Reprod Med Biol (2011) 10:43–49
DOI 10.1007/s12522-010-0069-y

ORIGINAL ARTICLE

Effect of bovine cumulus–oocyte complexes-conditioned medium on in-vitro maturation of canine oocytes

Mohammed A. Abdel-Ghani · Yasuyuki Abe · Tomoyoshi Asano · Seizo Hamano · Hiroshi Suzuki

Received: 16 June 2010 / Accepted: 1 October 2010 / Published online: 26 October 2010

Abstract
Purpose To investigate the ability of medium conditioned with bovine cumulus–oocyte complexes (COCs) to support nuclear maturation of canine oocytes recovered from domestic dog ovaries.
Methods Cumulus–oocyte complexes were obtained from ovaries of domestic bitches (8 months old to 7 years old), and in-vitro maturation was evaluated in TCM-199 supplemented with different concentrations (0, 20, 30 or 50%) of bovine COCs-conditioned medium (BCM). The canine COCs were cultured for 72 or 96 h at 38.5°C in 5% CO_2, 5% O_2 and 90% N_2. The bovine COCs-conditioned medium was obtained from culture of bovine COCs with TCM-199 supplemented with 5% FCS for 22 h at 38.5°C in 2% CO_2, 98% air.
Results The proportion of germinal vesicle breakdown (GVBD) after 72 h was significantly higher ($P < 0.05$) in medium supplemented with 30% BCM (20.7%) compared with the control group (13.4%). The rates of GVBD-MII stage were significantly higher ($P < 0.05$) when oocytes were matured with BCM at concentration of 30% (41.5%) compared with control (26.6%) after 72 h in-vitro culture. After 96 h in-vitro culture, the oocytes matured in medium supplemented with 30% BCM (5.5%) showed a significant increase ($P < 0.05$) in the proportion of MII compared with control (0.7%). However, increasing the cultivation time from 72 to 96 h resulted in an increase in oocyte degeneration rate.
Conclusions The results suggested that bovine COCs-conditioned medium supplementation significantly increased nuclear maturation of canine oocytes.

Keywords Bovine · Canine · Conditioned medium · Nuclear maturation · Oocytes

Introduction

Development of reproductive technologies is highly desirable for advancement of the breeding of domestic dogs, and for conservation programs of wild and endangered canid species. However, assisted reproduction technologies (ARTs), for example in-vitro maturation of oocytes (IVM), in-vitro fertilization (IVF), and embryo culture, are less developed for the dog than for other domestic livestock. Unique species-specific reproductive characteristics substantially affect development of ARTs in dogs. In contrast with most mammalian species, in which the oocyte completes its first meiotic division in the preovulatory follicle and matured oocytes are ovulated and ready for fertilization within the oviduct, the domestic bitch ovulates immature oocytes at the germinal vesicle stage and the oocytes undergo a 48–72-h period of postovulatory maturation in the upper regions of the oviduct, and during oocyte maturation in vivo oocytes are exposed to an ever-changing environment of gonadotropins, steroids, growth factors, and many other factors, any or all of which may interact to regulate maturational changes that occur in the oocyte and its surrounding cumulus cells during the preovulatory period [1–7]. Clearly, these factors are assumed to be beneficial and involved in nuclear and/or

M. A. Abdel-Ghani · Y. Abe · T. Asano · H. Suzuki (✉)
Research Unit for Functional Genomics, National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-Choe, Obihiro 080-8555, Japan
e-mail: hisuzuki@obihiro.ac.jp

S. Hamano
Animal Bio-Technology Center, Livestock Improvement Association, 1-9-8, Kyobashi, Chuo-Ku, Tokyo, Japan

cytoplasmic maturation of oocytes during IVM for several mammalian species, for example porcine [8, 9], human [10], mouse [11], equine [12], and bovine [13]. The peculiarities of reproductive physiology of the dog complicate the definition of a culture system for IVM, which is currently characterized by poor and highly variable results.

The first study on IVM and IVF of dog oocytes was over 30 years ago by Mahi and Yanagimachi [14]. Since that report, and despite numerous studies documenting and focusing on establishing a suitable system for IVM of canine oocytes [15–19], the efficiency is lower than for other domestic mammalian species, for example cattle [20, 21], sheep [22], pigs [23], mice [24], and cats [25]. Although puppies have been produced from somatic cell nuclear transfer of in-vivo matured oocytes [26], there has been no report of the production of live young after transfer of in-vitro maturation and fertilization-derived embryos [27].

Despite multiple publications discussing the addition of chemical and hormonal compounds to the IVM medium, for example fetal bovine serum [28, 29], estrus bitch serum or estrus cow serum [15, 30], gonadotropins [31], steroids [5, 32, 33], and oviductal fluid and oviductal tissue in co-culture [34, 35], to promote GV synchronization through meiotic inhibition or to promote meiotic resumption and increase the percentage of oocytes which undergo GVBD, or substances known to protect cells from oxidative damage, for example β-mercaptoethanol and insulin–transferrin–selenium combinations [16, 36], the meiotic response of in-vitro matured canine oocytes is still very unpredictable because it is difficult to identify the components lacking in the medium, or which compounds may be detrimental or suppressive [37], and which compounds in the in-vitro maturation system support the dynamic changes required for maturation of all components of the cumulus–oocyte complexes (COCs) [38].

