatory LH surge triggers meiosis and final oocyte maturation. LH and HCG act through the common receptors on the cumulus cells and they are equally effective in promoting IVM (Hreinsson et al., 2003). However, the actions of HCG in IVM are still controversial and may be different in different species. Anderiesz et al. (2000) showed that human and bovine IVM were improved by FSH in combination with LH, but inclusion of LH had no significant effect on murine IVM. On the contrary, another recent study showed that addition of HCG to the maturation medium did not improve the maturation rate or embryonic development in patients with polycystic ovary syndrome (Ge et al., 2008). Although rarely used in murine IVM, HCG is used in many human IVM systems, including this study centre's. The present study used murine oocytes to study human IVM systems, so HCG was added to the maturation media.

Most of the murine IVM studies obtained blastocyst rates of about 30% (Anderiesz et al., 2000; Wang et al., 2006), which are higher than those in the present study. Immature murine oocytes take 16–18 h to mature *in vitro* (Chin and Chye, 2004; Roberts et al., 2005), although some researchers cultured for 24 h (Andersen et al., 1999; Junk et al., 2003). The present study cultured the immature oocytes for 24 h in order to obtain more mature oocytes. Extended culture, however, may cause the oocytes to age *in vitro* and have adverse effects on IVM (Nagano et al., 2008), which may be the reason for lower blastocyst rates presented here.

As in most murine IVM studies, PMSG was administered before oocyte retrieval to increase the size of the follicles and the number of immature oocytes obtained (Chin and Chye, 2004). The role of FSH priming in IVM remains inconclusive. FSH priming has been shown to enhance oocyte maturation (Junk et al., 2003; Schramm and Bavister, 1994; Wynn et al., 1998); however, some studies have shown no differences in the oocyte recovery rate or oocyte maturation (Trounson et al., 1998; Mikkelsen et al., 1999). In the present study, the COC obtained were still at the germinal-vesicle stage, and hypoxanthine was added to the maturation medium to prevent spontaneous oocyte maturation as in most murine IVM studies. The results of the present study demonstrate that after in-vivo priming with PMSG, 2-h incubation with FSH is better than 24-h incubation for IVM. Further research is required to confirm the beneficial effects of 2-h FSH incubation in natural-cycle IVM.

Oocyte maturation is closely dependent on the surrounding granulosa cells. The cumulus cells, in particular, nurture the oocyte through the final stages of development. However, recent studies show that the oocyte is a central regulator of granulosa cell function and plays a critical role in the regulation of oogenesis, ovulation and fecundity (Gilchrist et al., 2008). The oocyte regulates granulosa cell and cumulus cell function by secreting certain oocyte-secreted factors, the most important being GDF-9 and BMP-15. The oocyte secretes these growth factors that act on the neighbouring granulosa cells, which in turn regulate oocyte development (Gilchrist et al., 2008). The present study found that oocyte BMP-15 mRNA concentrations were similar in all four groups. Further, in-vitro-matured oocytes after 2-h FSH incubation expressed similar concentrations of GDF-9 mRNA. The results further support that in the case of 2-h FSH incubation, 10 mIU/ml and 75 mIU/ml produced similar oocyte quality.

In conclusion, the present study demonstrated that exposure to FSH for just 2 h was sufficient to enhance IVM and produced a better outcome than 24-h exposure to FSH. Low- and high-dose FSH produced similar outcomes. Further studies are necessary to determine the best duration of FSH exposure for human IVM.

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References

Anderiesz, C., Ferraretti, A.P., Magli, C., Fiorentino, A., Fortini, D., Gianaroli, L., Jones, G.M., Trounson, A.O., 2000. Effect of recombinant human gonadotrophins on human, bovine and murine oocyte meiosis, fertilization and embryonic development in vitro. Human Reproduction 15, 1140–1148.

Andersen, C.Y., Westergaard, L.G., Byskov, A.G., Erb, K., 1997. Reduced in vitro fertilization of human oocytes correlates with raised circulating FSH levels during ovarian stimulation in normogonadotropic women downregulated with GnRH-analogues. Journal of Assisted Reproduction and Genetics 14, 581–588.

