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Full Length Research Paper

Effect of jasplakinolide on the *in vitro* maturation of bovine oocytes

Chunling Bai[1], Cheberi[1], Lei Cheng[1], Fengxia Yin[1], Dongsheng Wu[2], Xihe Li[2], Bou Shorgan[1] and Guang-Peng Li[1]*

[1]The Key Laboratory of National Education Ministry for Mammalian Reproductive Biology and Biotechnology, Inner Mongolia University, Hohhot 010021, China.
[2]Inner Mongolia Mengniu Sexing Reproductive Biotechnology CO. Ltd, Hohhot, China.

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Jasplakinolide (JAS), a cytotoxic natural product, induces actin polymerization and increases microfilament assembly. The knowledge about the effect of JAS on oocyte meiosis in mammals is limited. The present study was to investigate the effect of JAS on the events of oocyte meiosis such as spindle configuration, chromosome alignment and segregation and oocyte maturation. The results showed that (1) JAS affected oocyte maturation and haploid composition of matured oocytes in a dose-dependent manner. The maturation rates were 70.3, 53.1, 39.7 and 20.6% after culturing oocytes in JAS at 100, 200, 300 and 400 nM for 24 h, respectively, which were significantly lower than the control group (86.2%); (2) JAS treatment caused drastically irregular meiotic spindle formation and destroyed microfilament organization; (3) JAS altered the chromosomal alignment, homologous chromosomal segregation and resulted in failure of PB1 extrusion. The majority of the non-PB1 oocytes was arrested at the tetrad state in which homologous chromosomes and sister chromatids were clearly visible; (4) pretreatment of oocytes with JAS affected the oocyte maturation even after subsequent incubation in normal maturation medium; (5) oocytes at metaphase I were much more sensitive to JAS than oocytes at anaphase I stage. In conclusion, JAS affected polar body extrusion, spindle morphology, microfilament organization and chromosome composition in a dose dependent manner and oocyte meiotic stage dependent manner.

Key words: Jasplakinilide, oocyte meiosis, spindle, microfilament, chromosome.

INTRODUCTION

Mammalian oocyte meiosis is a complicated process and intricate regulatory mechanisms may be involve in it. The accomplishment of this process correlated with organizing changes of microtubules and microfilaments at different stages of cell cycle. Microfilament, a member of cyto-skeleton, take part in many cytokinetic events such as spindle migration, polar body extrusion, cortical granule exocytosis, sperm penetration and vicinity of pronucleus (Maro et al., 1984; Longo and Chen, 1985; Schatten et al., 1986; Dimaggio et al., 1997; Sutovsky et al., 1997; Connors et al., 1998), hence, is very important in oocyte maturation and fertilization. Drug treatments of cells with some reagents are usually used for the study of microtubules and microfilaments. Cytochalasin B (CB), cytochalasin D (CD) and latrunculins (LAT) are inhibitors of microfilament assembly and aggregation. In mouse oocytes, CB did not affect spindle migration and chromosome segaregation but inhibited spindle rotation and cytoplasmic division and finally, the polar body extrusion (Zhu et al., 2003). Latrunculins A (LAT A) and CD treatment also resulted in inhibition of spindle rotation and polar body extrusion (Navarro et al., 2005; Houssen et al., 2006).

Jasplakinolide (JAS) isolated from marine sponge is a

*Corresponding author. E-mail: guangpengli@yahoo.com. Tel: 86-10-471-4994329. Fax: 86-10-4992147.

Abbreviations: CB, Cytochalasin B; **LAT,** latrunculins; **JAS,** jasplakinolide; **MFs,** microfilaments; **COC,** cumulus oocyte complexes; **FBS,** fetal bovine serum; **HEPES,** N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic; **FSH,** follicle stimulating hormone; **PB1,** first polar body; **PBS,** phosphate buffered saline; **Ig G,** immunoglobulin G; **GVBD,** germinal vesicle breakdown.

cyclic peptide with a 15-carbon macrocyclic ring containing three amino acid and has fungicidal and antiproliferative activity. Contrary to CB and CD, JAS promotes polymerization of actin under non-polymerizing conditions and stabilizes actin filaments (Bubb et al., 1994). JAS treatment resulted in microfilaments (MFs) polymerization and stabilization in cultured rabbit muscle cells (Bubb et al., 2000). JAS could promote the metastasis of some kind of cancer cells, but CD and LAT exhibited inhibitory effects (Hayot et al., 2006). In yeast cells, JAS introduction caused actin aggregation, fragmentation of nucleus and increase of active oxygen concentration, which indicated that JAS induced apoptosis of yeast cells (Gourlay et al., 2004). Similar apoptotic phenomena were also observed in lymphocyte. JAS treatment caused cell morphology alteration, the uniform distribution of microfilaments under plasma membrane was substituted by non-uniform distribution and massive microfilaments surrounded the nucleus (Posey et al., 1999).

Most recently, JAS treatment displayed quicker release of Ca^{2+} in response to fertilizing sperm in starfish oocytes (Kyozuka et al., 2009), induced abnormal chromosomal alignment and lose of chromosomes (Lénárt, et al., 2005). Pre-treatment of starfish eggs with JAS resulted in polyspermy in which JAS completely blocked the vitelline layer elevation and failure of the fertilization cone formation occurred (Puppo et al., 2008).

The effect of JAS on mammalian oocytes was only reported in mouse. JAS, even at very low concentration, caused wide range accumulation of microfilaments at cortex and inhibited spindle migration and polar body extrusion (Terada et al., 2000). Though JAS did not inhibit sperm head penetration into oocytes, tail penetration was totally inhibited. Moreover, cortical granular exocytosis, formation and depolymerization of fertilization cone, pronuclear migration and PB2 extrusion were also inhibited (Terada et al., 2000). Treatment of zonae pellucidae-freed mouse oocytes with JAS resulted in a decrease in the percentage of eggs fertilized and the average number of sperm fused per egg (McAvey et al., 2002).

The present study was designed to investigate the effects of JAS on bovine oocyte maturation, spindle configuration, chromosomal composition and if JAS-treated oocytes could be rescued by subsequent culture in normal maturation medium.

