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Ovine cumulus cells estradiol-17ß production in the presence or absence of oocyte

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Abstract

The objective of the present study was to compare the in vitro production of estradiol-17ß (E_2) by cumulus cells in the presence or absence of ovine oocyte. Moreover, the relationship between the concentration of produced estradiol-17ß and oocyte nuclear maturation was assessed.

Ovaries collected from the local abattoir were transported to the laboratory in saline at 30–35 °C within 1–3 h after collection. The oocytes of follicles, 2–6 mm in diameter, were recovered by aspiration. The oocytes with evenly granulated cytoplasm and which were surrounded with at least three layers of cumulus cells were selected and subjected to culture in pre-incubated oocyte culture medium (OCM). Before culturing, the selected oocytes were randomly divided into five treatment groups: Group 1, cumulus enclosed oocytes cultured in OCM (Group COCs); Group 2, denuded oocytes cultured in OCM (Group D); Group 3, denuded oocytes co-cultured with a cumulus cell-monolayer in OCM (Group D + M); Group 4, denuded oocytes co-cultured with previously cultured (for 26 h) cumulus cell-monolayer (10^5 cells/ml) in refreshed OCM (Group $D + M_{26}$); Group 5, cumulus cell-monolayer (10^5 cells/ml) cultured in OCM (Group M). After an incubation period (26 h at 38.6 °C, 5% CO_2 and 100% humidity), the media were collected and kept at −20 °C until hormonal assay. The concentration of E_2 was determined by RIA method. For assessment of nuclear status, the completely denuded oocytes were subjected to DAPI staining. The highest percentage of metaphase II (MII) stage oocytes was observed in Group N (91%) and the lowest percentage was observed in Group D (6%) and Group $D + M_{26}$ (6%). The mean production of E_2 was highest and lowest in Group D + M (378.69 ± 54.34 pg/ml) and Group $D + M_{26}$ (109.15 ± 8.24 pg/ml), respectively. The production of E_2 was significantly ($P < 0.01$) higher in Group D ± M when compared with Groups M and $D \pm M_{26}$. Regarding the nuclear maturation, the percentage of MII stage oocytes was significantly ($P < 0.001$) higher in Group COCs compared to the other groups.

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The results suggest that steroidogenic activity of cumulus cells in in vitro condition can be influenced by the pattern of connection between cumulus cells and the oocyte. Moreover, the nuclear maturation of oocytes is not influenced by the different production levels of E_2.
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Keywords: Sheep; Oocyte; Cumulus; Estradiol-17ß; Nuclear maturation

1. Introduction

The presence of steroids in the follicular fluid at the time of oocyte maturation suggests that they may play a role in oocyte maturation (Anisworth et al., 1980). It has been suggested that estradiol acts directly on the oocyte because specific receptors have been identified in oocytes of various species (Danforth, 1995). In mouse, both testosterone and estradiol are able to overcome the inhibitory signal and promote germinal vesicle (GV) breakdown (Gill et al., 2004). In contrast, it has been demonstrated that estradiol as well as other steroids are involved in keeping oocytes in meiotic arrest (Barrett and Powers, 1993; Mingoti et al., 1995).

An established role of estradiol is to promote alteration in the reactivity of the calcium liberation system during cytoplasmic maturation (Tezarik and Mendoza, 1997). In this respect, those oocytes that were matured in the presence of estradiol-17ß (E_2) were more likely to develop two pronuclei and cleaved further than oocytes incubated in the absence of estradiol-17ß (Tezarik and Mendoza, 1995). By contrast, there is evidence indicating that the estradiol-17ß supplementation had no effect on the percent of oocytes that matured in vitro (Palter et al., 2001). These observations were taken to mean that the specific role of estrogens in oocyte maturation seems to be species dependent (Moudgal et al., 1996).

In sheep, estradiol receptor and its mRNA were observed in the cumulus cells surrounding oocytes in small (<2 mm) and medium size (2–4 mm) follicles (Tomanek et al., 1997). The presence of estrogen receptors (ER⟨alpha⟩ and ER⟨beta⟩) in ovine granulosa cells (GC) and other ovarian compartments during the early stages of follicular growth indicates that E_2 has the potential to regulate the function of a number of different ovarian cell types (Juengel et al., 2006).