Andersen, C.Y., Leonardsen, L., Ulloa-Aguirre, A., Barrios-De-Tomasi, J., Moore, L., Byskov, A.G., 1999. FSH-induced resumption of meiosis in mouse oocytes: effect of different isoforms. Molecular Human Reproduction 5, 726–731.

Andersen, C.Y., Leonardsen, L., Ulloa-Aguirre, A., Barrios-De-Tomasi, J., Kristensen, K.S., Byskov, A.G., 2001. Effect of different FSH isoforms on cyclic-AMP production by mouse cumulus–oocyte-complexes: a time course study. Molecular Human Reproduction 7, 129–135.

Byskov, A.G., Andersen, C.Y., Hossaini, A., Guoliang, X., 1997. Cumulus cells of oocyte–cumulus complexes secrete a meiosis-activating substance when stimulated with FSH. Molecular Reproduction and Development 46, 296–305.

Cha, K.Y., Chung, H.M., Lee, D.R., Kwon, H., Chung, M.K., Park, L.S., Choi, D.H., Yoon, T.K., 2005. Obstetric outcome of patients with polycystic ovary syndrome treated by in vitro maturation and in vitro fertilization-embryo transfer. Fertility and Sterility 83, 1461–1465.

Chian, R.C., Buckett, W.M., Tulandi, T., Tan, S.L., 2000. Prospective randomized study of human chorionic gonadotrophin priming before immature oocyte retrieval from unstimulated women with polycystic ovarian syndrome. Human Reproduction 15, 165–170.

Child, T.J., Abdul-Jalil, A.K., Gulekli, B., Tan, S.L., 2001. In vitro maturation and fertilization of oocytes from unstimulated normal ovaries, polycystic ovaries, and women with polycystic ovary syndrome. Fertility and Sterility 76, 936–942.

Chin, A.H., Chye, N.S., 2004. Investigations of oocyte in vitro maturation within a mouse model. Zygote 12, 1–18.

Combelles, C.M., Albertini, D.F., 2003. Assessment of oocyte quality following repeated gonadotropin stimulation in the mouse. Biology of Reproduction 68, 812–821.

Dube, J.L., Wang, P., Elvin, J., Lyons, K.M., Celeste, A.J., Matzuk, M.M., 1998. The bone morphogenetic protein 15 gene is X-linked and expressed in oocytes. Molecular Endocrinology 12, 1809–1817.

Durinzi, K.L., Wentz, A.C., Saniga, E.M., Johnson, D.E., Lanzendorf, S.E., 1997. Follicle stimulating hormone effects on immature human oocytes: in vitro maturation and hormone production. Journal of Assisted Reproduction and Genetics 14, 199–204.

Elvin, J.A., Clark, A.T., Wang, P., Wolfman, N.M., Matzuk, M.M., 1999. Paracrine actions of growth differentiation factor-9 in the mammalian ovary. Molecular Endocrinology 13, 1035–1048.

Eppig, J.J., 1989. The participation of cyclic adenosine monophosphate (cAMP) in the regulation of meiotic maturation of oocytes in the laboratory mouse. Journal of Reproduction and Fertility 38 (Suppl.), 3–8.

Filicori, M., Flamigni, C., Cognigni, G.E., Falbo, A., Arnone, R., Capelli, M., Pavani, A., Mandini, M., Calderoni, P., Brondelli, L., 1996. Different gonadotropin and leuprorelin ovulation induction regimens markedly affect follicular fluid hormone levels and folliculogenesis. Fertility and Sterility 65, 387–393.

Ge, H.S., Huang, X.F., Zhang, W., Zhao, J.Z., Lin, J.J., Zhou, W., 2008. Exposure to human chorionic gonadotropin during in vitro maturation does not improve the maturation rate and developmental potential of immature oocytes from? Patients with polycystic ovary syndrome. Fertility and Sterility 89, 98–103.

Gilchrist, R.B., Lane, M., Thompson, J.G., 2008. Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. Human Reproduction Update 14, 159–177.

Grondahl, C., Breinholt, J., Wahl, P., Murray, A., Hansen, T.H., Faerge, I., Stidsen, C.E., Raun, K., Hegele-Hartung, C., 2003. Physiology of meiosis-activating sterol: endogenous formation and mode of action. Human Reproduction 18, 122–129.

