Available online at www.sciencedirect.com
ScienceDirect
Theriogenology
Theriogenology 68 (2007) 640–645
www.theriojournal.com

Effects of caffeine on in vivo and in vitro oocyte maturation in mice☆

Yi-Liang Miao [a,b], Li-Hong Shi [a,b], Zi-Li Lei [a,b], Jun-Cheng Huang [a,b], Ji-Wen Yang [a,b], Ying-Chun OuYang [a], Qing-Yuan Sun [a,*], Da-Yuan Chen [a,*]

[a] State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, # 5 Da Tun Road, Chaoyang, Beijing 100101, PR China
[b] Graduate School, Chinese Academy of Sciences, Beijing 100039, PR China

Received 6 January 2007; accepted 18 April 2007

Abstract

The objective was to investigate, using a mouse model, the effects of caffeine on the number of ovulated oocytes, the rate of oocyte maturation, the susceptibility of oocytes to activating stimuli, spindle morphology, and distribution of cortical granules (CGs). Mice were given caffeine (150 mg/kg body weight ip) at various times relative to hCG (−2, 0, and +2 h); in an in vitro study, 1, 5 or 10 mM caffeine was added to the maturation culture. Caffeine had no effect on the quality of oocytes in vivo maturation, but caffeine was detrimental to the quality of oocytes matured in vitro. Further studies are needed to determine caffeine concentration in follicles relative to that in culture medium.
© 2007 Elsevier Inc. All rights reserved.

Keywords: Mouse oocyte; Caffeine; Spindle; Cortical granules; Maturation

1. Introduction

Caffeine (1,3,7-trimethylxanthine), a central nervous system stimulant, acts through adenosine receptors and monoamine neurotransmitters. It is used as a psychotropic drug [1] and is an additive in many foods and drugs. Maturation of oocytes is initiated by decreases in intracellular cAMP [2,3] and by subsequent activation of the M-phase promoting factor (MPF) and the mitogen-activated protein kinase (MAPK) pathway [4]. Caffeine can inhibit cAMP phosphodiesterase [5] and thus effectively inhibit meiotic resumption of oocytes [6–8]. Therefore, caffeine intake in people undergoing IVF and gamete intra-Fallopian transfer (GIFT) could affect oocyte maturation, sperm parameters, fertilization, and other outcomes of assisted reproductive technologies. In that regard, Klonoff–Cohen reported that typical female caffeine consumption could induce failure of a live birth and decrease infant gestational age [9].

Caffeine at concentrations ≥200 μg/mL (1.03 mM) suppressed mouse oocyte meiosis in vitro without production of any numerical or structural abnormalities [10]. Although acute administration of caffeine (0.25 mg/g body weight) prevented superovulation and oocytes in follicles that were still at the germinal vesicle stage, chronic administration of the same dose did not induce any cytologically detected meiotic abnormalities [10]. Caffeine neither retarded oocyte

☆ This work was supported by the “973” Project from the Ministry of Science and Technology of China (A1902164).
* Corresponding authors. Tel.: +86 10 64807052; fax: +86 10 64807099.
E-mail addresses: sunqy@ioz.ac.cn (Q.-Y. Sun), chendy@ioz.ac.cn (D.-Y. Chen).

0093-691X/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2007.04.061

meiotic maturation nor increased the incidence of aneuploidy in ovulated mouse oocytes [11]. In contrast, caffeine at concentrations >0.017 mM delayed initiation of meiotic maturation in hamster [11]. Furthermore, 2.5 mM caffeine effectively inhibited the meiotic resumption of porcine oocytes after 24 h of culture; 95.5% of oocytes were arrested at the germinal vesicle stage, whereas the withdrawal of caffeine after 24 h of culture resulted in the resumption of meiotic maturation [8]. However, the ability of caffeine-treated oocytes to develop to blastocysts after artificial activation was lower than that of the oocytes not given caffeine [8]. It was also reported that caffeine could inhibit the ageing of porcine oocytes by elevating MPF activity [12] increase not only the rate of chromosome condensation but also the developmental rate to the blastocyst stage of porcine NT embryos [13].