MATERIALS AND METHODS

Chemicals

All chemicals were purchased from Sigma Chemical Company (St. Louis, MO) except otherwise stated. Stock solutions of JAS (Invitrogen) was prepared to 100 µM with dimethyl sulfoxide and stored at -20°C. It was diluted with medium before use. The final concentrations of JAS in the maturation media were 0 (control), 100, 200, 300, 400, 600, 800 or 1000 nmol, respectively, according to the experimental design.

Oocyte maturation *in vitro* (IVM)

Maturation of oocytes was as previously described (Li et al., 2004a,b). Briefly, bovine cumulus oocyte complexes (COC) were aspirated from 3 to 8 mm diameter follicles on the surface of ovaries collected from a local slaughterhouse. Oocytes with at least four layers of intact, compact cumulus cells and with a homogenous ooplasm were selected. The maturation medium was TCM199 with Earle's salts, L-glutamine and sodium bicarbonate (Gibco Inc., Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) (Gibco BRL), 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic (HEPES) acid, 0.01 g/ml E2 (Sigma), 0.01 IU/ml follicle stimulating hormone (FSH) (NIH-FSH-S17) and 1 IU/ml LH (USDA-bLH-6). COC were incubated in maturation medium containing different concentrations of JAS in 4-well plates (Nunc, Roskilde, Denmark) with 0.5 ml medium. Fifty oocytes/per well were maintained under 5% CO_2 in atmosphere at 39°C and with high humidity.

Assessment of oocyte maturation

After incubation for 24 h, cumulus cells were removed by vortexing for 4 min in 0.5 ml 100 IU/ml hyaluronidase medium. The maturation rates were determined by the presence of the first polar body (PB1).

Immunofluorescence assays of microtubules and microfilaments

After removing the cumulus cells, oocytes were fixed and permeabilized at the same time with 4% (w/v) paraformaldehyde and 0.2% Triton-100 in PBS at room temperature for 1 h. For microtubule staining, after washing thrice with phosphate buffered saline (PBS) containing 3% bovine serum albumin (BSA), oocytes were incubated overnight at 4°C with a mouse monoclonal antibody against α-tubulin (Sigma, T-5168) diluted by PBS (1:500). On the second day, oocytes were washed thrice with PBS containing 0.3% BSA and incubated in fluorescein isothiocyanate-labeled goat-anti-mouse immunoglobulin G (Ig G) (Southern Biotechnology Associate, Inc. Birmingham, AL 35226. Cat no. 1030-02) diluted with PBS (1:500) for 1 h. After three washes in washing solution, nuclear state of oocytes were evaluated by staining with propidium iodide (PI, 10 µg/ml in PBS) for 10 min. Finally, oocytes were mounted on slides with glycerol: PBS (1:1). The samples were examined under a laser confocal scanning microscopy.

For microfilament staining, after washing with PBS containing 0.3% BSA, oocytes were stained with 1 µg/ml fluorescein isothiocyanate-phalloidin (Sigma, P-5282) diluted 1:200 with PBS for 1 h at 37°C. Chromatin staining and sample examination was as described earlier.

Chromosomal analysis

Oocytes incubated for different times, with or without PB1 from each group were separately transferred to 0.8% trisodium citrate for 5 to 8 min and then, fixed by fresh methanol: glacial acetic acid (3:1) on clean glass slides. After being dried for more than 24 h at room temperature, the slides were stained with 1% Giemsa for 15 to 20 min. Chromosome number were scored for each oocyte under a microscope (Nikon, Japan) fitted with an image system.

Experimental design

Experiment 1

This experiment was to examine the chromatin morphological

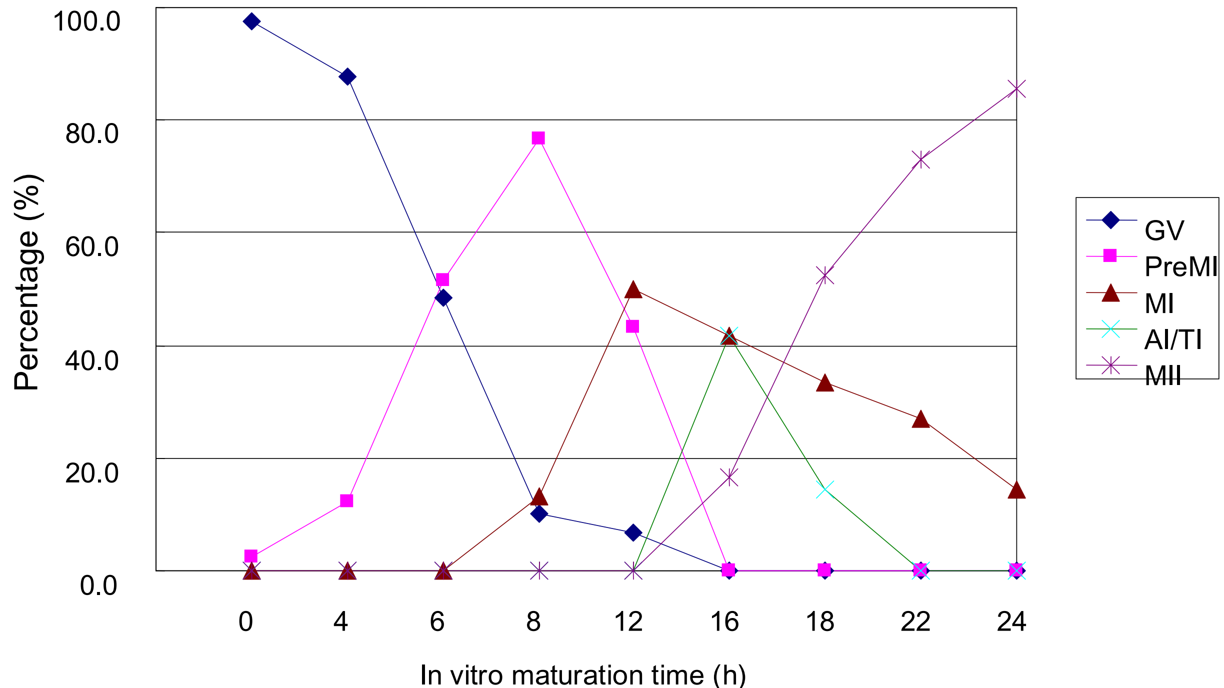

Figure 1. Bovine oocyte meiotic progression under normal maturation condition.

dynamic changes during oocyte IVM. COC were incubated for 0, 4, 6, 8, 12, 16, 18, 22 and 24 h, respectively and then the cumulus cells were removed and prepared cytogenetically to observe their chromatin (chromosome) state.