It has been shown that mouse oocytes secrete a factor that modulates E_2 and P_4 production in cultured granulosa cells (Vanderhyden et al., 1993). Further studies have shown that the pig oocytes are capable of directing the functional production of cumulus cells by changing their signals on the basis of their own biological conditions (Lucidi et al., 2003). There is evidence indicating that the oocyte plays an active role in the steroidogenic function of GC in many species (Vanderhyden et al., 1993; Coskun et al., 1995; Vanderhyden and MacDonald, 1998; Dhawan et al., 2000; Li et al., 2000). Secretion of estradiol by human and bovine cumulus cells has also been reported (Chian et al., 1999; Mingoti et al., 2002).

The maintenance of cumulus cell function, namely in terms of estradiol production, in response to gonadotropin (follicle-stimulating hormone, FSH) stimulation seems to be critical to the development of in vitro culture systems. Accordingly, estradiol has been traditionally added to the medium for in vitro maturation (IVM) of mammalian oocytes. However, it is not clear that the maturation medium should be supplemented with estradiol for oocyte maturation in vitro because estradiol biosynthesis has been demonstrated in cultures of bovine GC (Mingoti et al., 1995) and cumulus cells of various species, such as human (Chian et al., 1999), rats (Sherizly and Kraicer, 1980), and pigs (Channing et al., 1981).

The objective of the present study was to compare the in vitro production of estradiol-17ß by cumulus cells in the presence or absence of ovine oocytes. Moreover, the relationship between the produced amounts of estradiol-17ß and oocyte nuclear maturation was assessed.

2. Materials and methods

2.1. Oocyte collection and maturation

Adult and pre-pubertal sheep ovaries ($n = 2700$) of Lori–Bakhtiari breed were collected during the non-breeding season (April–July) from a local slaughterhouse in Shahrekord (latitude 32′17″N; longitude 50′51″E; altitude 2049 m) and transported to the laboratory in saline (30–35 °C) in a thermos flask, within 1–3 h following collection. Ovaries were washed three times with pre-warmed fresh saline (37 °C), and all visible follicles with a diameter of 2–6 mm were aspirated using gentle vacuum (40 mmHg) via a 20 gauge short beveled needle connected to the 2 ml syringe. Prior to aspiration, the syringe was filled with 0.5 ml pre-incubated hepes-modified TCM, supplemented with 50 IU/ml heparin. After aspiration, the content of the syringe was emptied into a collection tube. After sedimentation, only oocytes surrounded by more than three layers of unexpanded cumulus cells (COCs: cumulus oocyte complexes) were recovered and selected for in vitro maturation. The COCs were randomly distributed in maturation droplets (15 oocytes in 50 μl) and covered by sterile paraffin oil (M 8410; Sigma Chemical Co., St. Louis, MO) in a 35 mm petri dish (Falcon 1008; Becton & Dickinson, Lincoln Park, NJ). Oocytes were incubated in an atmosphere of 5% CO_2 in air, with 100% humidity, at 38.6 °C for 26 h. The oocyte culture medium (OCM) consisted of bicarbonate-buffered TCM 199 with L-glutamine (M 5017; Sigma Chemical Co., St. Louis, MO), supplemented with 0.05 IU/ml rhFSH (Organon International, Oss, The Netherlands), 1 IU/ml hCG, 100 μl/ml penicillin, 100 μg/ml streptomycin, 20% FCS (F 3018; Sigma Chemical Co.), and 0.2 mM Na-pyrovate.

2.2. Preparation of cumulus cells monolayer

The cumulus cells of the oocytes, derived from follicles of 2–6 mm in diameter, were detached from the oocyte with a pipette and then vortexed for 1 min in 1 ml hepes buffer M199 (M 2520; Sigma Chemical Co., St. Louis, MO), washed twice in OCM and transferred to the droplets of culture medium, of approximately 10^5 cumulus cells/ml. The viability of the cells was evaluated pre- and post-culture period using Trypan blue staining.

2.3. Assessment of the nuclear stage

At the end of maturation period (26 h), the cumulus cells of the oocytes were removed by vortexing (3–6 min). Completely denuded oocytes were recovered and transferred to glass slides. Vaseline and paraffine wax were used to keep the coverslip in contact with the oocytes without the use of excessive pressure. For fixation, the slides were immersed in ethanol:acetic acid (3:1) for at least 24 h (Sun et al., 2001). Prior to staining, the fixative solution was removed by two to three washings with 100% ethanol. The oocytes were stained in 2% (w/v) aceto-orcein and examined under a light microscope at 100× magnification. Oocytes were classified as germinal vesicle, germinal vesicle breakdown (GVBD), metaphase I (MI), or metaphase II (MII) stage of the maturation process (Kubelka et al., 1988).