Although caffeine has little cytogenetic effect on mouse oocytes, whether caffeine affects the quality of oocytes in vivo or in vitro has not been studied. Therefore, we assessed the effects of caffeine in vivo and in vitro on the number of ovulated oocytes, rate of oocyte maturation, the susceptibility of oocyte to activating stimuli, morphology of spindle, and distribution of CGs.

2. Materials and methods

2.1. Animals

Mice (Kunming breed) were kept in a room with 12 h/12 h light–dark cycles, with the dark starting from 7 p.m. The mice were handled in accordance with the rules stipulated by the Animal Care and Use Committee of Institute of Zoology, Chinese Academy of Science.

2.2. Caffeine treatment

2.2.1. In vivo

Caffeine was obtained from Sigma Chemical Co. St. Louis, MO, USA (Sigma, C-0750). We used physiological saline as a diluent. Based on earlier studies, the LD_{50} for an ip injection to mice was 168 mg/kg body [14] and 250 mg/kg body of caffeine could block induced ovulation in mice [10]. Thus, we used 150 mg/kg body weight, as reported by Mailhes [11], which did not cause toxicity or lethality in any of the mice. A 15 mg/mL solution was prepared no more than 30 min before injection; 150 mg caffeine/kg body weight (0.1 mL/10 g) was given by ip injection −2, 0, and +2 h relative to treatment with hCG. Concurrently, one group without caffeine treatments was used as a control. Caffeine was injected into three mice per group, and each group was repeated three times.

2.2.2. In vitro oocyte maturation

Oocytes collected from the follicles were incubated with culture media supplemented with 1, 5, or 10 mM caffeine.

2.3. Recovery of oocytes

2.3.1. Ovulated oocytes

To induce superovulation, female mice, ∼6–8 weeks old, were given an ip treatment of 10 IU PMSG (Tianjin Laboratory Animal Center, Tianjin, China) followed 48 h later by ip treatment of 10 IU hCG, Tianjin Laboratory Animal Center) [15]. Three superovulated mice in each group were killed 13 h after hCG injection and the oviductal ampullae were broken to release the cumulus–oocyte complexes (COCs). The COCs were denuded of cumulus cells by pipetting in M2 medium (Sigma, M7167), containing 0.1% hyaluronidase (Sigma, H-3506) and the oocytes counted.

2.3.2. In vitro matured oocytes

Procedures for in vitro oocyte maturation were the same as we previously reported [16]. Briefly, female mice, 3 week after birth, were killed 46 h after PMSG administration, and the large follicles on the ovary were punctured and follicular fluid (including COCs) were collected in M2. The COCs recovered were cultured (groups of ∼30) for 20 h in 60 μL microdrops of TCM-199 (Gibco, Grand Island, New York, NY, USA) supplemented with 10% fetal bovine serum (FBS, Gibco) and 10 IU/mL PMSG. At the end of maturation culture, well-expanded COCs were selected and denuded of cumulus cells by pipetting in M2 containing 0.1% hyaluronidase. In this study, only oocytes with first polar bodies were used for activation and observation of the morphology of spindle and distribution of cortical granules.

2.4. Oocyte activation and assessment

Oocytes were incubated in activating medium at 37 °C in a humidified atmosphere with 5% CO_2 in air. The activating medium was Ca^{2+}-free CZB supplemented with 10 mM $SrCl_2$ (Sigma Chemical Co., 439665). Six hours after the onset of activation treatment, oocytes were observed under a microscope; oocytes with one (1PN) or two pronuclei (2PN), or two cells each having a nucleus (2-cell) were considered activated.

2.5. Staining and observation of spindle and chromosomes

Oocytes were collected and fixed with 4% (w/v) paraformaldehyde in PBS (pH 7.4) for 40 min at room temperature (RT). Fixed samples were permeabilized by transferring into PBS supplemented with 0.1% (w/v) Triton-X100 and 0.3% BSA for 30 to 40 min (RT). After washing twice in PBS containing 0.01% Triton-X100, samples were incubated in block solution (PBS containing 1% BSA) for 1 h at RT. The microtubules were localized by incubation for 1 h at RT with a fluorescein isothiocyanate-labeled mouse monoclonal antibody against α-tubulin (Sigma Chemical Co., F-2168), which was diluted 1:100 in blocking solution. Nuclear status of samples was evaluated by staining with 10 mg/mL propidium iodide (PI) in PBS for 10 min. After extensive washing, samples were mounted on slides with antifluorescence-fade medium (1,4-diazobicyclo-[2,2,2]-octane, DABCO, Sigma, D-2522). Finally, the samples were observed under a Zeiss confocal laser scanning microscope (Zeiss LSM 510 META, Heidelberg, Germany).