Experiment 2

This experiment was to study the effect of JAS on bovine oocyte maturation. Oocytes were randomly selected and incubated in 100, 200, 300 and 400 nM JAS-containing media for 24 h and then, the maturation rates were evaluated, respectively. The chromosomal segregations of oocytes without PB1 from 200, 300 and 400 nM treated groups were cytogenetically analyzed, respectively.

Experiment 3

The results from experiment 2 showed that a high proportion of oocytes treated with JAS at 300 and 400 nM did not expel PB1. This experiment was designed to investigate the behavior of the meiotic spindles and chromosomal movement under the influence of JAS. After culturing the oocytes in JAS-containing media for 8, 12, 16, 20 and 24 h, the oocytes from each group were fixed and analyzed by microtubule and microfilament immunefluorescent staining, respectively.

Experiment 4

This experiment was designed to study if pretreatment of oocytes with JAS affected their maturation. Oocytes were pretreated with JAS at 200, 300, 400 nM for 12 and 16 h, respectively. Then, the treated oocytes were intensely washed and continued to incubate in normal maturation medium to 24 h and the maturation rates were evaluated.

Experiment 5

Pretreatment of COCs with JAS in experiment 4 significantly decreased oocyte maturation rates. This experiment was designed to study whether post-treatment of oocytes with JAS affected oocyte maturation. One group of oocytes were first cultured in normal maturation medium for 12 h, in which the oocytes were developed to MI stage then, transferred to JAS-media at 200, 300 and 400 nM, respectively and cultured for 24 h. A second group of oocytes were first cultured in normal maturation medium for 16 h, in which the oocytes were at AI stage, the oocytes were then transferred to JAS-media at 200, 300, 400, 600, 800 and 1000 nM, respectively and cultured for 24 h.

Statistical analysis

Differences in maturation rates were tested by analysis of variance (ANOVA). Proportions of chromosomal composition between experimental groups were analyzed using Chi-square and the Student's t-test. A probability of $P < 0.05$ was considered statistically significant.

RESULTS

Chromatin dynamic changes during bovine oocyte maturation

Bovine oocyte meiotic progression under normal maturation condition is shown in Figure 1. During the first 4 h, the oocytes were at GV stage, the chromatins were at diplotene or diakinesis (Figure 2a to d); after being cultured for 6 h, 51.6% of the oocytes showed germinal

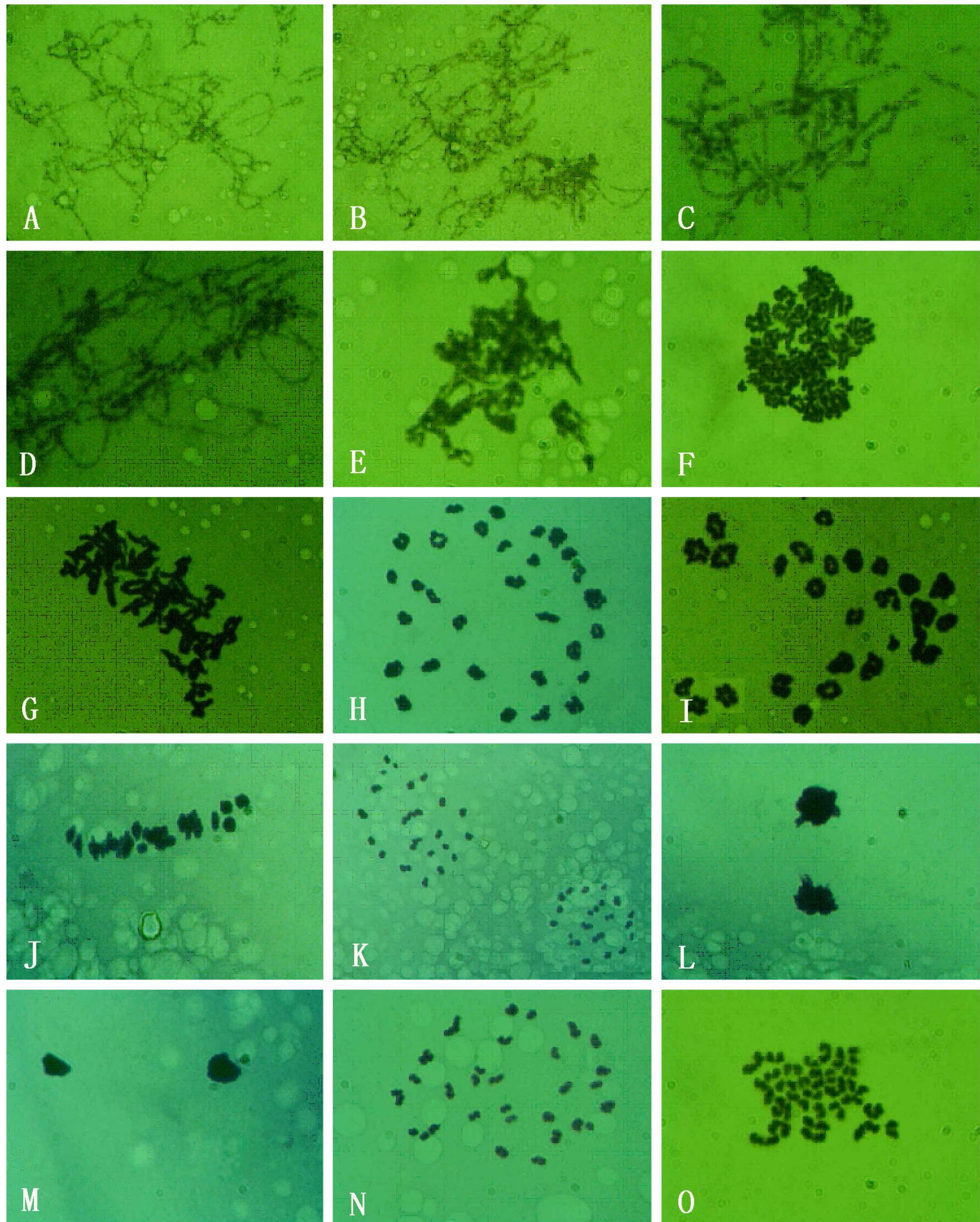

Figure 2. Chromosomal state of bovine oocyte meiosis from metaphase I to metaphase II (1000×). A-B: GV oocytes, IVM 0 h; C-D: GV oocytes, IVM 4 h; F-G.: prometaphase I oocytes, IVM 12 h; H-J: M I s oocytes, IVM 14 to 16 h; K: early anaphase I oocytes, IVM 14 to 16 h; L: late anaphase I oocytes, IVM 16h; M: telophase I oocytes, IVM 16 to 18 h; N-O: metaphase II oocytes (IVM 18 to 24 h).

