Short Communications

Indian Journal of Animal Sciences **83** (4): 377–378, April 2013/short communication

Use of extract egg for parthenogenetic embryos development in caprine

KULDEEP KUMAR[1], RENU SINGH[2], R RANJAN[3], YASOTHA T[4], R K SINGH[5], MANISH KUMAR[6], S K BHANJA[7], B C DAS[8], N H MOHAN[9] and SADHAN BAG[10]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

Received: 1 February 2012; Accepted: 3 November 2012

Key words: Caprine, Extract egg, Parthenogenesis embryo

Foetal bovine serum has been widely used for oocyte maturation *in vitro* in many species such as goat (Crozet *et al*. 2000), sheep (Ghasemzadeh-Nava and Tajik 2000), pig (Wang *et al.* 1997), buffalo (Goorani-Nejad *et al*. 1999), horse (Li *et al.* 2000) and llama (Del Campo *et al.* 1992). However, in some experiments, the serum of the same species is utilized for *in vitro* maturation of oocytes. The examples are oestous goat serum for caprine oocytes (Keskintepe *et al.* 1994) and equine follicular fluid for equine oocytes (Hinrichs *et al.* 1995). In all the experiments, maturation media contain foetal bovine serum (FBS) (Martin-Lunas *et al.* 1996), calf serum (Crozet *et al.* 1993), oestrous goat serum (EGS) (Keskintepe *et al.* 1994) and bovine serum albumin + EGS (Rajikin *et al.* 1994) as protein supplement. However, in nearly all cases, different hormones have been added to the tissue culture media for *in vitro* maturation of this species. The present experiment was undertaken to compare the efficiency of different concentrations of egg yolk and FBS on the *in vitro* maturation of caprine oocytes.

Goat ovaries were collected from local abattoir and carried to the laboratory in normal saline solution (NSS) (0.85%) fortified with antibiotic in a thermos flask at 35°–37°C within 2 h of slaughter. Cumulus oocyte complex (COCs) were aspirated from all visible non atretic follicles by an 18 gauge needle attached to 5 ml syringe containing oocyte collection media (OCM) (Puri 2011). Only excellent (i.e., more than 5 layers of cumulus cells and evenly granulated cytoplasm) and good (more than three layers of cumulus cells and evenly granulated cytoplasm) COCs were selected and drop washed several times in OCM and further drop-washed 5–6 times with *in vitro* maturation (IVM) medium. Groups of 15–20 selected oocytes were placed in 60 µl droplets of IVM medium. IVM media were TCM 199, supplemented with 10% foetal bovine serum (FBS), bovine serum albumin (BSA) (3 mg/ml), FSH (0.5 µg/ml); LH (10 IU/ml); Goat follicular fluid (5%); Na pyruvate 2 mg/ml; L- glutamine (0.1 mg/ml) and gentamycin (50 µg/ml). On the other hand 10% FBS were replaced by 2 and 4% extract egg in *in vitro* maturation medium. The droplets were covered with warm non-toxic mineral oil and cultured at 37°C, in an atmosphere of 5% CO_2 and 20% oxygen under humidified air (95%) for 27 h in CO_2 incubator. The evaluation of maturation were based on the visual assessment of degree of cumulus expansion under inverted microscope described by Kobayashi *et al.* (1994). After maturation, the oocytes were treated with 0.1% hyaluronidase in TCM-199 followed by pipetting to remove the cumulus cells. Finally, cumulus free oocytes were activated using 5µm ionomycin in mSOF for 5 min followed by 4 h incubation with 2 mM 6-DMAP in mSOF. After activation treatment, oocytes were taken out of 6-DMAP drop, washed several times in modified synthetic oviductal fluid (mSOF) (Puri 2011) supplemented with 0.8% BSA and essential and non-essential amino acids and cultured in 100 µl of same media in CO_2 incubator at 37°C, 5% CO_2 and 95% relative humidity until assessment to determine cleavage. For embryo development, cleaved oocytes were subsequently transferred in fresh drop of mSOF and were further cultured.

Table 1. Embryo development percentage in different maturation media

Maturation media with	No. of oocytes	Cleavage%	8–16 cell%	Morula%
10% FBS	61	(85.24%) 52	(65.30)34	(34.61%) 18
2% extract egg	52	(57.69%) 30	(66.66) 20	(10.00) 3
4% extract egg	43	(69.76%) 30	(50.00) 15	(26.66) 8

The cleavage rate percentages of parthenogenetic embryo development are given in Table 1. There was no difference in the formation of 8–16 cell stage embryos between 10% FBS and 2% extract egg while in 4% it was 50.00% (Table 1). This result was higher to the earlier reports of Puri (2011). The morula stage embryos between FBS and in 2 and 4% extract egg were 34.61%, 10.00% and 26.66%,

Present address: [1,2]Research Associate, [7-10]Senior Scientist, [3-6]Ph.D. Scholar, Physiology and Climatology Division, ([10]bag658@gmail.com).

respectively. The higher cleavage rate and embryo development are related to the selection of protein supplements and hormones for *in vitro* maturation media (IVM) that play an important role in embryo development (Pawshe *et al.* 1996).