It has been reported that epidermal growth factor (EGF) stimulates the IVM of oocytes in mice [39], cattle [40], humans [41] and pigs [42]. Activin, transforming growth factor beta (TGF-β), and basic fibroblast growth factors (bFGF) have been shown to stimulate the mitotic activity of bovine granulosa cells and/or the growth of granulosa cells or follicles in culture [43, 44]. It has been shown that granulosa cells of several species, for example the cow and hamster, produce some of these growth factors [45].

In addition, during conditioning the culture secreted embryotrophic components including proteins into the media that support blastocyst formation in early bovine embryo fertilized in vitro [46].

Therefore, the ability of bovine COCs-conditioned medium to support nuclear maturation of canine oocytes recovered from domestic dog ovaries in random reproductive states was examined.

Materials and methods

Collection and preparation of cumulus–oocyte complexes (COCs)

Ovaries were obtained from healthy domestic bitches at random stages of estrus cycle (8 months old to 7 years old) undergoing routine ovariohysterectomy in local veterinary clinics in Obihiro. The dogs were of different breeds and the total number was 32. Both ovaries were transported to the laboratory within 1 h in a vacuum flask containing physiological sterile saline supplemented with 100 IU/mL penicillin (Calbiochem, La Jolla, CA, USA) at 37°C. After transportation, the fat, ligaments and medulla were trimmed off carefully and discarded. The COCs were released by repeatedly slicing the ovarian cortex with a scalpel blade (Feather, Osaka, Japan) at room temperature. These COCs were placed in 35-mm Petri dishes (Falcon # 3001; Becton–Dickinson, Lincoln Park, NY, USA) containing PB1 medium [47] supplemented with 3 mg/mL BSA (Sigma, St Louis, MO, USA) and 100 μg/mL streptomycin (Meiji, Tokyo, Japan), and examined under a dissecting microscope (SMZ1500; Nikon Instech, Tokyo, Japan). After three washes in the same medium, the COCs were selected according to the criteria previously described by De los Reyes et al. [18], Hewitt and England [48], and Otoi et al. [49] under an inverted microscope (DMIR/E, Leica, Wetzlar, Germany). The conditions were those reported to favor meiotic competence based on the uniformity of ooplasm and cumulus cell complement, homogeneous dark cytoplasm with three or more layers of compact cumulus cells, and oocytes >110 μm in diameter. The vitelline diameter of COCs was measured using a calibrated ocular micrometer.

Culture media

Collected COCs were cultured in TCM-199 (Earle's salt + 2.2 g/L sodium bicarbonate, buffered with 25 mM Hepes; Gibco-Invitrogen Life Technologies, NY, USA) supplemented with 10% canine serum containing 18 ng/mL progesterone, 50 ng/mL EGF (Sigma), 10 μg/mL estradiol-17β (Sigma), 0.1 IU/mL hCG (Sankyo, Tokyo, Japan), 0.1 IU/mL FSH (Sigma), 0.25 mM pyruvic acid (Wako, Tokyo, Japan), 100 μM β-mercaptoethanol (Sigma) and antibiotics (12.2 mg/mL penicillin and 20 mg/mL streptomycin) [50]. After preparation of TCM-199, the bovine COCs-conditioned medium (BCM) was added. The bovine medium was TCM-199 supplemented with 5% fetal calf serum (Sigma) and 50 μg/mL gentamycin (Sigma). The bovine medium was used for cultivation of bovine COCs for 22 h at 38.5°C, in 2% CO_2 [51] in air, and was denoted bovine BCM. The canine serum used for culture was

collected from healthy bitches, heat-inactivated for 30 min at 56°C, and stored at −78°C until it was used. The concentration of progesterone was measured using a BioMerieux Vidas® progesterone kit (Spotchem Vidas sv-5020; Arkray, Kyoto, Japan).

In-vitro maturation of oocytes

To investigate the effects of TCM-199 supplemented with different concentrations of BCM (0, 20, 30 or 50%), COCs were allocated randomly to four treatment groups: (A) TCM-199 (control); (B) TCM-199 + 20% BCM; (C) TCM-199 + 30% BCM; (D) TCM-199 + 50% BCM. The four groups of COCs were cultured for 72 or 96 h. COCs from each treatment were placed in 35-mm Petri dishes that contained 10 oocytes/100 μL medium and covered with mineral oil (Nacalai Tesque, Kyoto, Japan) at 38.5°C in a humidified atmosphere of 5% CO_2, 5% O_2, and 90% N_2.