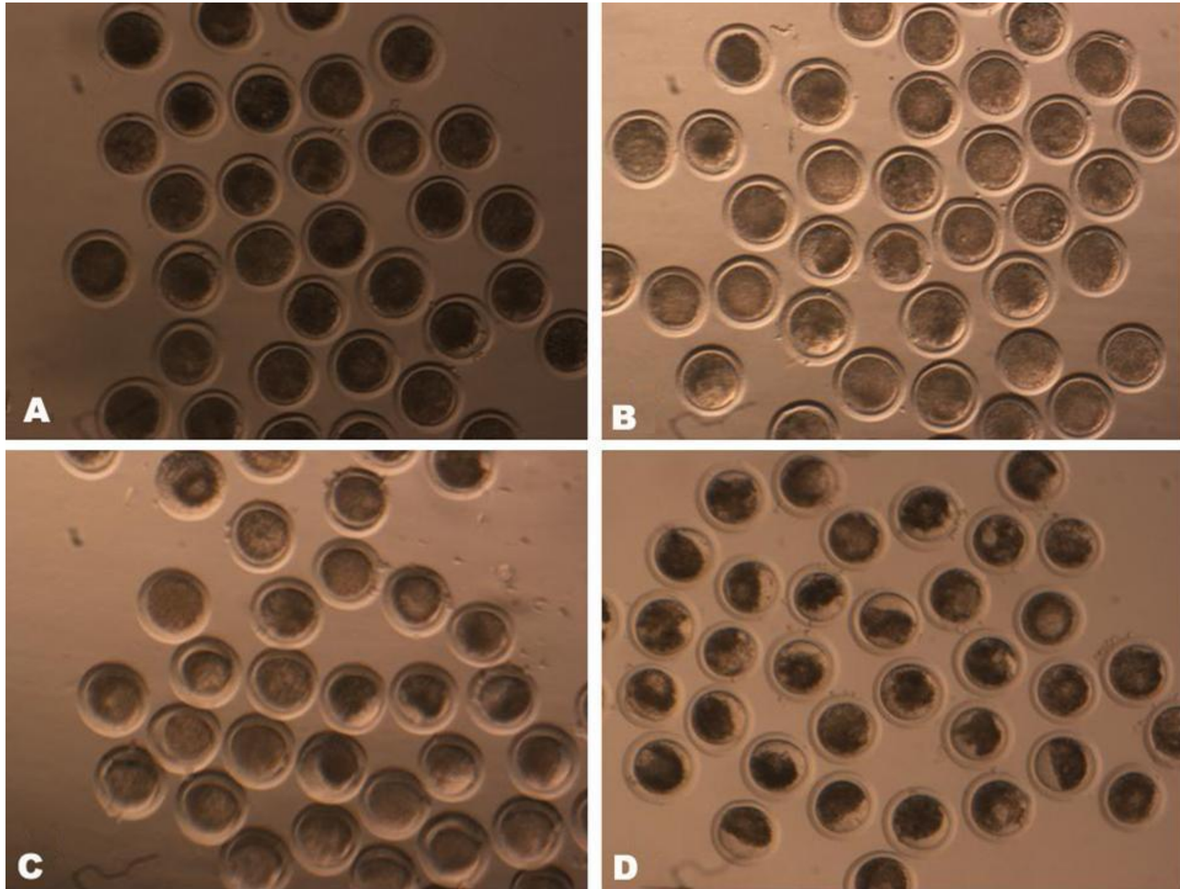

Figure 3. Morphology of oocytes incubated in control (A), 200 nM (B), 300 nM (C) and 400 nM (D) JAS-containing media for 24 h. (100 ×).

vesicle breakdown (GVBD), and almost all of the oocytes showed GVBD at IVM 8 h, the chromatins gradually condensed (Figure 2e); 12 h later, the oocytes reached to pre-metaphase I and metaphase I (M I) stages, chromatins became bivalent chromosomes in visible tetrad state (4c) (Figure 2f to g and Figure 2h to j); more than 40% of oocytes developed to anaphase I and telophase I (AI/TI) stages and 18% to metaphase II (M II) at IVM 16 h, the homologous chromosomes separated in very early AI stage (Figure 2k). The chromosomes condensed together and solid mass-like structure formed in late AI and TI stages (around IVM 18 h, Figure 2l to m); the chromosomes re-decondensed and became countable when oocytes developed to M II (18 to 24 h IVM).

JAS affected oocyte maturation and oocyte haploid composition in a dose-dependent manner

After culturing oocytes in JAS-containing medium, the oocyte morphology changed. In the control oocytes, the ooplasm were in dark and homogenously distributed (Figure 3a), while the cytoplasm became pale and the ooplasm were distributed in a polarized and much more polarization occurred with the increase of JAS concentrations (Figure 3b to d).

Treatments of oocytes with JAS at 100, 200, 300 and 400 nM for 24 h drastically decreased the maturation rates to 70.3, 53.1, 39.7and 20.6%, respectively, when compared with the control (86.2%). Analysis of the PB1 oocytes showed that, about 80% of the oocytes derived from 100 and 200 nM JAS contained haploid composition (n=30), with no difference to the control (86%). When JAS concentrations were increased to 300 and 400 nM, significantly lower haploid complements (61%) were obtained (Table 1).

Data in Table 1 shows that, more than half of the oocytes incubated in JAS at or over 200 nM did not expel PB1. Chromosomal analyses of these non-PB1 oocytes revealed that most of the oocytes from 200 and 300 nM groups were arrested at the tetrad stage (from 59.6 to 73.9%) (Figure 4b to d). While in the 400 nM group, oocytes were mainly diploid containing two 30-chromosome-plates (Figure 4e to f) or a 60-chromosome-plate (Figure 4g to h) and the proportion of the 60-plates

Table 1. Maturation of bovine oocytes incubated in JAS-containing media for 24 h.

