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A comparison of the effects of estrus cow serum and fetal calf serum on *in vitro* nuclear maturation of bovine oocytes

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INTRODUCTION

The use of *in vitro* matured (IVM) oocytes in *in vitro* fertilization (IVF) programs requires an exact knowledge of the timing of nuclear maturation events. In cattle, the timing of IVM prior to IVF has been variously reported to be 19–22 h (Goto *et al*, 1988) up to 27 h (Xu *et al*, 1987). Correct IVM is necessary to avoid fertilization of premature or aging oocytes. In IVF programs the criteria used for oocyte maturation are cumulus cell expansion and extrusion of the first polar body. Neither are direct assessments of cytoplasm maturation, which is one of the most important factors for successful IVF, but since fertilization *in vivo* occurs when the oocyte is at metaphase II (MII) it is usually assumed that completion of meiosis is accompanied by acquisition of developmental competence by the oocyte. Thus, *in vitro*, it is also assumed that complete oocyte maturation is acheived by the MII nuclear stage.

In this study the timing of the meiotic process during IVM of oocytes is examined under conditions similar to those used for IVF programs.

MATERIALS AND METHODS

Ovaries were collected from adult cows and heifers approximately 20 min after slaughter and transported to the laboratory in sterile phosphate-buffered saline (PBS) or normal saline supplemented with 100 IU/ml penicillin and 100 μg/ml streptomycin at between 30–38°C. Ova were collected from follicles 1–6 mm in diameter by aspiration with an 18 gauge needle within 2–3 h of the animal's death. The cumulus–oocyte complexes (COC) were washed twice in TCM-199 with Hepes containing 20% fetal calf serum (FCS) and 50 μg/ml gentamicin and then

transferred into the culture medium. The culture medium was TCM-199 with 2.2 g/l sodium bicarbonate plus 50 μg/ml gentamicin supplemented with either 20% FCS or 20% estrus cow serum (ECS). A total of 479 oocytes were cultured, 320 with added granulosa cells and 159 without.

Oocyte culture was either in droplets of medium under sterile paraffin oil in a Petri dish or in multiwell plates under paraffin oil, and in 5% CO_2 in air at 39°C for between 8 and 25 h.

To harvest, the cumulus cells were removed either by putting the COC in a 0.1% hyaluronidase:PBS solution for 2–10 min and flushing through a fine pipette or by a quick spin with a whirlimixer. Chromosome preparations were made from the denuded oocytes using either Tarkowski's method (1966) or a modification of the method of Dyban (1983). The modification consisted of placing the oocyte in Tyrode's acid (pH 2.5) for a few seconds after the hypotonic treatment. This softens the zona pellucida. Next the oocyte is placed in fixative (3:1, acetic acid–methanol) for 1–2 min and then transferred to a wet, ice-cold slide. The fixative is allowed to evaporate and the slides to dry in air. Staining was with a 10% solution of Giemsa at pH 6.8–7.0

The ECS was obtained by collecting blood from cows as soon as possible after behavioral estrus was first noted. The serum was inactived by heating at 56°C for 30 min before use.

RESULTS

Cumulus–oocyte complexes with added cumulus cells achieved better and more extensive cumulus cell expansion than those without added cells, but this was not correlated with the stage of meiotic division after a given culture time or the quality of the chromosome preparations.

Denuding the oocytes by fine pipetting was much better than the use of the whirlimixer because many oocytes were lost using the latter technique, presumably by sticking to the wall of the tube.

The modified Dyban's method for chromosome preparations (Dyban, 1983) was preferred to that of Tarkowski (1966) because the spreading was more controllable and there was less chance of losing chromosomes, thus making assessment of chromosomal abnormalities more reliable.

The meiotic stage of oocytes after different culture times are shown in table I.

Table I. Comparison of the effects of culture supplementation with fetal calf serum (FCS) or estrus cow serum (ECS) on bovine oocyte maturation time *in vitro*.