2.6. Staining and observation of CGs and chromosomes

The zonae pellucidae were removed by treating the oocytes with 0.5% pronase (Roche Diagnostics GmbH, Mannheim, Germany; Roche Diagnostic Corporation, Indianapolis, IN, USA) in M2. After being washed three times in a washing solution (M2 supplemented with 0.3% BSA and 0.01% TritonX-100), oocytes were fixed with 3.7% paraformaldehyde in M2 for 30 min at room temperature. The oocytes were then blocked three times (5 min each time) in a blocking solution (M2 containing 0.3% BSA and 100 mM glycine). After permeabilization for 5 min in M2 containing 0.1% Triton X-100 (Sigma Chemical Co.), oocytes were washed two additional times (5 min each) in blocking solution and then cultured in 100 μg/mL fluorescein isothiocyanate (FITC)-labeled lens culinaris agglutinin (LCA; Sigma Chemical Co.) in M2 for 30 min in dark. Finally, the oocytes were washed three times in washing solution. The DNA was stained in the final incubation for at least 10 min with M2 containing 10 μg/mL PI. After washing, oocytes were mounted on glass slides and observed with a laser scanning confocal microscope.

2.7. Data analysis

At least four replicates were conducted for each treatment. Data, including the rate of oocytes with GV and FPB and the rate of oocytes activated, were arc-sine transformed and analyzed with ANOVA; a Duncan's multiple comparison test was used to locate differences (Statistics Package for Social Science, SPSS Inc., Chicago, IL, USA). Data are expressed as means ± S.E.M. and $P < 0.05$ was considered significant.

3. Results

3.1. Effect of caffeine on the number of ovulated oocytes

The average numbers of ovulated oocytes were 30 ± 5, 33 ± 6, and 37 ± 4 after caffeine was injected at −2, 0,+2 h relative to hCG; there was no significant difference between the control (30 ± 6) and any of the caffeine-treated groups.

3.2. Effect of caffeine on mouse oocyte nuclear maturation in vitro

When concentration of caffeine was increased from 1 to 10 mM, GV rates increased, whereas maturation rates decreased significantly (Table 1); caffeine concentrations ≥5 mM suppressed meiosis. Furthermore, exposure to 50 mM caffeine resulted in oocyte shrinkage and death.

3.3. Effect of caffeine on the activation of ovulated and IVM mouse oocytes

For in vivo groups, there were no significant differences between the control and each of the caffeine groups. Activation rates in all groups were more than 80%. However, in the in vitro groups, activation rates decreased with increasing concentration of caffeine from 1 to 10 mM. When the concentration of caffeine reached 10 mM, less than 10% of oocytes were activated (Table 2).

Table 1
Effects of caffeine on the maturation of mouse oocytes (COC) in vitro

Caffeine concentration (mM)	No. of oocytes	GV (%)	FPB (%)
0	142	0 ± 0 a	100 ± 0 a
1	137	0 ± 0 a	100 ± 0 a
5	206	33.1 ± 4.9 b	58.3 ± 6.1 b
10	195	27.0 ± 7.5 b	65 ± 7.9 b

Within a column, values without a common letters differ ($P < 0.05$).
GV: germinal vesicle; FPB: first polar body.

Table 2
Activation of ovulated and IVM mouse oocytes after treatment with caffeine

Time of treatment relative to hCG (h)	No. of oocytes treated	No. of oocytes activated (%)
In vivo		
−2	137	123 (89.0 ± 0.8 a)
Control	143	127 (86.8 ± 4.0 a)
0	163	124 (80.4 ± 6.6 a)
+2	206	178 (87.4 ± 5.0 a)
Concentration of caffeine (mM)	**No. of oocytes treated**	**No. of oocytes activated (%)**
In vitro		
0	74	64 (86.3 ± 3.0 a)
1	73	62 (84.8 ± 1.5 a)
5	84	38 (47.3 ± 7.5 b)
10	50	4 (8.3 ± 0.9 c)

Within a production system (in vivo and in vitro, respectively), values without a common letters differ ($P < 0.05$).