JAS (nM)	No. of oocytes	Oocytes with PB1 (%)	Chromosome status of PB1 oocytes		
			No.	n=30	Non haploid
Control	232	200 (86.2)[a]	121	105 (86.8)[a]	16(13.2)[b]
100	293	206 (70.3)[b]	136	109 (80.1)[a]	27(19.9)[b]
200	369	196 (53.1)[c]	119	93(78.1)[a]	26(21.8)[b]
300	397	158 (39.7)[c]	120	74 (61.7)[b]	46(38.3)[a]
400	559	115 (20.6)[c]	98	60 (61.2)[b]	38(38.8)[a]

Different letters in a column indicate significant difference ($p < 0.05$).

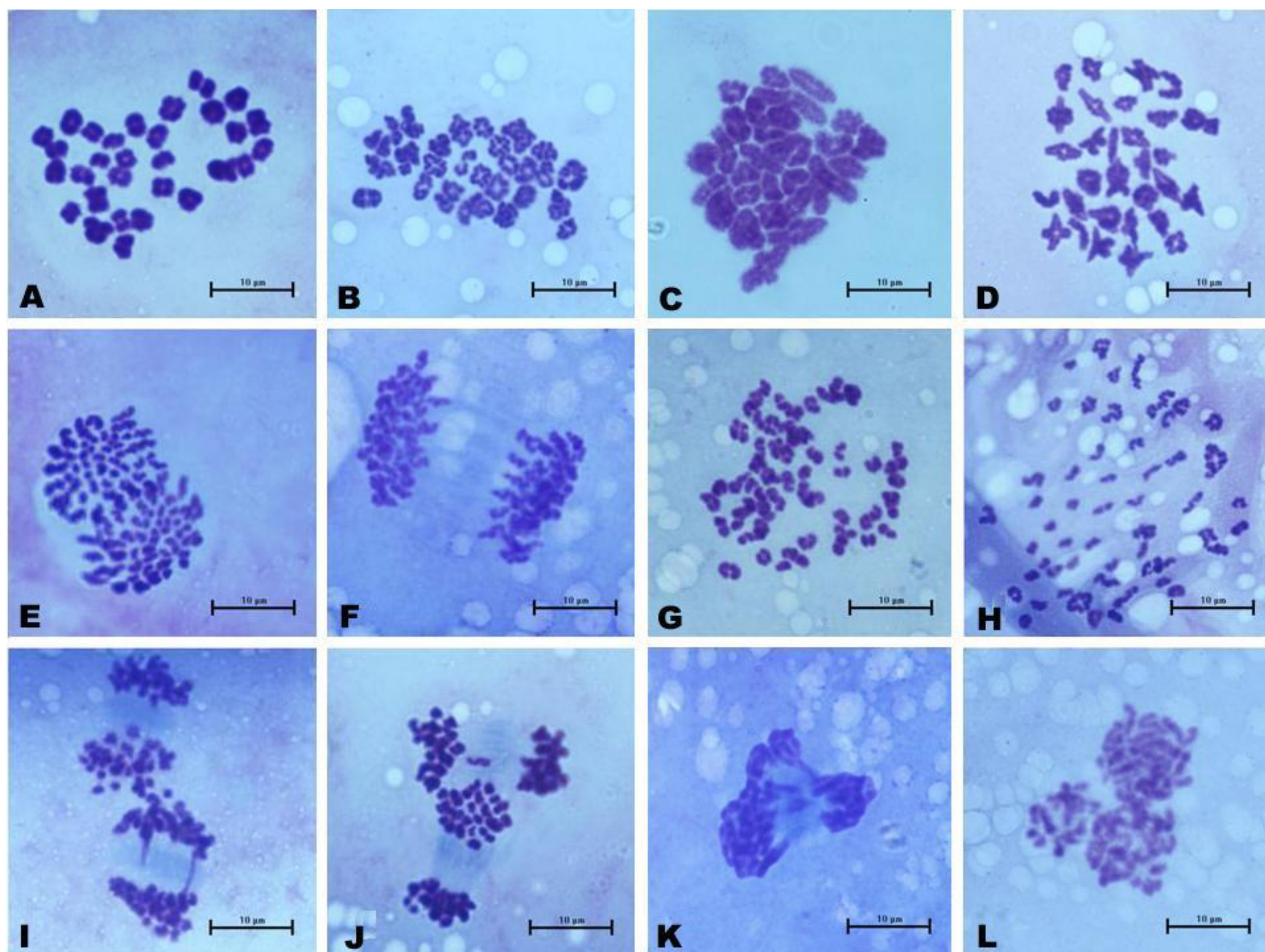

Figure 4. Chromosomal state of oocytes did not expel PB1 after incubation in JAS media. A: MI chromosomes from the arrested control oocytes; B-D: tetrad spreads in which 4 chromatids visible; E-F: two 30-chromosome plates in a single oocyte; G-H: one large diploid, 2n (4c) = 60; I-J: two sets of anaphase spindles in a single oocyte; K-L: Abnormal chromosome separation (bar =10 µm).

was significantly higher than the two 30-plates (Table 2). In addition, some treated oocytes formed two sets of anaphase spindles (Figure 4i to j) leading to the formation of abnormal chromosomal segregation (Figure 4k to l).

JAS induced changes of meiotic spindle pattern and chromosome segregation

After treatment of oocytes with JAS, the morphology and

Table 2. Chromosomal analysis of the non-PB1 oocytes treated by JAS for 24 h.

JAS (nM)	Oocytes with no PB1	Tetrad (%)	Diploidy (%) *			MI	>60-chr.
			2 to 30-chr	60-chr	Total		
200	46	34(73.9)[a]	2(4.3)	7(15.2)[a]	9(19.5)	3(6.5)	0
300	47	28(59.6)[b]	3(6.4)	14(29.8)[b]	17(36.2)	0	2(4.3)
400	47	12(25.5)[c]	8(17.0)	17(36.2)[c]	25(53.2)	9(19.1)	1(2.2)

*2 to 30-chro: two 30-chromosome-plate; 60-chro: 60-chromosome-plate; >60-chro: chromosome number over 60. Different letters in a column indicate a significant difference ($p < 0.05$).