Culture time (h)	*Serum*	*No of oocytes*	*Diplotene*	*Diakinesis*	*MII*	*Undiagnosed chromosomes*	*Ooplasm but no chroms*
8–12	FCS	73	9 (12.3)[a]	37 (50.7)	6 (8.2)	8 (11)	13 (17.8)
8.5–12.5	ECS	100	1	62	1	23	13
19–25	FCS	260	1 (0.4)	14 (5.4)	207 (79.6)	13 (5)	25 (9.6)
22.5–23.5	ECS	46	0	4 (8.7)	49 (87.0)	2 (4.4)	0

[a] Percentages are given between parentheses.

There was no statistical difference between the percentage of oocytes at diakinesis (fig 1) after 8–12 h of culture ($\chi^2 = 1.76$; $P = 0.1$) or at metaphase II (fig 2) after 19–25 h of culture ($\chi^2 = 0.74$; $P = 0.1$) with FCS or ECS.

Fig 1. Chromosomes from a bovine oocyte at diakinesis after *in vitro* culture for approximately 10 h.

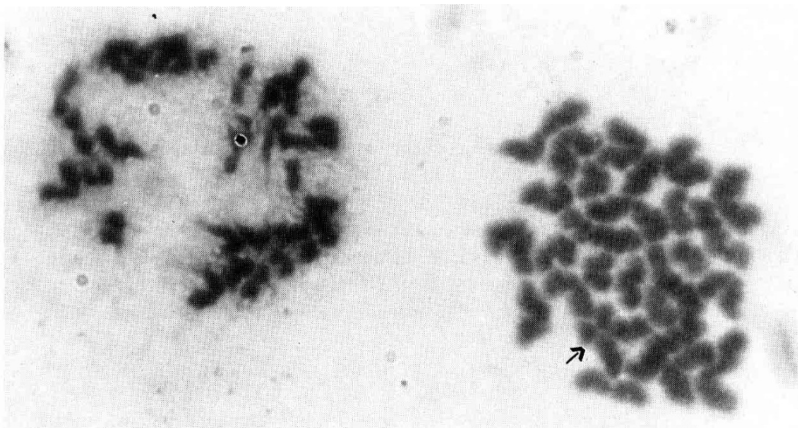

Fig 2. Chromosomes from a bovine oocyte at the second metaphase after *in vitro* culture for approximately 22 h. The arrow indicates the X chromosome. On the left is the degenerating chromatin of the first polar body.

DISCUSSION

An interesting finding in the present study was that the extent of the expansion of the cumulus cells was not found to correlate with the stage of nuclear maturation. Therefore, it would seem unwise to use this phenomenon as a criterion of IVM for IVF.

No difference was found in nuclear maturation time using the two different sources of serum supplementation. Lu *et al* (1987) reported higher IVF rates when ECS was used as the supplement. Therefore, it may be that the ECS contributes to cytoplasmic maturation.

The results from the present study of timing of nuclear maturation are in broad agreement with those previously reported. Sus *et al* (1988) found that 62% of bovine COCs in serum-free TCM-199 progressed to diakinesis after 8.25–12.5 h of culture and 72% reached MII after 19.5–25.75 h of culture. Similar percentages reached MII after 24–27 h of culture in TCM-199 supplemented with 15% FCS (61.8%) (Leibfried and First, 1979) or when supplemented with 20 mg/ml of bovine serum growth protein (71.4%) (Liehman *et al*, 1986). A somewhat longer maturation time of 30 h was reported by Xu *et al* (1986) when they cultured COCs in Ham's F12 with 20% FCS.

In vivo, the oocyte resumes meiosis after the luteinizing hormone (LH) peak and, in cattle, reaches MI after 8–19 h and MII after 20.5–25 h (Kruip *et al*, 1983). Therefore, *in vitro* maturation times are very similar to those occurring *in vivo*. It seems likely, therefore, that, once the oocyte resumes meiosis, progress to MII is spontaneous and at a fixed rate. *In vivo*, ovulation takes place 24–30 h after the LH peak (Hopkins, 1989), that is, some time after the oocyte will have achieved nuclear maturation. It is possible that this extra time allows for the completion of cytoplasmic maturation.

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