Assessment of nuclear status

Cumulus–oocyte complexes were denuded by exposure to 0.1% hyaluronidase (Sigma) for 10 min with gentle pipetting to remove cumulus cells. The denuded oocytes were fixed and permeabilized in Dulbecco's phosphate-buffered saline (PBS) containing 3.7% (*w*/*v*) paraformaldehyde for 20 min. They were washed three times in PB1 supplemented with 3 mg/mL BSA and then transferred to 7.5 μg/mL bis-benzimide (Hoechst 33342; Sigma) for 15 min; afterwards they were washed again three times in PB1 and placed on glass slides. The oocytes were subsequently overlaid with a coverslip. The chromatin state was evaluated using a fluorescence microscope with UV light (C-SHG1, Nikon) to determine the meiotic stage as described by Hewitt and England [48] and De los Reyes et al. [18]:

1 immature or germinal vesicle (GV, Fig. 1a), when the nuclear envelope was visible;
2 resumption of meiosis or germinal vesicle breakdown (GVBD, Fig. 1b), when the nuclear envelope was no longer visible and the chromatin was dispersed;
3 metaphase I (MI, Fig. 1c), when chromosomes were condensed and present in equatorial view or when the chromosomes were migrating to the poles; and
4 mature (metaphase II; MII, Fig. 1d), when chromosomes were in second metaphase with extrusion of the first polar body.

Oocytes with loss of membrane integrity or showing either dispersed chromosomes or unidentifiable chromatin are counted as degenerated (Fig. 1e).

Statistical analysis

All COCs were randomly distributed within each experimental group and each experiment was repeated at least three times. Data were subjected to ANOVA (Statistical Analysis System, SPSS, USA); followed by post-HOC multiple comparisons using the least significant difference (LSD) test. Differences of $P < 0.05$ were regarded as significant. The proportions of GV, GVBD, MI, MII, degenerated, and GVBD-MII oocytes cultured in different media are presented as mean ± standard deviations.

All experiments were carried out in accordance with the guidelines for the care and use of animals approved by Obihiro University of Agriculture and Veterinary Medicine.

Results

The meiotic status of canine oocytes (a total of 384 oocytes were used from 17 bitches) cultured in medium supplemented with different concentrations of BCM after 72 h is shown in Table 1. The proportion of GVBD was significantly higher ($P < 0.05$) when medium was supplemented with 30% BCM (20.7%) compared with the control group (13.4%). Similarly, the rates of MI stage were significantly higher ($P < 0.05$) when oocytes were matured with BCM at a concentration of 30% (14.0%) compared with controls (7.9%). Although there were no significant differences between the experimental groups and period, the proportion of MII was highest when COCs were cultured in TCM-199 with 30% BCM. Significantly higher rates ($P < 0.05$) of GVBD-MII were found in treatment with BCM at a concentration of 30% (41.5%) compared with control (26.6%). None of the oocytes matured to MII in 50% BCM and overall meiotic resumption was lower in 50% BCM than in any of the other treatment groups.

The meiotic status of canine oocytes (a total of 357 oocytes were used from 15 bitches) after 96 h of culture is shown in Table 1. The rates of GV and GVBD did not differ significantly for oocytes matured with or without BCM. However, compared with control (0.5%), the inclusion of 20% (5.1%) and 30% (11.2%) BCM during IVM significantly ($P < 0.05$) increased the proportion of MI. The oocytes matured in medium supplemented with 30% BCM (5.5%) showed a significant increase ($P < 0.05$) in the proportion of MII compared with that of oocytes without BCM (0.7%). The oocytes cultured with 50% BCM failed to progress beyond GVBD.

Treatments with 30% BCM concentration showed significant ($P < 0.05$) reduction of oocyte degeneration rate than in other experimental groups.