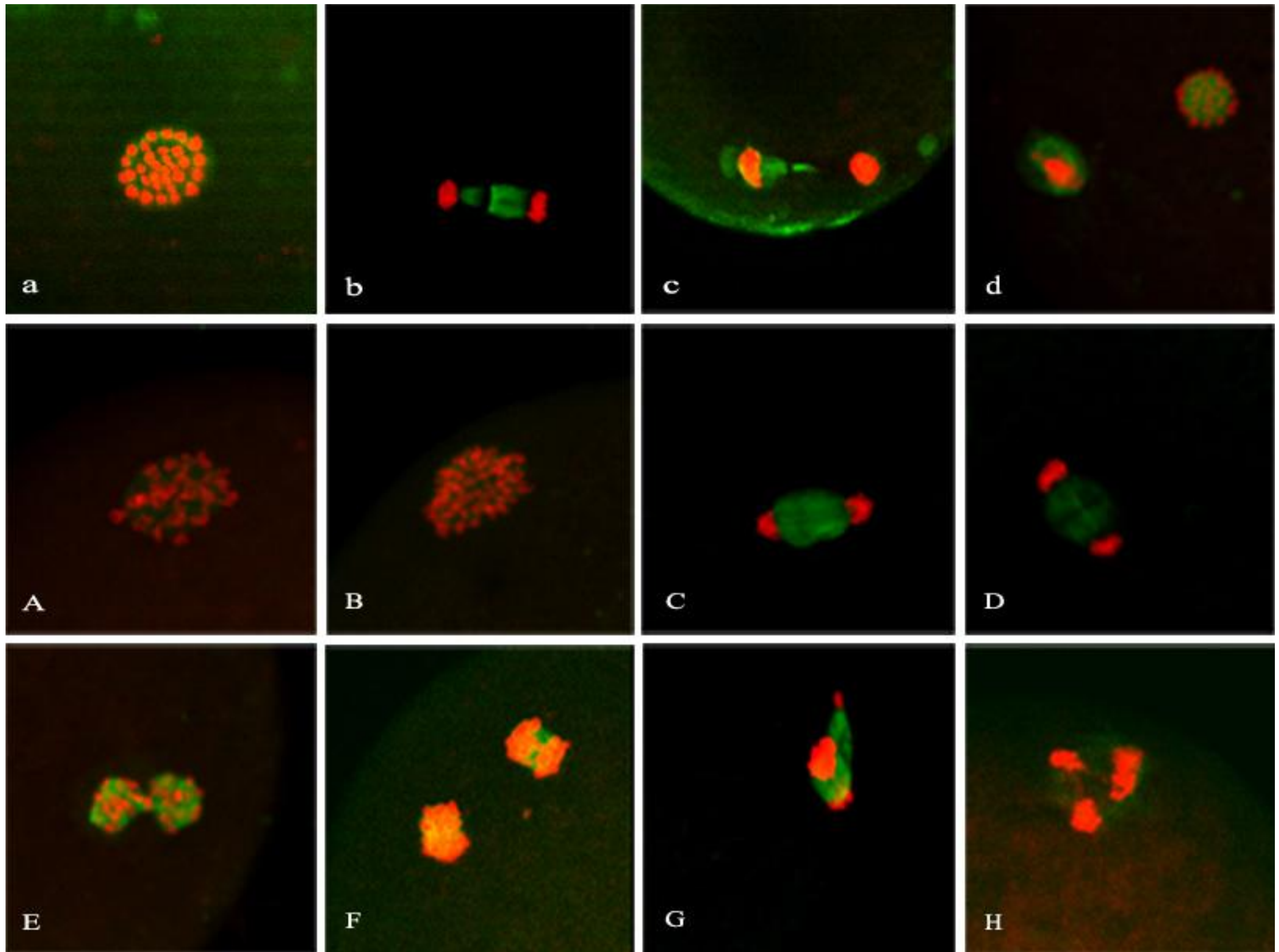

Figure 5. The effects of JAS on microtubule organization and chromosome morphology in bovine oocytes. A-D: Control oocytes at MI, AI/TI and MII stage; A-H: oocytes treated by JAS for 24 h. A: tetrad oocyte (bivalent); B: a large spindle; C-D: AI stage oocytes; E: 2-spindle oocytes; F: tow sets of anaphase spindles; G-H: tri-polar spindles (all images 400×).

pattern of the spindle structures were visibly altered. In the control group, spindle microtubules began to asymmetrically distribute into two separate chromosome groups in early AI. The spindle microtubules were triangularly shaped and the majority of them were moved towards the forming PB1 (Figure 5a to d). In JAS-treated group, a high proportion of oocytes were with abnormal spindles (Figure 5a to h), especially in 400 nM group. These disfigured spindles including: (1) Arrested tetrad chromosomes (Figure 5a); (2) although homologous chromosome segregated, the oocytes did not develop to anaphase, the number of chromosomes duplicated and