3.4. Effect of caffeine on spindle morphology of ovulated and IVM mouse oocytes

All ovulated oocytes displayed bipolar spindles with focused poles in the control and the caffeine groups (Fig. 1A–D). When IVM was carried out in the presence of caffeine, the oocytes had different spindle characteristics in response to various different concentrations of caffeine. When exposed to 0 or 1 mM caffeine, oocytes had a convergent array of microtubules at the spindle poles (Fig. 1E and 1F), but oocytes displayed a large barrel configuration when treated by 5 or 10 mM caffeine (Fig. 1G and 1H). Furthermore, chromosomes in oocytes treated by 10 mM caffeine became dispersed (Fig. 1H).

3.5. Effect of caffeine on the distributions of CGs of ovulated and IVM mouse oocytes

In all oocytes examined, cortical granules were densely populated in a line just beneath the oolemma, with typical normal CG-free domains (Fig. 2). There was no difference in CG distribution between ovulated and IVM oocytes treated by caffeine.

4. Discussion

Early studies reported that there were limited cytogenetic effects of caffeine on in vivo and in vitro mouse oocyte maturation [10,11], in the present study caffeine had no effect on the in vivo maturation of mouse oocytes, whereas 5 mM caffeine suppressed the meiotic maturation of mouse oocytes during in vitro maturation. Furthermore, the suppressive effects of caffeine on in vitro oocyte maturation were dose-dependent; 50 mM was toxic to oocytes. In the absence of any information regarding the concentration of caffeine in follicular fluid after ip treatment, we can not reconcile the concentration of caffeine in follicles with that in culture medium. Therefore, the in vivo effects of caffeine need further study.

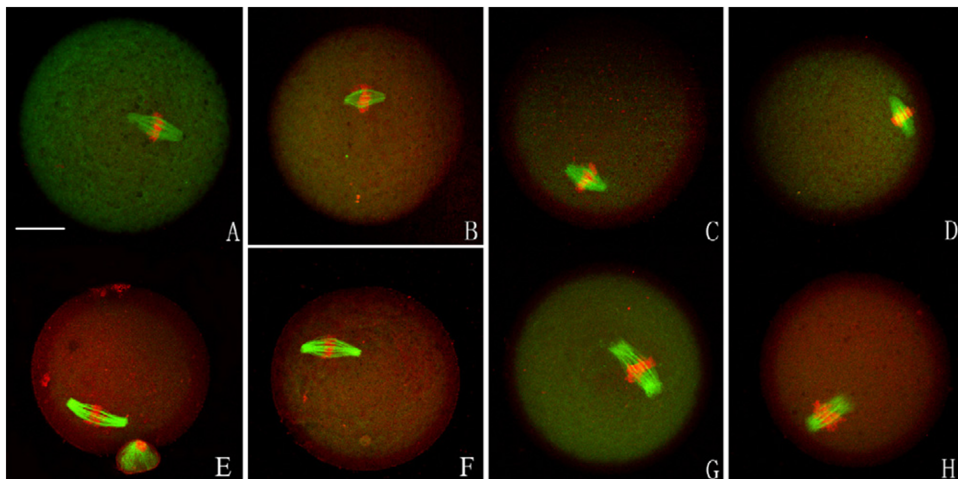

Fig. 1. Confocal micrographs of ovulated and IVM mouse oocytes treated by caffeine showing the morphology of spindle (green) and chromosomes (red). (A) Spindle morphology of ovulated oocytes without treatment of caffeine (control); (B–D) spindle morphologies after caffeine was injected at −2, 0,+2 h relative to hCG; (E) spindle morphology of IVM oocytes without caffeine treatment (control); (F–H) in vitro matured oocytes treated by 1, 5 and 10 mM caffeine, respectively. Bar, 20 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