Hreinsson, J., Rosenlund, B., Friden, B., Levkov, L., Ek, I., Suikkari, A.M., Hovatta, O., Fridstrom, M., 2003. Recombinant LH is equally effective as recombinant hCG in promoting oocyte maturation in a clinical in-vitro maturation programme: a randomized study. Human Reproduction 18, 2131–2136.

Jinno, M., Sandow, B.A., Hodgen, G.D., 1989. Enhancement of the developmental potential of mouse oocytes matured in vitro by gonadotropins and ethylenediaminetetraacetic acid (EDTA). Journal of In Vitro Fertility and Embryo Transfer 6, 36–40.

Junk, S.M., Dharmarajan, A., Yovich, J.L., 2003. FSH priming improves oocyte maturation, but priming with FSH or hCG has no effect on subsequent embryonic development in an in vitro maturation program. Theriogenology 59, 1741–1749.

Lambert, A., Harris, S.D., Knaggs, P., Robertson, W.R., 2000. Improved FSH sensitisation and aromatase assay in human granulosa-lutein cells. Molecular Human Reproduction 6, 677–680.

Lin, Y.H., Hwang, J.L., 2006. In vitro maturation of human oocytes. Taiwanese Journal of Obstetrics and Gynecology 45, 95–99.

Lin, Y.H., Hwang, J.L., Huang, L.W., Mu, S.C., Seow, K.M., Chung, J., Hsieh, B.C., Huang, S.C., Chen, C.Y., Chen, P.H., 2003. Combination of FSH priming and hCG priming for in-vitro maturation of human oocytes. Human Reproduction 18, 1632–1636.

Lu, Z., Xia, G., Byskov, A.G., Anderson, C.Y., 2000. Effects of amphotericin B and ketoconazole on mouse oocyte maturation: implications on the role of meiosis-activating sterol. Molecular and Cellular Endocrinology 164, 191–196.

Mazerbourg, S., Hsueh, A., 2003. Growth differentiation factor-9 signaling in the ovary. Molecular and Cellular Endocrinology 202, 31–36.

McGrath, S.A., Esquela, A.F., Lee, S.J., 1995. Oocyte-specific expression of growth/differentiation factor-9. Molecular Endocrinology 9, 131–136.

McNatty, K.P., 1978. Cyclic changes in antral fluid hormone concentrations in humans. Journal of Clinical Endocrinology and Metabolism 7, 577–600.

Mikkelsen, A.L., Smith, S.D., Lindenberg, S., 1999. In-vitro maturation of human oocytes from regularly menstruating women may be successful without follicle stimulating hormone priming. Human Reproduction 14, 1847–1851.

Morgan, P.M., Warikoo, P.K., Bavister, B.D., 1991. In vitro maturation of ovarian oocytes from unstimulated rhesus monkeys: assessment of cytoplasmic maturity by embryonic development after in vitro fertilization. Biology of Reproduction 45, 89–93.

Nagano, M., Uchikura, K., Takahashi, Y., Hishinuma, M., 2008. Effect of duration of in vitro maturation on nuclear maturation and fertilizability of feline oocytes. Theriogenology 69, 231–236.

Otsuka, F., Yao, Z., Lee, T., Yamamoto, S., Erickson, G.F., Shimasaki, S., 2000. Bone morphogenetic protein-15. Identification of target cells and biological functions. Journal of Biological Chemistry 275, 39523–39528.

Padmanabhan, V., McFadden, K., Mauger, D.T., Karsch, F.J., Midgley Jr., A.R., 1997. Neuroendocrine control of follicle-stimulating hormone (FSH) secretion. I. Direct evidence for separate episodic and basal components of FSH secretion. Endocrinology 138, 424–432.

Roberts, R., Iatropoulou, A., Ciantar, D., Stark, J., Becker, D.L., Franks, S., Hardy, K., 2005. Follicle-stimulating hormone affects metaphase I chromosome alignment and increases aneuploidy in mouse oocytes matured in vitro. Biology of Reproduction 72, 107–118.

Rose, T.A., Bavister, B.D., 1992. Effect of oocyte maturation medium on in vitro development of in vitro fertilized bovine embryos. Molecular Reproduction and Development 31, 72–77.