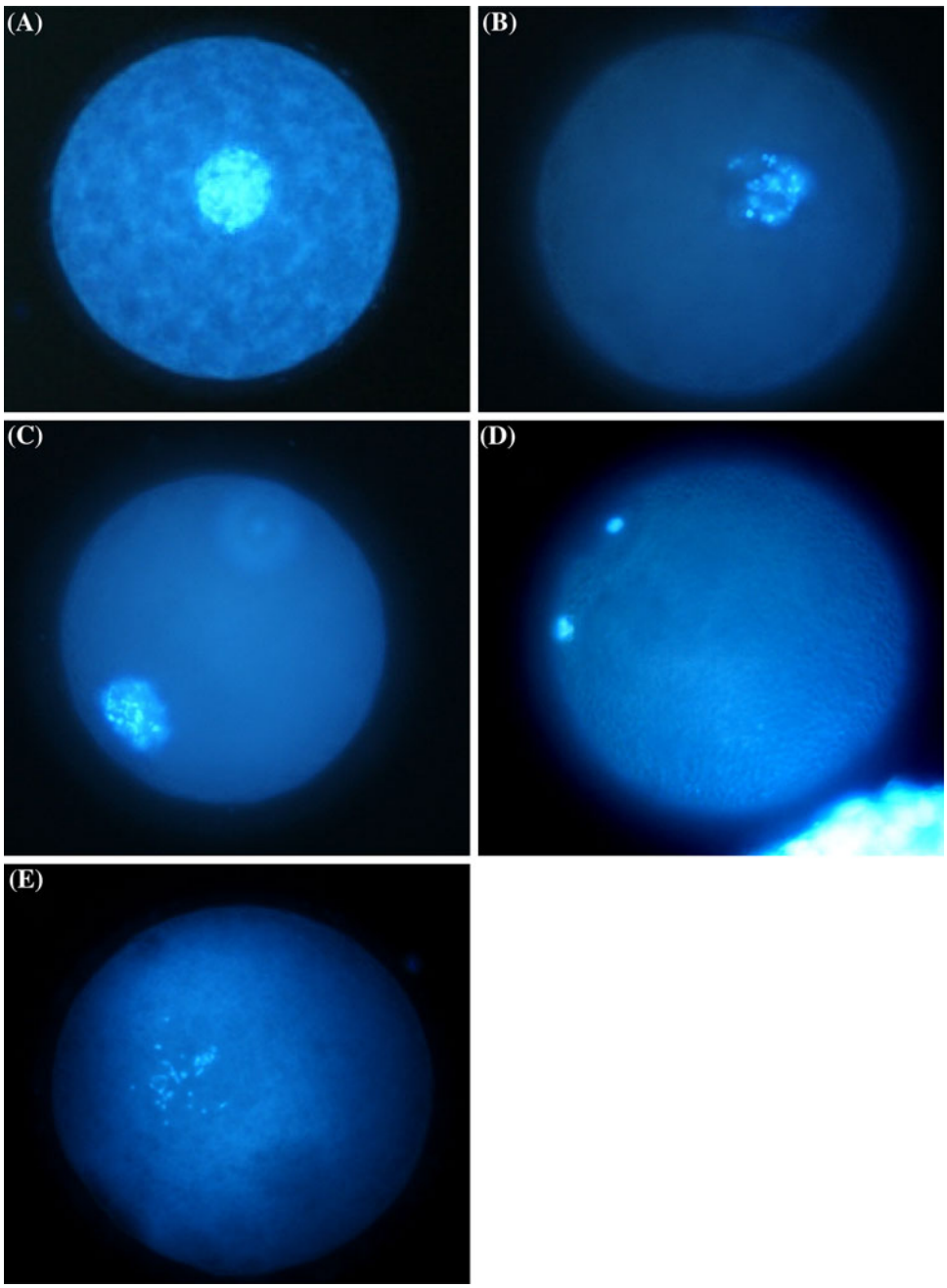

Fig. 1 Fluorescence photomicrographs showing chromatin configuration in canine oocytes stained with Hoechst 33342: **a** germinal vesicle (GV), **b** germinal vesicle breakdown (GVBD), **c** metaphase I (MI), **d** metaphase II (MII), **e** Degenerated (×400)

Increasing the cultivation time from 72 to 96 h resulted in a significant ($P < 0.05$) decrease in nuclear maturation rates and an increase in oocyte degeneration.

Discussion

The rates of meiotic completion of canine oocytes have varied because the oocytes used for IVM experiments have been collected from different sources, and cultured with a variety of different culture systems and media [52].

Our result showed that supplementation of maturation medium with BCM at a concentration of 30% during IVM significantly increased the MII rate (7%) and significantly reduced degeneration rates compared with control. Although the percentage of MII stage in our experiment is not higher than that achieved in some previous studies, that ranged from 1.9 to 20% [15–19, 31, 33, 34, 53–55], it can be argued that the presence of BCM during IVM resulted in a higher percentage of oocytes completing MI synchronously to reach the MII stage, and that the BCM medium was used for the first time and might have to be modified to increase the nuclear maturation rate.

Table 1 Nuclear status of canine oocytes after 72 or 96 h in-vitro culture in TCM-199 supplemented with different concentrations of bovine COCs-conditioned medium