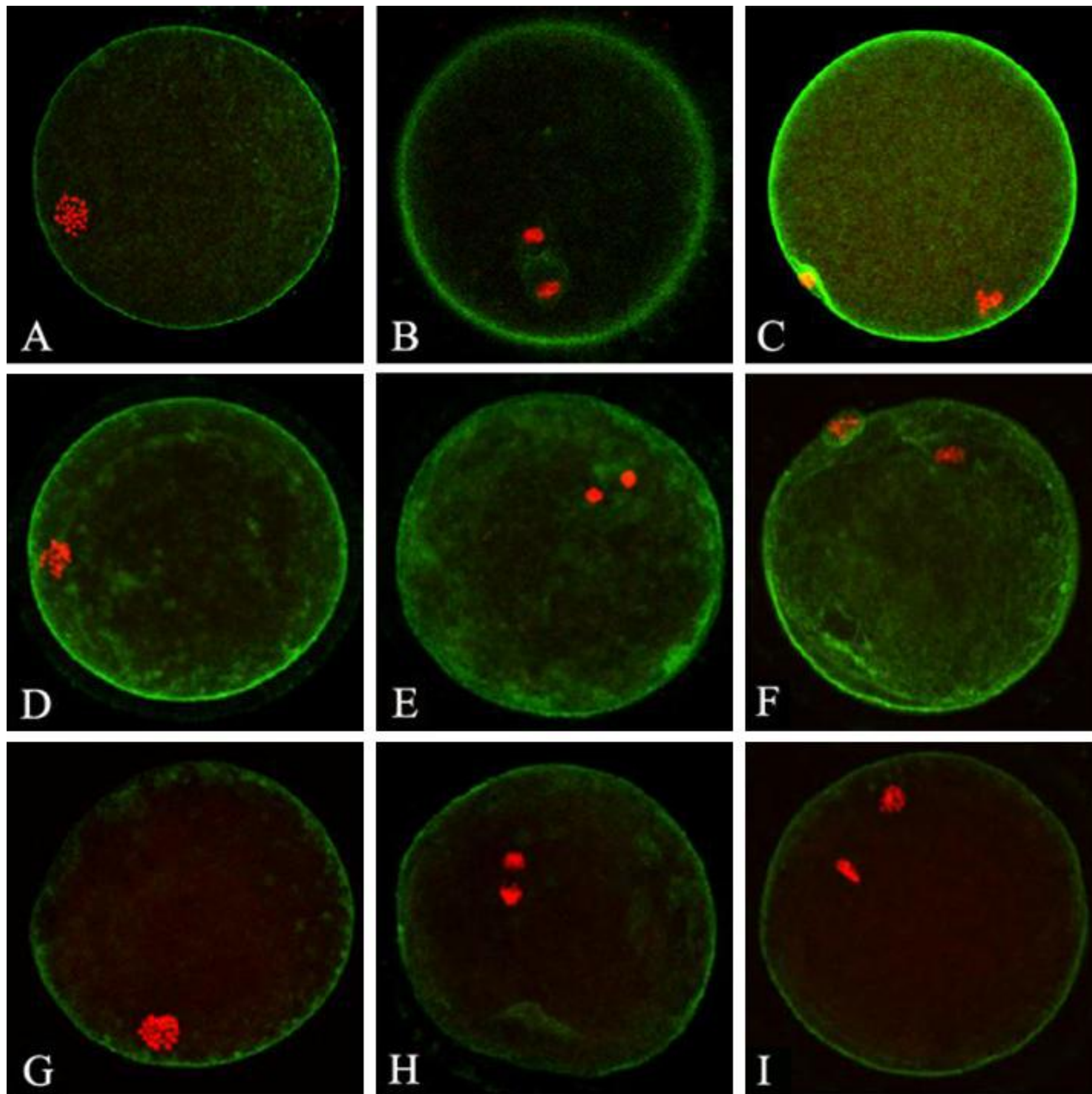

Figure 6. The effects of JAS on microfilament organization in bovine oocytes. A-C: Control oocytes at MI, AI and MII stages, respectively; D-I: oocytes incubated in JAS containing medium at concentration of 200 nM (D-F) or 400 nM (G-I). A, D, G: metaphase I; B, E, H: anaphase I; C, F, I: metaphase II. (All images 200×).

formed spindles much larger than normal (Figure 5b); (3) the spindle microtubules were almost equally distributed into two separated groups of chromosomes during AI to late TI stage (Figure 5c and d); (4) two spindles existed in a single oocytes (Figure 5e to f); (5) tri-polar spindles occurred (Figure 5g and h). As to microfilaments, JAS treatment affected their organization. In the control (Figure 6a to c), the microfilaments were evenly distributed under plasma membrane. No obvious microfilaments distributed around MI chromosomes and rich microfilaments surrounded the separated two masses of chromatin in AI or TI oocytes. In JAS-treated oocytes, the microfilaments were disorganized as follows: (1) Microfilaments under plasma membrane became non-uniform or disappeared in some or entire cortex region (Figure 6e to h); (2) microfilaments became disorganized into various

Table 3. Maturation of bovine oocytes in JAS-containing media in the first 12 h then into normal maturation medium.

First culture in JAS(nM) (h)	Total IVM time (h)	No. oocytes	Oocytes with PB1 (%)
Control (24)	24	147	123 (83.7)[a]
200 JAS, 12	24	169	106 (62.7)[b]
300 JAS, 12	24	180	78(43.3)[c]
400 JAS, 12	24	183	59(32.2)[d]

At the same column, ab, ac, ad, bc and bd: $P < 0.01$; cd: $P < 0.05$.

Table 4. Maturation of bovine oocytes in JAS-containing media in the first 16 h then into normal maturation medium to 24 h.

First culture in JAS(nM) (h)	Total IVM time(h)	No. oocytes	Oocytes with PB1 (%)
Control (24)	24	185	159 (85.9)[a]
200 JAS, 16	24	224	135 (60.3)[b]
300 JAS, 16	24	215	78 (36.3)[c]
400 JAS, 16	24	243	68 (28.0)[c]

At the same column, ab, ac and bc: $P < 0.01$.

Table 5. Maturation of bovine oocytes in JAS-containing media after normally cultured for 12 h.

JAS(nM)	No. oocytes	Oocytes with PB1 (%)
Control	156	133 (85.2)[a]
200	143	100 (69.9)[b]
300	185	138 (74.6)[b]
400	200	143 (71.5)[b]

At the same column, a, b: $P < 0.05$.

Masses scattered around the cytoplasm (Figure 6d to f); (3) when JAS concentration reached 200 nM, the microfilaments were not distributed around chromatin masses but out of chromatin region in AI and TI stage oocytes (Figure 6e); (4) in the 400 nM treated oocytes, the microfilaments almost disappeared (Figure 6g to i).

Pretreatment of oocytes with JAS affected their maturation

Tables 3 and 4 show that, the pretreatment of oocytes with JAS-containing media for 12 or 16 h significantly decreased the maturation rates when compared with the controls.

Post-treatment of oocytes with JAS decreased oocyte maturation

When oocytes were incubated in normal maturation medium for 12 or 16 h, the majority of the oocytes developed to M I or A I stages, respectively. Treatment of M I oocytes with JAS at 200, 300 and 400 nM resulted in 69.9, 74.6 and 71.5% of the oocytes developed to MII, respectively, which were significantly lower than the control group (85.2%) (Table 5). When oocytes at A I stage were treated with JAS at 200 to 400 nM, similar maturation rates were obtained when compared to the control. However, when JAS concentrations reached 600, 800 and 1000 nM, the oocyte maturation rates significantly decreased ($p < 0.01$) (Table 6).