2.4. Hormone assay

After incubation the media was collected and stored at −20 °C. Estradiol-17ß concentration was assayed by coated tube RIA (Spectria, Orion Diagnostica, Finland). Intra- and inter-assay coefficients of variation were less than 6.7 and 13.2%, respectively.

2.5. Experimental groups

The selected oocytes ($n = 1538$) were randomly allocated to four treatment groups. Each treatment consisted of at least six replicates.

Group 1: cumulus enclosed oocytes cultured in OCM (COCs group, $n = 388$).
Group 2: denuded oocytes cultured in OCM (D group, $n = 393$).
Group 3: denuded oocytes co-cultured with cumulus cells monolayer in OCM (D + M group, $n = 435$).
Group 4: denuded oocytes co-cultured with previously cultured (for 26 h) cumulus cell-monolayer (10^5 cells/ml), in refreshed OCM (D + M_{26} Group, $n = 322$).
Group 5: cumulus cell-monolayer (10^5 cells/ml), cultured in OCM (M Group, six replicates).

The duration and conditions of incubation were similar in all experimental groups with oocytes being incubated in an atmosphere of 5% CO_2 in air with 100% humidity, at 38.6 °C for 26 h.

2.6. Statistical analysis

Data was collected over at least six replicates. Comparison of the produced concentrations of estradiol-17ß between experimental groups was analyzed using Kruskal–Wallis one-way ANOVA on ranks followed by Dunn's method. The difference in the number of MII stage oocytes between groups was analyzed using Chi-square test. The possible relationship between concentrations of estradiol-17ß and number of MII stage oocytes was analyzed using logistic regression. A $P < 0.05$ level was considered significant (SPSS version 11.5).

3. Results

The produced amounts of estradiol-17ß at the end of the culture period (26 h), were significantly different ($P < 0.001$) between Group D + M and Groups M and D + M_{26} (Table 1).

As set out in Table 1, the highest concentration of estradiol-17ß (378.69 ± 54.34 pg/ml) was detected in the Group D + M, where the pattern of communication between cumulus cells and oocytes was in a paracrine fashion. In contrast, the lowest concentration (109.15 ± 8.24 pg/ml) was observed in the Group D ± M_{26} in which the denuded oocytes were co-cultured with cumulus cell-monolayer that had been previously cultured for 26 h. The percentage of oocytes presenting an extruded first polar body (MII oocytes) for each treatment group after culture period was shown in Table 2.

The highest percentage of MII oocytes (91%) was observed in Group COCs and the lowest percentage (6%) was observed in Groups D and D + M_{26} (Table 2). The lowest and the highest percentage of germinal vesicle stage oocytes were detected in Groups COCs and D, respectively (0.0% versus 58%). There was no relationship between the concentrations of estradiol-17ß and the number of MII stage oocytes.

Table 1
Mean ± S.E.M. concentrations of estradiol-17ß (pg/ml) produced by cumulus cells cultured in presence or absence of sheep oocytes (Lori–Bakhtiari breed) in different conditions

Groups	Replicates (*n*)	Estradiol-17ß	Max	Min
COC	7	$215.643 \pm 46.9^{a,b}$	377	82
D	8	$182.37 \pm 27.07^{a,b}$	341	95.5
D + M	8	378.69 ± 54.34^{b}	597.5	145.5
$D + M_{26}$	6	109.15 ± 8.24^{a}	140.4	85.5
M	6	133.25 ± 7.55^{a}	163	107

COC: cumulus enclosed oocytes cultured in OCM; D: denuded oocytes cultured in OCM; D + M: denuded oocytes co-cultured with cumulus cells monolayer in OCM; $D + M_{26}$: denuded oocytes co-cultured with previously cultured (for 26 h) cumulus cell-monolayer (10^5 cells/ml), in refreshed OCM; M: cumulus cell-monolayer (10^5 cells/ml) cultured in OCM in the absence of oocytes. Numbers with different superscripts (a, b) in the same column differ significantly ($P < 0.01$).