Culture time (h)	Treatment	No. of oocytes examined	% Meiotic stage (mean ± SD)[A]					
			GV	GVBD	MI	MII	Deg.	GVBD-MII
72	TCM-199[B]	119	18.51 ± 8.27^{a}	13.43 ± 6.04^{a}	7.87 ± 5.72^{a}	3.09 ± 5.29^{a}	57.04 ± 14.09^{a}	26.62 ± 13.09^{a,b}
	80%TCM-199 + 20%BCM	112	14.96 ± 7.08^{a}	18.07 ± 12.77^{a,b}	11.95 ± 6.51^{a,b}	5.07 ± 6.62^{a}	47.92 ± 11.73^{a}	35.38 ± 7.81^{a,d}
	70% TCM-199 + 30%BCM	112	16.10 ± 7.84^{a}	20.68 ± 7.26^{b}	13.98 ± 4.08^{b}	6.82 ± 7.69^{a}	43.65 ± 16.23^{a}	41.48 ± 9.74^{c,d}
	50% TCM-199 + 50%BCM	41	19.61 ± 2.02^{a}	9.80 ± 1.01^{ab}	4.88 ± 0.53^{a}	0.0 ± 0.0	65.66 ± 3.55^{a}	17.89 ± 2.98^{b}
96	TCM-199	105	9.86 ± 9.30^{a}	12.86 ± 12.82^{a}	0.45 ± 1.43^{a}	0.7 ± 2.25^{a}	72.75 ± 10.26^{a}	17.36 ± 12.66^{a}
	80% TCM-199 + 20%BCM	106	10.28 ± 10.11^{a}	15.15 ± 10.53^{a}	5.12 ± 5.66^{b}	4.33 ± 10.66^{a,b}	66.87 ± 24.54^{a,b}	20.55 ± 9.25^{a}
	70% TCM-199 + 30%BCM	101	10.73 ± 7.43^{a}	12.79 ± 7.35^{a}	11.24 ± 9.23^{b}	5.49 ± 6.38^{b}	58.11 ± 9.61^{b}	29.54 ± 8.74^{a}
	50% TCM-199 + 50%BCM	45	6.71 ± 3.35^{a}	6.52 ± 9.22^{a}	0.0 ± 0.0	0.0 ± 0.0	86.75 ± 5.86^{a}	6.52 ± 9.22^{a}

GV germinal vesicle, *GVBD* germinal vesicle break down, *MI* metaphase I, *MII* metaphase II, *Deg* degenerated, *BCM* bovine COCs-conditioned medium

[A] Values with different superscripts (a–d) are significantly different ($P < 0.05$) in the same column for each cultivation time

[B] TCM-199 containing 10% canine serum, 18 ng/mL progesterone, 50 ng/mL EGF, 10 μg/mL estradiol-17β, 0.1 IU/mL hCG, 0.1 IU/mL FSH, 0.25 mM pyruvic acid, 100 μM β-mercaptoethanol, and antibiotics

Granulosa cells of the cow produce some of growth factors, for example activin, EGF, TGF-β, and bFGF [45]. These factors have been shown to stimulate the mitotic activity of bovine granulosa cells and/or the growth of granulosa cells in culture [43, 44], and in-vitro studies showed that TGF-β stimulated oocyte maturation in rats [56], mice [57], and pigs [58]. Furthermore, cumulus cells of bovine [59] or human [60] COCs can secrete high concentrations of E_2 and progesterone when supplemented with fetal bovine serum or bovine serum albumin.

Kim et al. [16] and Willingham-Rocky et al. [33] have demonstrated a positive effect of E_2 and progesterone on IVM in dogs. Furthermore, cumulus cells were involved in mediating the stimulatory effects of E_2 and EGF on nuclear maturation of canine oocytes, and that E_2 and EGF acted to complete oocyte meiotic maturation [61]. Thus, when canine oocytes were cultured in BCM, secretions by BCM probably participated in the regulation mechanism and facilitated the maturation progress of oocytes by modulating intracellular cooperation during IVM. It is more likely that one of these factors, or several of them acting in concert, are responsible for the nuclear maturation, probably through specific receptor activation on the oocyte vitelline membrane.

Likewise, it is possible that the growth factors may play critical roles in oocyte maturation by stimulating the pattern of proteins neosynthesized during oocyte maturation [40], and play a regulatory role in oocyte maturation in a paracrine/autocrine manner, or it might be one of the signaling factors for the resumption of meiosis in oocytes [62]. The growth factors have been shown to accelerate progression of meiosis [63] and the meiotic cell cycle in bovine oocytes, possibly by increasing H1 and MAP kinase activity during the early stages of maturation [64]. Additionally, the factors secreted by cumulus cells that regulate the disruption of gap junction and cumulus expansion, as shown for pig oocytes [65], may be stimulated by these growth factors [66].

In contrast, a prolonged incubation time is associated not only with an increased rate of degeneration of canine oocytes but also with potential decreases in the proportion of GVBD-MII. The detrimental effects of extending the culture time to nuclear maturation are consistent with other reports [30, 31, 38] that a more protracted incubation time increases oocyte degeneration, and with reports by Otoi et al. [67] and Suzukamo et al. [68] of a greater frequency of nuclear maturation of oocytes when they were cultured for 72 h. However, the optimum culture time for IVM of canine oocytes is still controversial [27]. We speculate that higher rates of degeneration of canine oocytes observed on extending the culture time are particularly due to the combined interaction of aging in oocytes and the assumed consumption of medium nutrients such as growth factors and serum.

Thus, it might be important to optimize the balance between culture system and incubation time as accurately as possible to improve nuclear maturation and limit the rate of degeneration.

In conclusion, the results of this study indicate that supplementing culture medium with bovine COCs-conditioned medium significantly increased nuclear maturation of canine oocytes and presumably reduced oocyte degeneration compared with maturation medium alone, but the media might have to be modified to increase the nuclear maturation rate. Moreover, the incubation time might be an important factor for successful nuclear maturation.