DISCUSSION

In mouse, treatment of oocytes with CB resulted in the arrest of oocytes at M I stage (Wassarman et al., 1976), inhibited PB1 and also PB2 extrusion, but without any effect on the formation and movement of pronuclei (Kim, 1997). CB at 1.0 μg/ml concentration was enough to inhibit PB1 extrusion in pig (Somfai et al., 2006). Cytochalasin D did not affect segregation of homologous chromosomes but inhibited PB1 extrusion, resulting in two meiotic spindles in the mouse oocytes (Kubiak et al., 1991). In starfish, treatment of oocytes with LAT B resulted in 75% of oocytes, displayed abnormal chromosome alignment and severe chromosome lose, which indicated that chromosome progression not only require microtubules but also the involvement of microfilaments

Table 6. Maturation of bovine oocytes in JAS-containing media after normally cultured for 16 h.

JAS (nm)	No. oocytes	Oocytes with PB1 (%)
Control	135	118 (87.4)[a]
200	120	102 (85.0)[a]
300	132	111 (84.0)[a]
400	135	110 (81.5)[a]
600	263	166 (63.1)[b]
800	286	174 (60.8)[b]
1000	266	108 (40.6)[c]

At the same column, a, b and b, c and a, c: $P < 0.01$.

(Lénárt et al., 2005).

As contrary to CB, CD and LAT B, JAS promote actin filament assembly and stabilize their movement (Bubb et al., 1994; Holzinger et al., 1997). JAS acts to decrease the apparent critical concentration of G-actin, enhancing nucleation and inhibiting subunit dissociation from filaments (Bubb et al., 2000). The knowledge about the effect of JAS on oocyte meiosis in mammals is very limited, only one report in mouse. Treatment of mouse oocytes with JAS at 100 nM caused 90.5% of the oocytes arrested at M I stage and the spindles were localized at cell center with no PB extrusion (Terada et al., 2000).

Consistent with the findings in mouse, JAS did not affect bovine oocyte GVBD, but affected oocyte maturation in dose dependent manner and oocyte-stage dependent manner. When JAS concentration increased from 100 to 400 nM, the PB1 extrusion decreased from 70.3 to 20.3%. Pretreatment of oocytes with JAS caused significantly lower number of oocytes developing to M II and the meiotic process did not resume even after removal of JAS (Tables 3 and 4). When oocytes at M I stage were treated with JAS, there was a significant decreased in maturation. However, similar concentrations of JAS did not affect the maturation rate when anaphase I oocytes were treated. These results indicated that, the destructive influence of JAS on oocyte maturation was irreversible, M I-stage oocytes were more sensitive to JAS than A I-stage oocytes. It is noted that, JAS treatment caused significantly lower haploid composition of the matured oocytes which was consistent with the observation in starfish. JAS treatment of starfish oocytes induced abnormal chromosomal alignment and loses of chromosomes (Lénárt et al., 2005; Kyozuka et al., 2009). The results from CB-, CD- or JAS-treatment suggest that, either inhibition or promotion of actin polymerization have impact on polar body formation and inhibit PB1 extrusion.

When mouse oocytes were exposed to CD, segregation of homologous chromosomes occurred without extrusion of PB, resulting in two meiotic spindles in the oocytes. Later, these two spindles merged into one single spindle (Kubiak et al., 1991). The use of CB instead of CD caused almost the same effect in pig (Somfai et al., 2006). In the present study, we found that JAS did not inhibit homologous chromosome segregation, but inhibited PB1 extrusion as occurred in mouse and pig. When JAS was at low concentration (200 nM), the majority of the oocytes were arrested at the tetrad state; two pairs of homologous (4 sister chromatids) were clearly observed. If JAS concentration reached 400 nM, most of the non-PB1 oocytes were diploid containing either two-30-chromosome plates or a 60-chromosome plate (Figure 1) and 60-plates were at least two times more than two-30-plates. Analyses of the treated oocytes by both immunostating and cytogenetical techniques suggested that, JAS inhibited anaphase chromosome movement to telophase and caused the oocytes arrested at anaphase I. The arrested anaphase chromosomes gradually move towards each other and then merged together to form 60 chromosome arrangement. Similar phenomena were observed in nicotine-treated bovine oocytes (Liu et al., 2008; Liu et al., 2009), CB-treated porcine oocytes (Samfai et al., 2006) and bovine oocytes (the author's unpublished data). The mechanisms involved in it are not clear.

Normally, in bovine, oocyte spindles presented barrel shape at MI and MII stage, column shape at AI, triangular shape at TI and almost all of the microtubule and microfilament were destined to polar body (Li et al., 2005). The present study showed that JAS treatment led to formation of abnormal spindle configuration, such as two spindles, one large spindle, multi-polar spindles and symmetric but not triangular AI/TI spindles. Abnormality of reassembled spindles and chromosomes caused occurrence of aneuploidy. In the control oocytes, microfilaments evenly distributed in the cortex area beneath the oocyte membrane at GV, GVBD, premetaphase I and MI stages and did not appear in the chromatin area until early anaphase stage. Rich microfilaments surrounded the separated two masses of chromatin in AI or TI oocytes (Li et al., 2005). In JAS-treated oocytes, the microfilaments became non-uniform or disappeared in some or entire cortex region (Figure 6e to h) and masses of microfilaments scattered around the cytoplasm (Figure 6d to f). Similar phenomena were also observed in CB-treated oocytes in bovine (authors' unpublished data). In mouse, CB did not change cortical microfilament distribution

(Longo and Chen, 1984), while cortical actin filament aggregated into bundles in JAS-treated oocytes (Terada et al., 2000). JAS treatment of somatic cells also induced aggregation and stabilization of actin filaments and caused the polymerization of actin to undefined crumb structure in live individual (Bubb et al., 2000). These results suggest that, the co-operation of microtubules and microfilaments is important for oocyte meiosis.

In conclusion, JAS an actin polymerization promoter and stabilizer altered the normal spindle microtubule and microfilament distribution, thereafter, affected the meiotic process, inhibited the polar body extrusion and resulted in decreased haploid composition. Both pretreatment and post-treatment of oocytes with JAS decreased oocyte maturation. The dynamic destruction between actin polymerization and depolymerization not only influence the function of microfilaments but also the spindle pattern and chromosome composition.

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