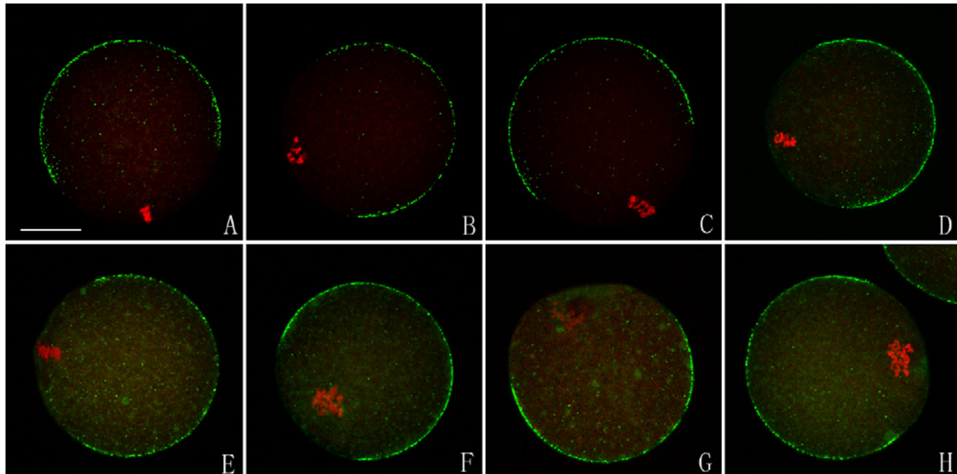

Fig. 2. Confocal micrographs (equatorial sections through the spindle) of ovulated and IVM mouse oocytes treated by caffeine showing distributions of cortical granules (green) and chromosomes (red). (A) Distributions of CGs of ovulated oocytes without caffeine treatment (control); (B–D) CG distribution after caffeine was injected at −2, 0,+2 h relative to hCG; (E) distribution of CGs of IVM oocytes without caffeine treatment (control); (F–H) in vitro matured oocytes treated by 1, 5, and 10 mM caffeine, respectively. In all micrographs, CGs were densely populated in a line just beneath the oolemma, with typical normal CG-free domains. Bar, 20 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Oocyte maturation is initiated by decrease in intracellular cAMP [2,3] and by subsequent activation of the MPF and MAPK pathways [4]. As caffeine can inhibit cAMP phosphodiesterase [5], it can effectively inhibit the meiotic resumption of oocytes in many species, including the mouse, hamster, and pig [6–8,10]. In that regard, we reported that caffeine had the same function as IBMX [17] and forsklin [18,19] in blocking oocyte maturation. Furthermore, few in vitro matured oocytes treated by 10 mM caffeine were stimulated by $SrCl_2$; therefore, we inferred that higher caffeine concentrations inhibited molecular, cytoplasmic, or nuclear events during oocyte maturation in vitro.

Recently, many researchers used spindle analysis to assess oocyte quality [20–23] and to assess the effects of toxicants and drugs on oocytes [24–26]. Although ovulated oocytes had normal bipolar spindles with focused poles, IVM oocytes had barrel-shaped spindles [27]. Rossi showed that most oocytes had spindles characterized by a large barrel configuration when IVM was carried out in the presence of hypoxanthine (HX) and FSH, whereas the percentage of oocytes with a convergent array of microtubules at the spindle poles increased in the presence of HX and epidermal growth factor (EGF) [28]. In our study, all ovulated oocytes displayed bipolar spindles with focused poles in the control and the caffeine groups, but the IVM oocytes had various spindle characteristics when treated by different concentrations of caffeine. In control groups, most oocytes had a pole-focused spindle, whereas Sanfins et al. showed that IVM oocytes had barrel-shaped spindles; this apparent difference was attributed to the lack of hormones in the culture media in the previous study [27]. Most COCs had a convergent array of microtubules at the spindle poles when the culture media included added hormone (our unpublished data). The barrel-shaped spindle resulted from the incorporation in the spindle of an excessive number of MTOCs, which substantially reduced the stores of cytoplasmatic γ-tubulin in the oocytes [23,27,29,30]. Therefore, high concentrations of caffeine may help to incorporate more MTOCs into the spindle and reduce the stores of cytoplasmatic γ-tubulin in the oocytes.

The distribution of CGs during oocyte maturation has been used as an important criterion to evaluate cytoplasmic maturation [31–33]. Cortical granules of mature oocytes migrate to the cortex and form a continuous layer under the oolemma [34–36]. In our experiments, there was no difference between ovulated and IVM oocytes treated by caffeine in the distribution of CGs. However, oocytes treated with 5 or 10 mM caffeine had low susceptibility to activating stimuli and abnormal spindle morphology.