References

1. Holst PA, Phemister RD. The prenatal development of the dog: preimplantation events. Biol Reprod. 1971;5:194–206.
2. Andersen AC, Simpson ME. The ovary and reproductive cycle of the dog (Beagle). Los Altos: Geron-X Press; 1973.
3. Concannon PW, McCann JP, Temple M. Biology and endocrinology of ovulation, pregnancy and parturition in the dog. J Reprod Fertil Suppl. 1989;39:3–25.
4. Tsutsui T. Gamete physiology and timing of ovulation and fertilization in the dogs. J Reprod Fertil Suppl. 1989;39:269–75.
5. Bolamba D, Russ KD, Harper SA, Sandler JL, Durrant BS. Effects of epidermal growth factor and hormones on granulosa expansion and nuclear maturation of dog oocytes in vitro. Theriogenology. 2006;65:1037–47.
6. Kim MK, Fibrianto YH, Oh HJ, Jang G, Kim HJ, Lee KS, Kang SK, Lee BC, Hwang WS. Effects of estradiol-17β and progesterone supplementation on in vitro nuclear maturation of canine oocytes. Theriogenology. 2005;63:1342–53.
7. Hanna C, Long C, Hinrichs K, Westhusin M, Kraemer D. Short communication. Assessment of canine oocyte viability after transportation and storage under different conditions. Anim Reprod Sci. 2008;105:451–6.
8. Mito T, Yoshioka K, Nagano M, Suzuki C, Yamashit S, Hoshi H. Transforming growth factor-α in a defined medium during in vitro maturation of porcine oocytes improves their developmental competence and intracellular ultrastructure. Theriogenology. 2009;72:841–50.
9. Ding J, Foxcroft GR. Epidermal growth factor enhances oocyte maturation in pigs. Mol Reprod Dev. 1994;39:30–40.
10. Goud PT, Goud AP, Qian C, Laverge H, Van Der Elst J, De Sutter P, Dhont M. In vitro maturation of human germinal vesicle stage oocytes: role of cumulus cells and epidermal growth factor in the culture medium. Human Reprod. 1998;13:1638–44.
11. Fuente RDL, O'Brien M, Eppig JJ. Epidermal growth factor enhances preimplantation developmental competence of maturing mouse oocytes. Hum Reprod. 1999;14:3060–8.
12. Carneiro G, Lorenzo P, Primentel C, Pegoraro L, Bertolini M, Ball M, Anderson G, Liu I. Influence of insulin-like growth factor-I and its interaction with gonadotropins, estradiol, and fetal calf serum on in vitro maturation and parthenogenic development in equine oocytes. Biol Reprod. 2001;65:899–905.
13. Ali A, Sirard MA. The effects of 17 beta-estradiol and protein supplement on the response to purified and recombinant follicle stimulating hormone in bovine oocytes. Zygote. 2002;10:65–71.
14. Mahi CA, Yanagimachi R. Maturation and sperm penetration of canine ovarian oocytes in vitro. J Exp Zool. 1976;196:189–96.
15. Otoi T, Fujii M, Tanaka A, Ooka A, Suzuki T. Effect of serum on the in vitro maturation of canine oocytes. Reprod Fertil Dev. 1999;11:387–90.
16. Kim MK, Fibrianto YH, Oh HJ, Jang G, Kim HJ, Lee KS, Kang SK, Lee BC, Hwang WS. Effect of β-mercaptoethanol or epidermal growth factor supplementation on in vitro maturation of canine oocyte collected from dogs with different stage of the estrus cycle. J Vet Sci. 2004;5:253–8.
17. Rodrigues BA, Dos Santos LC, Rodrigues JL. Embryonic development of in vitro matured and in vitro fertilized dog oocytes. Mol Reprod Dev. 2004;67:215–23.
18. De los Reyes M, De Lange J, Miranda JP, Claudio B. Effect of human chorionic gonadotrophin supplementation during different culture periods on in vitro maturation of canine oocytes. Theriogenology. 2005;64:1–11.
19. Luvoni GC, Chigioni S, Beccaglia M. Embryo production in dogs: from in vitro fertilization to cloning. Reprod Domest Anim. 2006;41:286–90.
20. Kitiyanant Y, Thonabulsombat C, Tocharus C, Sanitwong B, Pavasuthipaisit K. Co-culture of bovine embryos from oocytes matured and fertilized in vitro to the blastocyst stage with oviductal tissue. J Sci Soc Thailand. 1989;15:215–20.
21. Barnes FL, Eyestone WH. Early cleavage and the maternal zygotic transition in bovine embryos. Theriogenology. 1990;33:141–52.
22. Brown BW, Radziewic T. Production of sheep embryos in vitro and development of progeny following single and twin embryo transfers. Theriogenology. 1998;49:1525–36.
23. Ocampo MB, Ocampo LC, Ryu IS, Mori T, Ueda J, Kanagawa H. Effect of culture time, ovarian activity, cumulus cells and sera on the nuclear and cytoplasmic maturation of pig oocytes in vitro. Anim Reprod Sci. 1993;34:135–46.
24. Schroeder AC, Eppig JJ. The developmental capacity of mouse oocytes that matured spontaneously is normal. Dev Biol. 1984;102:493–7.
25. Kitiyanant Y, Saikhun J, Pavasuthipaisit K. Somatic cell nuclear transfer in domestic cat oocytes treated with IGF-I for in vitro maturation. Theriogenology. 2003;59:1775–86.
26. Lee BC, Kim MK, Jang G, Oh HJ, Yuda F, Kim HJ, Shamin MH, Kim JJ, Kang SK, Schatten G, Hwang WS. Dogs cloned from adult somatic cells. Nature. 2005;436:641.
27. Songsasen N, Wildt DE. Oocyte biology and challenges in developing in vitro maturation systems in the domestic dog. Anim Reprod Sci. 2007;98:2–22.
28. Yamada S, Shimazu Y, Kawano Y, Nakazawa M, Naito K, Toyoda Y. In vitro maturation and fertilization of preovulatory dog oocytes. J Reprod Fertil Suppl. 1993;47:227–9.
29. Hewitt DA, Watson PF, England GC. Nuclear staining and culture requirements for in vitro maturation of domestic bitch oocytes. Theriogenology. 1998;49:1083–101.
30. Nickson DA, Boyd JS, Eckersall PD, Ferguson JM, Harvey MJ, Renton JP. Molecular biological methods for monitoring oocyte maturation and in vitro fertilization in bitches. J Reprod Fertil Suppl. 1993;47:231–40.
31. Songsasen N, Yu I, Gomez M, Leibo SP. Effects of meiosis-inhibiting agents and equine chorionic gonadotropin on nuclear maturation of canine oocytes. Mol Reprod Dev. 2003;65:435–45.
32. Hewitt DA, England GC. Effect of preovulatory endocrine events on maturation of oocytes of domestic bitches. J Reprod Fertil Suppl. 1997;51:83–91.
33. Willingham-Rocky LA, Hinrichs K, Westhusin ME, Kraemer DC. Effects of stage of oestrous cycle and progesterone supplementations during culture on maturation of canine oocytes in vitro. Reprod. 2003;126:501–8.
34. Hewitt DA, England GC. Synthetic oviductal fluid and oviductal cell coculture for canine oocyte maturation in vitro. Anim Reprod Sci. 1999;55:63–75.