Table 2
In vitro nuclear maturation of sheep oocytes (Lori–Bakhtiari breed) cultured in presence or absence of cumulus cells in four different conditions

Treatment groups	Cultured oocytes *n*	Nuclear status			
		GV[1] *n* (%)	GVBD[2] *n* (%)	MI[3] *n* (%)	MII[4] *n* (%)
COCs	388	$0.0\ (0.0)^{a}$	$16\ (4)^{a}$	$17\ (4)^{a}$	$355\ (91)^{a}$
D	393	$229\ (58)^{b}$	$113\ (29)^{b}$	$28\ (7)^{a,c}$	$23\ (6)^{b}$
D + M	435	$103\ (24)^{c}$	$200\ (46)^{c}$	$60\ (14)^{b}$	$72\ (16)^{c}$
$D + M_{26}$	322	$46\ (14)^{d}$	$226\ (70)^{d}$	$30\ (9)^{c,b}$	$20\ (6)^{b}$

COC: cumulus enclosed oocytes cultured in OCM; D: denuded oocytes cultured in OCM; D + M: denuded oocytes co-cultured with cumulus cells monolayer in OCM; $D + M_{26}$: denuded oocytes co-cultured with previously cultured (for 26 h) cumulus cell-monolayer (10^5 cells/ml), in refreshed OCM. Numbers with different superscripts (a, b, c, d) in the same column differ significantly ($P < 0.001$). (1) Germinal vesicle; (2) germinal vesicle breakdown; (3) metaphase I; (4) metaphase II.

4. Discussion

As indicated, the cumulus cells have the potential to produce estradiol in detectable amounts during culture, probably as a consequence of the action of gonadotropin, in many species (Chian et al., 1999; Dode and Graves, 2002; Mingoti et al., 2002). These findings support the idea that estradiol may have an effect during oocyte maturation, where its effect can be exerted indirectly via cumulus cells or directly in the oocyte. In sheep, granulose cells secrete estradiol during culture in a steroid free medium, as a consequence of the action of FSH, and that E_2 production will be increased by addition of high dose of bone morphogenetic protein 2 (BMP2) to the culture media (Campbell et al., 1996; Souza1 et al., 2002).

As investigated in the current study, the sheep COCs, cumulus cells and even the denuded oocytes were capable to produce E_2 in detectable amounts in a steroid free medium. As shown, the highest concentration of estradiol-17ß (378.69 ± 54.34 pg/ml) was detected in the D + M group, where the communication between cumulus cells and oocytes was occurred in a paracrine fashion. Interestingly, the lowest concentration (109.15 ± 8.24 pg/ml) was observed in the $D \pm M_{26}$ group in which the denuded oocytes were co-cultured with cumulus cell-monolayer that had been previously cultured for 26 h. One possibility may be that oocyte removal during the first 26 h of cumulus cells culture encourages cumulus cells luteinization which in turn decreases the potential secretion of E_2 in cumulus cells (Group $D + M_{26}$). Another possibility must be made of the fact

that the first 26 h of cumulus cells culture is a crucial time in which steroidogenesis has to occur and that some of the normal functions of cumulus cells are compromised when these cells are devoid from the oocyte.

In mouse, the normal steroid hormone production is altered within 24 h after oocyte loss (Vanderhyden and Tonary, 1995). In contrast to the most mammals (Vanderhyden et al., 1993; Coskun et al., 1995; Vanderhyden and Tonary, 1995; Vanderhyden and MacDonald, 1998; Lucidi et al., 2003), it seems in sheep the presence of oocyte has a positive effect on E_2 production by cumulus cells in in vitro condition. In this context, the mouse oocytes secrete a factor(s) that inhibits progesterone and stimulates estradiol production by cumulus granulosa cells (Vanderhyden et al., 1993).

In the current study, if the oocyte had a positive effect on E_2 production of cumulus cells why the production of E_2 was higher in D + M than COCs group (378.69 ± 7.55 pg/ml versus 215.64 ± 46.9 pg/ml)?

One possibility may be that the oocyte has a dual effect on production of E_2 by cumulus cells. As such, where the pattern of connection between oocyte and cumulus cells is in the manner of cell–cell contact (Group COCs), the oocyte could exert its both inhibitory and stimulatory effects on production of E_2 by cumulus cells. While in Group D + M, where the denuded oocyte was cultured in vicinity of cumulus cells, in the absence of cell–cell contact, the oocyte could exert just its stimulatory effect. In pig, however, the response to oocyte signals does not require a physical contact between the cells, and the active substance must be a soluble factor secreted in the medium by the germ cell (Buccione et al., 1990; Vanderhyden et al., 1993; Tirone et al., 1997; Li et al., 2000; Nagyova et al., 2000; Lucidi et al., 2003). The pig oocytes prove to be capable of directing the functional production of cumulus cells by changing their signals on the basis of their own biological conditions (Lucidi et al., 2003). That being said, it seems in sheep the positive effect of oocyte on cumulus cells E_2 production is, probably, exerted via soluble factor while for its moderate inhibitory effect the cell–cell contact is needed.