In summary, in vivo versus in vitro exposure to caffeine had different effects on oocyte maturation. Caffeine had no effect on the number of ovulated oocytes, the susceptibility of oocyte to activating stimuli, morphology of spindle and distribution of CGs during in vivo oocytes; however, higher concentrations of caffeine inhibited oocyte maturation in vitro. Few in-vitro-matured oocytes treated by caffeine were stimulated by $SrCl_2$, and the spindle morphology of these oocytes became disrupted.

However, caffeine had no effect on the distribution of CGs of both ovulated and IVM oocytes.

References

[1] Eskenazi B. Caffeine during pregnancy: grounds for concern? J Am Med Assoc 1993;270:2973–4.

[2] Dekel N, Galiani D, Sherizly I. Dissociation between the inhibitory and the stimulatory action of cAMP on maturation of rat oocytes. Mol Cell Endocrinol 1988;56:115–21.

[3] Horner K, Livera G, Hinckley M, Trinh K, Storm D, Conti M. Rodent oocytes express an active adenylyl cyclase required for meiotic arrest. Dev Biol 2003;258:385–96.

[4] Fan HY, Sun QY. Involvement of mitogen-activated protein kinase cascade during oocyte maturation and fertilization in mammals. Biol Reprod 2004;70:535–47.

[5] Butcher RW. Sutherland EW. Adenosine 3′,5′-phosphate in biological materials. I. Purification and properties of cyclic 3′,5′-nucleotide phosphodiesterase and use of this enzyme to characterize adenosine 3′,5′-phosphate in human urine.. J Biol Chem 1962;237:1244–50.

[6] Jagiello GM. Caffeine effects on meiotic maturation in hamster oocytes in vitro. Reprod Toxicol 1993;7:183.

[7] Prather AL, Racowsky C. Caffeine effects on meiotic maturation in hamster oocytes in vitro. Reprod Toxicol 1992;6:309–18.

[8] Kren R, Ogushi S, Miyano T. Effect of caffeine on meiotic maturation of porcine oocytes. Zygote 2004;12:31–8.

[9] Klonoff-Cohen H, Bleha J, Lam-Kruglick P. A prospective study of the effects of female and male caffeine consumption on the reproductive endpoints of IVF and gamete intra-Fallopian transfer. Hum Reprod 2002;17:1746–54.

[10] Jagiello G, Ducayen M, Lin JS. Meiosis suppression by caffeine in female mice. Mol Gen Genet 1972;118:209–14.

[11] Mailhes JB, Young D, London SN. Cytogenetic effects of caffeine during in vivo mouse oocyte maturation. Mutagenesis 1996;11:395–9.

[12] Kikuchi K, Naito K, Noguchi J, Shimada A, Kaneko H, Yamashita M, et al. Maturation/M-phase promoting factor: a regulator of aging in porcine oocytes. Biol Reprod 2000;63:715–22.

[13] Kawahara M, Wakai T, Yamanaka K, Kobayashi J, Sugimura S, Shimizu T, et al. Caffeine promotes premature chromosome condensation formation and in vitro development in porcine reconstructed embryos via a high level of maturation promoting factor activity during nuclear transfer. Reproduction 2005;130: 351–7.

[14] Senda S, Hirota K. Pyrimidine derivatives and related compounds XXII. Synthesis and pharmacological properties of 7-deazaxanthine derivatives.. Chem Pharm Bull (Tokyo) 1974;22:1459–67.

[15] Jagiello GM. A method for meiotic preparations of mammalian ova. Cytogenetics 1965;4:245–50.

[16] Miao Y, Ma S, Liu X, Miao D, Chang Z, Luo M, et al. Fate of the first polar bodies in mouse oocytes. Mol Reprod Dev 2004;69: 66–76.

[17] Luciano AM, Pocar P, Milanesi E, Modina S, Rieger D, Lauria A, et al. Effect of different levels of intracellular cAMP on the in vitro maturation of cattle oocytes and their subsequent development following in vitro fertilization. Mol Reprod Dev 1999;54:86–91.