35. Bogliolo L, Zedda MT, Ledda S, Leoni G, Naitana S, Pau S. Influence of co-culture with oviductal epithelial cells on in vitro maturation of canine oocytes. Reprod Nutr Dev. 2002;42:265–73.
36. Rota A, Cabianca G. In vitro maturation rates of canine oocytes from anoestrous bitches in simple media. Reprod Nutr Dev. 2004;44:105–9.
37. Rodrigues BA, Rodrigues JL. Responses of canine oocytes to in vitro maturation and in vitro fertilization outcome. Theriogenology. 2006;66:1667–72.
38. Luvoni GC, Chigioni S. Culture strategies for maturation of carnivore oocytes. Theriogenology. 2006;66:1471–5.
39. Downs SM. Specificity of epidermal growth factor action on maturation of the murine oocyte and cumulus oophorus in vitro. Biol Reprod. 1989;41:371–9.
40. Lonergan P, Carolan C, Van Langendonckt A, Donnay I, Khatir H, Mermillod P. Role of epidermal growth factor in bovine oocyte maturation and preimplantation embryo development in vitro. Biol Reprod. 1996;54:1420–9.
41. Goud PT, Goud AP, Qian C, Laverge H, Van Der Elst J, De Sutter P, Dhont M. In vitro maturation of human germinal vesicle stage oocytes: role of cumulus cells and epidermal growth factor in the culture medium. Hum Reprod. 1998;13:1638–44.
42. Prochazka SV, Nagyova E, Miyano T, Flechon JE. Developmental regulation of effect of epidermal growth factor on porcine oocyte–cumulus cell complexes: nuclear maturation, expansion, and F-actin remodeling. Mol Reprod Dev. 2000;56:63–73.
43. Wandji SA, Eppig JJ, Fortune JE. FSH and growth factors affect the growth and endocrine function in vitro of granulosa cells of bovine preantral follicles. Theriogenology. 1994;45: 817–32.
44. Schotanus K, Hage WJ, Vanderstichele H, Van Den Hurk R. Effects of conditioned media from murine granulosa cell lines on the growth of isolated bovine preantral follicles. Theriogenology. 1997;48:471–83.
45. Van Wezel IL, Umapathhysivam K, Tiley WD, Rodgers RJ. Immunohistochemical localization of bFGF in bovine ovarian follicles. Mol Cell Endocrinol. 1995;115:133–40.
46. Maeda J, Kotsuji F, Negami A, Kimtani N, Tominaga T. In vitro development of bovine embryos in conditioned media from bovine granulosa cells and Vero cells cultured in exogenous protein and amino acid-free chemically defined human tubal fluid medium. Biol Reprod. 1996;54:930–6.
47. Whitingham DG. Embryos banks in the future of developmental genetics. Genetics. 1974;78:395–402.
48. Hewitt DA, England GC. The effect of oocyte size and bitch age upon oocyte nuclear maturation in vitro. Theriogenology. 1998;49: 957–60.
49. Otoi T, Fujii M, Tanaka M, Ooka A, Suzuki T. Canine oocyte diameter in relation to meiotic competence and sperm penetration. Theriogenology. 2000;54:535–42.
50. Magnusson V, Feitosa WB, Goissis MD, Yamada C, Tavares LM, D'Avila Assumpção ME, Visintin JA. Bovine oocyte vitrification: effect of ethylene glycol concentrations and meiotic stages. Anim Reprod Sci. 2008;106:265–73.
51. Quero JM, Moreno Millán M, Córdoba VM, Franganillo RA. The influence of different types of media supplement on the meiotic maturation of bovine oocytes in vitro. Theriogenology. 1994;41: 405–11.
52. Farstad W. Current state in biotechnology in canine and feline reproduction. Anim Reprod Sci. 2000;60–61:375–87.