Salustri, A., Petrungaro, S., De Felici, M., Conti, M., Siracusa, G., 1985. Effect of follicle-stimulating hormone on cyclic adenosine monophosphate level and on meiotic maturation in mouse cumulus cell-enclosed oocytes cultured in vitro. Biology of Reproduction 33, 797–802.

Schramm, R.D., Bavister, B.D., 1994. Follicle-stimulating hormone priming of rhesus monkeys enhances meiotic and developmental competence of oocytes matured in vitro. Biology of Reproduction 51, 904–912.

Schroeder, A.C., Downs, S.M., Eppig, J.J., 1988. Factors affecting the developmental capacity of mouse oocytes undergoing maturation in vitro. Annals of the New York Academy of Sciences 541, 197–204.

Su, Y.Q., Wu, X., O'Brien, M.J., Pendola, F.L., Denegre, J.N., Matzuk, M.M., Eppig, J.J., 2004. Synergistic roles of BMP15 and GDF9 in the development and function of the oocyte–cumulus cell complex in mice: genetic evidence for an oocyte-granulosa cell regulatory loop. Developmental Biology 276, 64–73.

Themmen, A.P., Blok, L.J., Post, M., Baarends, W.M., Hoogerbrugge, J.W., Parmentier, M., Vassart, G., Grootegoed, J.A., 1991. Follitropin receptor down-regulation involves a cAMP-dependent post-transcriptional decrease of receptor mRNA expression. Molecular and Cellular Endocrinology 78, R7–R13.

Thomas, F.H., Ethier, J.F., Shimasaki, S., Vanderhyden, B.C., 2005. Follicle-stimulating hormone regulates oocyte growth by modulation of expression of oocyte and granulosa cell factors. Endocrinology 146, 941–949.

Tornell, J., Hillensjo, T., 1993. Effect of cyclic AMP on the isolated human oocyte–cumulus complex. Human Reproduction 8, 737–739.

Trounson, A., Wood, C., Kausche, A., 1994. In vitro maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. Fertility and Sterility 62, 353–362.

Trounson, A., Anderiesz, C., Jones, G.M., Kausche, A., Lolatgis, N., Wood, C., 1998. Oocyte maturation. Human Reproduction 13 (Suppl. 3), 52–62.

van de Sandt, J.J., Schroeder, A.C., Eppig, J.J., 1990. Culture media for mouse oocyte maturation affect subsequent embryonic development. Molecular Reproduction and Development 25, 164—171.

Vermeiden, J.P., Roseboom, T.J., Goverde, A.J., Suchartwatnachai, C., Schoute, E., Braat, D.D., Schats, R., 1997. An artificially induced follicle stimulating hormone surge at the time of human chorionic gonadotrophin administration in controlled ovarian stimulation cycles has no effect on cumulus expansion, fertilization rate, embryo quality and implantation rate. Human Reproduction 12, 1399—1402.

Wang, Y., Oct, S.A., Chian, R.C., 2006. Effect of gonadotrophin stimulation on mouse oocyte quality and subsequent embryonic development *in vitro*. Reproductive BioMedicine Online 12, 304—314.

Webb, R.J., Marshall, F., Swann, K., Carroll, J., 2002. Follicle-stimulating hormone induces a gap junction-dependent dynamic change in [cAMP] and protein kinase a in mammalian oocytes. Developmental Biology 246, 441—454.

Wynn, P., Picton, H.M., Krapez, J.A., Rutherford, A.J., Balen, A.H., Gosden, R.G., 1998. Pretreatment with follicle stimulating hormone promotes the numbers of human oocytes reaching metaphase II by in-vitro maturation. Human Reproduction 13, 3132—3138.

Xia, G.L., Kikuchi, K., Noguchi, J., Izaike, Y., 2000. Short time priming of pig cumulus—oocyte complexes with FSH and forskolin in the presence of hypoxanthine stimulates cumulus cells to secrete a meiosis-activating substance. Theriogenology 53, 1807—1815.

Yoshimura, Y., Nakamura, Y., Ando, M., Jinno, M., Oda, T., Karube, M., Koyama, N., Nanno, T., 1992. Stimulatory role of cyclic adenosine monophosphate as a mediator of meiotic resumption in rabbit oocytes. Endocrinology 131, 351—356.

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