The other interesting point was the production of E_2 in Group D, where the denuded oocytes were cultured in a steroid free medium. Whereas, serum and serum albumin (BSA) are two commonly used protein sources in media for in vitro culture of mammalian oocytes, these components may be contaminated with several defined and undefined molecules, such as steroids, cholesterol, peptides, and others (Keskintepe and Brackett, 1996; Wang et al., 1997). Therefore, the detected amounts of E_2 in Group D could be derived by the presence of FCS in culture media. However, there are sparse evidence indicating that human and rat's oocytes are able to synthesize steroid hormones (Tsutsumi et al., 1982; Suzuki et al., 1983).

As expected, the number of MII stage oocytes was higher ($P < 0.001$) in Group COCs compared with other groups, where the pattern of cell–cell communication (COCs) was in the best and most efficient manner (two-way communication between these two cell types, in a paracrine fashion and via GJC). One possibility for the higher ($P < 0.001$) number of MII stage oocytes in Group D + M than Group D + M_{26} is that during the first 26 h of culture of cumulus cells in Group D + M_{26}, in the absence of oocyte, some of the normal functions of cumulus cells are compromised. Therefore, after this period of time, the cumulus cells are not able to support the normal process of oocyte nuclear maturation.

In the current study, there was no relationship between the concentration of E_2 and oocyte nuclear maturation. There are controversial reports regarding the effect of E_2 on oocyte maturation in several species. In pig, estradiol is not involved in nuclear and cytoplasmic maturation of the oocytes (Dode and Graves, 2003). It has been shown that estradiol as well as other steroids are involved in keeping the oocytes in meiotic arrest (Barrett and Powers, 1993; Mingoti et al., 1995).

Supplementation of the culture medium with E_2 or P4 alone significantly increased maturation of canine oocyte to MII (Kim et al., 2005). In bovine, the presence of E_2 during IVM has a detrimental effect (both in denuded and COCs) on the nuclear maturation, including abnormal dispersion of chromosomes and on subsequent embryo development (Beker et al., 2002). The adverse effects of E_2 on nuclear maturation might be due to an improper spindle organization during IVM (Can and Semiz, 2000). In rhesus monkey, formation of morula nad blastocysts is greatest in oocytes matured in medium containing estradiol and/or progesterone (Zheng et al., 2003). Together, the specific role of estrogens in follicular and oocyte maturation, ovulation and embryo development seems to be species dependent (Moudgal et al., 1996).

5. Conclusion

The sheep COCs and cumulus cells are capable to produce E_2 in detectable amounts in a steroid free medium. It seems, the positive effect of oocyte on production of E_2 in cumulus cells exerted, probably, via the soluble factor. Moreover, the ability of cumulus cells in production of E_2 will be decreased in the absence of oocyte and that this ability will not be returned even if the cumulus cells are exposed to the oocyte afterward. There is no relationship between the produced amounts of E_2 and the number of MII oocytes. However, clarification of the physiological mechanisms that underline these observations will require further studies.

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References

Anisworth, L., Tsang, B.K., Downey, B.R., Marcus, G.J., Armstrong, D.T., 1980. Interrelationship between follicular fluid steroid levels, gonadotrophic stimuli and oocyte maturation during preovulatory development of porcine follicles. Biol. Reprod. 23, 621–627.

Barrett, C.B., Powers, R.D., 1993. Progestin inhibit murine oocyte meiotic maturation in vitro. J. Exp. Zool. 265, 231–239.

Beker, A.R., Colenbrander, B., Bevers, M.M., 2002. Effect of 17ß-estradiol on the in vitro maturation of bovine oocytes. Theriogenology 58, 1663–1673.

Buccione, R., Vanderhyden, B.C., Caron, P.J., Eppig, J.J., 1990. FSH-induced expansion of the mouse cumulus oophorous in vitro is dependent upon a specific factor(s) secreted by the oocyte. Dev. Biol. 138, 16–25.