53. Otoi T, Willingham L, Shin T, Kraemer DC, Westhusin M. Effects of oocyte culture density on meiotic competence of canine oocytes. Reproduction. 2002;124:775–81.
54. Luvoni GC, Modina S, Gandolfi F. Influence of different stages of the oestrous cycle on cumulus–oocyte communications in canine oocytes: effects on the efficiency of in vitro maturation. J Reprod Fertil Suppl. 2001;57:410–4.
55. Saint-Dizier M, Renard JP, Chastant-Maillard S. Induction of final maturation by sperm penetration in canine oocytes. Reproduction. 2001;121:97–105.
56. Tsafriri W, Vale W, Hsueh AJW. Effects of transforming growth factors and inhibin-related proteins on rat preovulatory graafian follicles in vitro. Endocrinology. 1989;125:1857–62.
57. Brucker C, Alexander NJ, Hodgen GD, Sandow BA. Transforming growth factor-alpha augments meiotic maturation of cumulus cell-enclosed mouse oocytes. Mol Reprod Dev. 1991;28:94–8.
58. Coskun S, Lin YC. Effect of transforming growth factors and activin-A on in vitro porcine oocyte maturation. Mol Reprod Dev. 1994;38:153–9.
59. Mingoti GZ, Garcia JM, Rosa-e-Silva AA. Steroidogenesis in cumulus cells of bovine cumulus–oocyte-complexes matured in vitro with BSA and different concentrations of steroids. Anim Reprod Sci. 2002;69:175–86.
60. Chian RC, Asangla A, Clarke HJ, Tulandi T, Tan SL. Production of steroids from human cumulus cells treated with different concentrations of gonadotropins during culture in vitro. Fertil Steril. 1999;71:61–6.
61. Hatoya S, Sugiyama Y, Nishida H, Okuno T, Torii R, Sugiura K, Kida K, Kawate N, Tamada H, Inaba T. Canine oocyte maturation in culture: significance of estrogen and EGF receptor gene expression in cumulus cells. Theriogenology. 2009;71:560–7.
62. Coskun S, Sanbuissho A, Lin YC, Rikihisa Y. Fertilization and subsequent developmental ability of bovine oocytes matured in medium containing epidermal growth factor EGF. Theriogenology. 1991;36:485–94.
63. Sakaguchi M, Dominko T, Leibfried Rutledge ML, Nagai T, First NL. A combination of EGF and IGF-1 accelerates the progression of meiosis in bovine follicular oocytes in vitro and fetal calf serum neutralizes the acceleration effect. Theriogenology. 2000;54:1327–42.
64. Sakaguchi M, Dominko T, Yamauchi N, Leibfried Rutledge ML, Nagai T, First NL. Possible mechanism for acceleration of meiotic progression of bovine follicular oocytes by growth factors in vitro. Reproduction. 2002;123:135–42.
65. Isobe N, Terada T. Effect of the factor inhibiting germinal vesicle breakdown on the disruption of gap junctions and cumulus expansion of pig cumulus–oocyte complexes cultured in vitro. Reproduction. 2001;121:249–57.
66. Purohit GN, Brady MS, Sharma SS. Influence of epidermal growth factor and insulin-like growth factor 1 on nuclear maturation and fertilization of buffalo cumulus oocyte complexes in serum free media and their subsequent development in vitro. Anim Reprod Sci. 2005;87:229–39.
67. Otoi T, Shin T, Kraemer DC. Influence of maturation culture period on the development of canine oocytes after in vitro maturation and fertilization. Reprod Nutrit Dev. 2004;44:631–7.
68. Suzukamo C, Hoshina M, Moriya H, Hshiyama N, Nakamura S, kawai F, Sato H, Ariga M, Ito J, Kashiwazaki N. Kinetics of nuclear status and kinase activities during in vitro maturation of canine oocytes. Reprod Dev. 2009;55:116–20.