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Influence of Day-0 and Day-7 Superovulated Cow Serum During Development of Bovine Oocytes *in vitro*

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Abstract. Oocytes were matured in medium supplemented with 5% serum collected from superovulated cows at oestrus (Day-0 SCS) or at the time of embryo collection (Day-7 SCS), or in medium supplemented with fetal calf serum (FCS). After insemination using frozen–thawed sperm, oocytes were cultured *in vitro* with medium supplemented with 5% Day-0 SCS or 5% Day-7 SCS or 5% FCS. The proportions of embryos that cleaved were not significantly different among treatments, whereas development of the embryo to a blastocyst was significantly higher in the presence of SCS than FCS. When the four possible combinations of Day-0 SCS and Day-7 SCS were used in the maturation and culture media, there were no differences among treatments, except that the cleavage rate was significantly higher ($P < 0{\cdot}05$) with Day-0 SCS in the maturation medium and Day-7 SCS in the culture medium than with Day-7 SCS in the maturation medium and Day-0 SCS in the culture medium. The proportions of embryos that cleaved and developed to blastocysts were not related with the level of progesterone and luteinizing hormone in the serum added to the maturation and culture media. However, the use of serum with low concentrations of glucose, fatty acids and cholesterol in the maturation medium and the culture medium tended to be associated with a higher rate of cleavage and blastocyst development.

Extra keywords: IVM, IVF, IVC, superovulated cow serum.

Introduction

In many laboratories, bovine blastocysts are routinely obtained from oocytes after *in vitro* maturation, fertilization and culture (IVM, IVF, IVC). Oocytes collected from ovarian follicles can undergo spontaneous nuclear maturation in medium containing serum. Bovine oocytes have matured in medium containing either serum or a more defined protein source such as bovine serum albumin (BSA) or fetal calf serum (FCS) (Liebfried-Rutledge *et al.* 1986). Although nuclear events characterizing maturation can occur in the absence of serum (Suss *et al.* 1988), fertilization and development of oocytes into blastocysts was superior after IVM and IVC in the presence of serum. Blastocyst development from bovine follicular oocytes was stimulated following IVM and IVC in medium supplemented with cow serum obtained at pro-oestrus (Younis and Brackett 1991), at oestrus (Schellander *et al.* 1990; Peli and Schellander 1993) or from Day-7 superovulated cows (Suzuki and Shimohira 1985; Matsuoka *et al.* 1992; Geshi *et al.* 1993). The establishment of ideal culture conditions for early embryonic development is important for the study of factors involved in this process as well as for technologies such as IVF, nuclear transplantation and gene transfer.

We examined the development *in vitro* of fertilized bovine oocytes into blastocysts and cultured in medium supplemented with superovulated cow serum (SCS) collected on Day 0 (oestrus) and on Day 7 (when embryos developed *in vivo* were collected).

Materials and Methods

IVF Bovine Embryos

Maturation of oocytes. Bovine ovaries obtained from a local abattoir were placed in a physiological saline solution (0·9% (w/v) NaCl) containing penicillin-G (100 I.U. mL^{-1}) and streptomycin sulfate (0·2 $\mu g\ mL^{-1}$). Ovaries were held at 30–32°C and transferred to the laboratory within 3 h. Oocytes within follicles 2–5 mm in diameter were aspirated with an 18G needle connected to a 5-mL syringe. The resultant oocyte suspensions were mixed with modified-PBS (m-PBS; Embryotec, Nihon Zenyaku, Fukushima, Japan) supplemented with 50 $\mu g\ mL^{-1}$ gentamycin sulfate (Sigma, St Louis, MO, USA). The oocyte preparations were then pooled into a 10-mL plastic tube and maintained at 37°C.

Oocytes were rinsed once with m-PBS and three times in maturation medium. This medium consisted of medium-199 (TCM-199 with Earle's salts, L-glutamine, 2200 mg mL^{-1} sodium bicarbonate and 25 mM HEPES buffer; Gibco, Grand Island, NY, USA) supplemented with: 5% SCS (collected on Day 0 or Day 7) or 5% heat-inactivated fetal calf serum (FCS; Gibco); 0·01 mg mL^{-1} follicle-stimulating hormone (FSH; Denka Pharmaceutical, Kawasaki, Japan); and 50 $\mu g\ mL^{-1}$ gentamycin sulfate. Oocytes (100–200) that were surrounded by cumulus cells for more than one-third of

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