Campbell, B.K., Scaramuzzi, R.J., Webb, R., 1996. Induction and maintenance of oestradiol and immunoreactive inhibin production with FSH by ovine granulosa cells cultured in serum-free media. J. Reprod. Fertil. 106, 7–16.

Can, A., Semiz, O., 2000. Diethylstilbestrol (DES)-induced cell cycle delay and meiotic spindle disruption in mouse oocytes during in vitro maturation. Mol. Hum. Reprod. 6, 154–162.

Channing, C.P., Bae, I.H., Stone, S.L., Anderson, L.D., Edelson, S., Fowler, S.C., 1981. Porcine granulosa and cumulus cell properties. LH/hCG receptors, ability to secrete progesterone and ability to respond to LH. Mol. Cell. Endocrinol. 22, 359–370.

Chian, R.C., Ao, A., Clarck, H.J., Tulandi, T., Tan, S.L., 1999. Production of steroids from human cumulus cells treated with different concentrations of gonadotrophins during culture in vitro. Fertil. Steril. 71, 61–66.

Coskun, S., Uzumcu, M., Lin, Y.C., Friedman, C.I., Alak, B.M., 1995. Regulation of cumulus cell steroidogenesis by the porcine oocyte and preliminary characterization of oocyte-produced factor(s). Biol. Reprod. 53, 670–675.

Danforth, D.R., 1995. Endocrine and paracrine control of oocyte development. Am. J. Obstet. Gynecol. 172, 747–752.

Dhawan, A., Léveillé, M.C., Vanderhyden, B.C., 2000. Inhibition by human embryos of mouse granulosa cell progesterone production: development of a sensitive bioassay. Hum. Reprod. 15, 917–924.

Dode, M.A.N., Graves, C.N., 2002. Involvement of steroid hormones on in vitro maturation of pig oocytes. Theriogenology 57, 811–821.

Dode, M.A.N., Graves, C.N., 2003. Role of estradiol-17beta on nuclear and cytoplasmic maturation of pig oocytes. Anim. Reprod. Sci. 78, 99–110.

Gill, A., Jamnongjit, M., Hammes, S.R., 2004. Androgens promote maturation and signaling in mouse oocytes independent of transcription: a release of inhibition model for mammalian oocyte meiosis. Mol. Endocrinol. 18, 97–104.

Juengel, J.L., Heath, D.A., Quirke, L.D., McNatty, K.P., 2006. Oestrogen receptor alpha and beta, androgen receptor and progesterone receptor mRNA and protein localisation within the developing ovary and in small growing follicles of sheep. Reproduction 131, 81–92.

Keskintepe, L., Brackett, B.G., 1996. In vitro developmental competence of in vitro matured bovine oocytes fertilized and cultured in completely defined media. Biol. Reprod. 55, 333–339.

Kim, M.K., Fibrianto, Y.H., Oh, H.J., Jang, G., Kim, H.J., Lee, K.S., Kang, S.K., Lee, B.C., Hwang, W.S., 2005. Effects of estradiol-17beta and progesterone supplementation on in vitro nuclear maturation of canine oocytes. Theriogenology 63, 1342–1353.

Kubelka, M., Motlik, J., Fulka, J.J., Prochazka, R., Rimkevikova, Z., Fulka, J., 1988. Time sequence of germinal vesicle breakdown in pig oocytes after cycloheximide and *p*-aminobenzamidine block. Gamete Res. 19, 423–431.

Li, R., Noran, R.J., Armestrong, D.T., Gilchrist, R.B., 2000. Oocyte-secreted factor(s) determine functional differences between bovine mural granulosa cells and cumulus cells. Biol. Reprod. 63, 839–845.

Lucidi, P., Bernab, N., Turriani, M., Barboni, B., Mattioli, M., 2003. Cumulus cell steroidogenesis is influenced by the degree of oocyte maturation. Reprod. Biol. Endocrinol. 1, 45.

Mingoti, G.Z., Garcia, J.M., Rosa-e-Silva, A.A., 1995. The effect of serum on in vitro maturation, in vitro fertilization and steroidogenesis of bovine oocytes co-cultured with granulose cells. Braz. J. Med. Res. 28, 213–217.

Mingoti, G.Z., Garcia, J.M., Rosa-e-Silva, A.A.M., 2002. Steroidogenesis in cumulus cells of bovine cumulus-oocyte complexes matured in vitro with BSA and different concentrations of steroids. Anim. Reprod. Sci. 69, 175–186.