The results of overall cleavage rate and different stages of embryo development in 4% extract egg was similar to 10% FBS. The effect of egg yolk extract may be due to the fact that it is a natural media, contains a variety of growth factors and nutrients which is required for development of embryos. Previously Gaspodarowitz (1974) has reported the growth promoting action of egg yolk extract. The results indicated that 4% extract egg can be used for generation of parthenogenetic embryos (morula stage and up to blastocyst) and FBS can be replaced with egg yolk for embryo culture in caprine.

SUMMARY

The present experiment was carried out to study the effect of extract egg - a patented product on parthenogenetic embryo development in caprine. Extract egg can be used for parthenogenetic embryo development instead of FBS. A total of 156 oocytes were taken for *in vitro* maturation having 10% FBS, 2% and 4% extract egg in maturation media. Cumulus free oocytes were activated using 5µm ionomycin in mSOF for 5 min followed by 4 h incubation with 2 mM 6-DMAP in mSOF. Finally activated oocyte were cultured in mSOF media in CO_2 incubator at 37°C, 5% CO_2 and 95% relative humidity until assessment to determine cleavage and further embryo development. For embryo development, cleaved oocytes were subsequently transferred in fresh drop of mSOF. The cleavage percentages were 85.24, 57.69 and 69.76% in 10% FBS, 2% and 4% of extract egg, respectively. However, there was no significant difference in the formation of 8–16 cell stage embryos between FBS (65.3%) and 2% extract egg (66.66%), however, in 4% it was 50.0%. The morula stage embryos between FBS and extract egg were 34.61, 10.00 and 26.66%, respectively. The results of overall cleavage rate and different stages of embryo development in 4% extract egg was similar of 10% FBS. The results indicated that 4% extract egg can be used for generation of parthenogenetic embryos and FBS can be replaced for embryo culture in caprine.

ACKNOWLEDGEMENTS

The authors are thankful to NAIP, ICAR and Director, IVRI for providing the facilities to carry out the present research work.

REFFERENCES

Crozet N, Dahirel M and Gall L. 2000. Meiotic competence of *in vitro* grown goat oocytes. *Journal of Reproduction and Fertility* **118**: 367–73.

Del Campo M R, Donoso M X, Del Campo C H, Rojo R, Barros C, Parrish J J and Mapletoft R J. 1992. *In vitro* maturation of llama *(Lama glama)* oocytes. *Proceedings of the 12th International Congress on Animal Reproduction,* vol. **1**, pp. 324–26.

Ghasemzadeh-Nava H and Tajik P. 2000. *In vitro* maturation of ovine follicular oocytes in different concentrations of foetal calf serum and oestous sheep serum. *Theriogenology* **53**: 435.

Goorani-Nejad S, Tajik P, Vodjgani M and Daliri M. 1999. Maturation *in vitro* of water buffaloes oocytes in TCM-199 with different concentrations of FCS. *Proceedings of the 26th World Veterinary Congress,* 23–26 September, Lyon, France.

Gaspodarowitz D. 1974. Localisation of a fibroblast growth factor and its effect alone and with hydrocortisone on $3T_3$ cell growth. *Nature, London* **249**: 123–27.

Hinrichs K, Martin M G, Schmidt A L and Friedman P P. 1995. Effect of follicular components on meiotic arrest and resumption in horse oocytes. *Journal of Reproduction and Fertility* **104**: 149–56.

Keskintepe L, Darwish G M, Kenimer A T and Brackett B G. 1994. Term development of caprine embryos derived from immature oocytes *in vitro*. *Theriogenology* **42**: 527–35.

Kobayashi K, Yamashitu S and Hushi H. 1994. Influence of epidermal growth factors and transforming growth factors on *in vitro* maturation of cumulus enclosed bovine oocytes in defined medium. *Journal of Reproduction and Fertility* **12**: 439–46.

Li X, Morris L H A and Allen W R. 2000. Effects of different activation treatments on fertilization of horse oocytes by intracytoplasmic sperm injection. *Journal of Reproduction and Fertility* **119**: 253–60.

Martin-Lunas M, Martino A, Paramino M T, Palomo M J, Mogas M T, Bielsa M A, Andols P and Martinez P. 1996. Effect of oocyte-sperm co-incubation on acrosome reaction in the goat. *Theriogenology* **46**: 320–30.

Puri G. 2011. 'Development of embryonic stem cells from early stage buffalo embryos and their molecular characterization.' Ph.D. Thesis submitted IVRI Deemed University.

Rajikin M H, Yusoff M A and Bdullah R B. 1994. Ultrastractural studies of developing goat oocytes *in vitro. Theriogenology* **42**: 1003–16.

Wang W H, Hosoe M and Shioya Y. 1997. Induction of cortical granule exocytosis of pig oocytes by spermatozoa during meiotic maturation. *Journal of Reproduction and Fertility* **109**: 247–55.