[18] Racowsky C. Effect of forskolin on maintenance of meiotic arrest and stimulation of cumulus expansion, progesterone and cyclic AMP production by pig oocyte–cumulus complexes. J Reprod Fertil 1985;74:9–21.

[19] Rose-Hellekant TA, Bavister BD. Roles of protein kinase A and C in spontaneous maturation and in forskolin or 3-isobutyl-1-methylxanthine maintained meiotic arrest of bovine oocytes. Mol Reprod Dev 1996;44:241–9.

[20] Albertini DF, Sanfins A, Combelles CM. Origins and manifestations of oocyte maturation competencies. Reprod Biomed Online 2003;6:410–5.

[21] Eichenlaub-Ritter U, Peschke M. Expression in in-vivo and in-vitro growing and maturing oocytes: focus on regulation of expression at the translational level. Hum Reprod Update 2002;8:21–41.

[22] Hu Y, Betzendahl I, Cortvrindt R, Smitz J, Eichenlaub-Ritter U. Effects of low O_2 and ageing on spindles and chromosomes in mouse oocytes from pre-antral follicle culture. Hum Reprod 2001;16:737–48.

[23] Plancha CE, Sanfins A, Rodrigues P, Albertini D. Cell polarity during folliculogenesis and oogenesis. Reprod Biomed Online 2005;10:478–84.

[24] Can A, Semiz O, Diethylstilbestrol. (DES)-induced cell cycle delay and meiotic spindle disruption in mouse oocytes during in-vitro maturation. Mol Hum Reprod 2000;6:154–62.

[25] Eichenlaub-Ritter U, Boll I. Nocodazole sensitivity, age-related aneuploidy, and alterations in the cell cycle during maturation of mouse oocytes. Cytogenet Cell Genet 1989;52:170–6.

[26] Rossi G, Palmerini MG, Macchiarelli G, Buccione R, Cecconi S. Mancozeb adversely affects meiotic spindle organization and fertilization in mouse oocytes. Reprod Toxicol 2006;22: 51–5.

[27] Sanfins A, Lee GY, Plancha CE, Overstrom EW, Albertini DF. Distinctions in meiotic spindle structure and assembly during in vitro and in vivo maturation of mouse oocytes. Biol Reprod 2003;69:2059–67.

[28] Rossi G, Macchiarelli G, Palmerini MG, Canipari R, Cecconi S. Meiotic spindle configuration is differentially influenced by FSH and epidermal growth factor during in vitro maturation of mouse oocytes. Hum Reprod 2006;21:1765–70.

[29] Combelles CM, Albertini DF. Microtubule patterning during meiotic maturation in mouse oocytes is determined by cell cycle-specific sorting and redistribution of gamma-tubulin. Dev Biol 2001;239:281–94.

[30] Sanfins A, Plancha CE, Overstrom EW, Albertini DF. Meiotic spindle morphogenesis in in vivo and in vitro matured mouse oocytes: insights into the relationship between nuclear and cytoplasmic quality. Hum Reprod 2004;19:2889–99.

[31] Damiani P, Fissore RA, Cibelli JB, Long CR, Balise JJ, Robl JM, et al. Evaluation of developmental competence, nuclear and ooplasmic maturation of calf oocytes. Mol Reprod Dev 1996;45:521–34.

[32] Liu XY, Mal SF, Miao DQ, Liu DJ, Bao S, Tan JH. Cortical granules behave differently in mouse oocytes matured under different conditions. Hum Reprod 2005;20:3402–13.

[33] Miyara F, Aubriot FX, Glissant A, Nathan C, Douard S, Stanovici A, et al. Multiparameter analysis of human oocytes at metaphase II stage after IVF failure in non-male infertility. Hum Reprod 2003;18:1494–503.

[34] Ducibella T, Duffy P, Buetow J. Quantification and localization of cortical granules during oogenesis in the mouse. Biol Reprod 1994;50:467–73.

[35] Hoodbhoy T, Talbot P. Mammalian cortical granules: contents, fate, and function. Mol Reprod Dev 1994;39:439–48.

[36] Zamboni L. Ultrastructure of mammalian oocytes and ova. Biol Reprod 1970;2:44–63 (Suppl 2).