Moudgal, N.R., Shetty, G., Selvaraj, N., Bhatnagar, A.S., 1996. Use of a specific aromatase inhibitor for determining whether there is a role for oestrogen in follicle/oocyte maturation, ovulation and preimplantation embryo development. J. Reprod. Fertil. Suppl. 50, 69–81.

Nagyova, E., Vanderhyden, B.C., Prochazka, R., 2000. Secretion of paracrine factors enabling expansion of cumulus cells is developmentally regulated in pig oocytes. Biol. Reprod. 63, 1149–1156.

Palter, S.F., Tavares, A.B., Hourvitz, A., Veldhuis, J.D., Adashi, E.Y., 2001. Are estrogens of import to primate/human ovarian folliculogenesis? Endocr. Rev. 22, 389–424.

Sherizly, I., Kraicer, P.F., 1980. Progesterone secretion by the post-ovulatory rat cumulus oophorus. Gamete Res. 3, 115–119.

Souza1, C.J.H., Campbell, B.K., McNeilly, A.S., Baird, D.T., 2002. Effect of bone morphogenetic protein 2 (BMP2) on oestradiol and inhibin A production by sheep granulosa cells, and localization of BMP receptors in the ovary by immunohistochemistry. Reproduction 123, 363–369.

Sun, Q.Y., Lai, L., Bonk, A., Prather, R.S., Schatten, H., 2001. Cytoplasmic changes in relation to nuclear maturation and early embryo developmental potential of porcine oocytes: effects of gonadotropins, cumulus cells, follicular size, and protein synthesis inhibition. Mol. Reprod. Dev. 59, 192–198.

Suzuki, S., Endo, Y., Fujiwara, T., Tanaka, S., Iizuka, R., 1983. Cytochemical study of steroid-producing activities of human oocytes. Fertil. Steril. 39, 683–689.

Tezarik, J., Mendoza, C., 1995. Nongenomic effects of the 17 beta-estradiol on maturing human oocytes: relationship to oocyte developmental potential. J. Clin. Endocrinol. Metab. 80, 1438–1443.

Tezarik, J., Mendoza, C., 1997. Direct non-genomic effects of follicular steroids on maturing human oocytes: oeastrogen versus androgen antagonism. Hum. Reprod. Update 3, 95–100.

Tirone, E., D'Alessandris, C., Hascall, V.C., Siracusa, G., Salustri, A., 1997. Hyaluronan synthesis by mouse cumulus cells is regulated by interactions between follicle-stimulating hormone (or epidermal growth factor) and a soluble oocyte factor (or transforming growth factor β_1). J. Biol. Chem. 272, 4787–4794.

Tomanek, M., Pisselet, C., Monget, P., Madigou, T., Thieulant, M.L., Monniax, D., 1997. Estrogen receptor protein and mRNA expression in the ovary of the sheep. Mol. Reprod. Dev. 48, 53–62.

Tsutsumi, O., Satoh, K., Sakamoto, S., 1982. Studies on steroidogenesis in the oocyte. Nippon Naibunpi Gakkai Zasshi. 58, 1321–1332 (in Japanese).

Vanderhyden, B.C., Cohen, J.N., Morley, P., 1993. Mouse oocyte regulate granulosa cell steroidogenesis. Endocrinology 133, 423–426.

Vanderhyden, B.C., MacDonald, E., 1998. Mouse oocytes regulate granulosa cell steroidogenesis throughout follicular development. Biol. Reprod. 59, 1296–1301.

Vanderhyden, B.C., Tonary, A.M., 1995. Differential regulation of progesterone and estradiol production by mouse cumulus and mural granulosa cells by a factor(s) secreted by the oocyte. Biol. Reprod. 53, 1243–1250.

Wang, S., Liu, Y., Holyoak, G.R., Bunch, T.D., 1997. The effects of bovine serum albumin and fetal bovine serum on the development or pre- and post-cleavage stage bovine embryos cultured in modified CR2 and M199 media. Anim. Reprod. Sci. 48, 37–45.

Zheng, P., Si, W., Bavister, B.D., Yang, J., Ding, C., Ji, W., 2003. 17 beta-estradiol and progesterone improve in vitro cytoplasmic maturation of oocytes from unstimulated prepubertal and adult rhesus monkeys. Hum. Reprod. 18, 2137–2144.