

Effect of additives in maturation medium on *in-vitro* maturation, fertilization and cleavage rate of prepubertal goat oocytes

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Caprine *in-vitro* embryo production plays important role in the development of reproductive biotechnologies especially in gene transfer by microinjection. Harvesting of oocytes from prepubertal animal can decrease the generation interval; increase the number of offspring for selected does and thus optimizes exploitation of the female genetic material for propagation of valuable goats (Mogas *et al.* 1997). The present study is aimed to evaluate the effects of different supplementation in the basic media (TCM–199) on *in-vitro* maturation, fertilization and cleavage rate of prepubertal goat oocytes.

Collection and processing of abattoir ovaries: Ovaries from prepubertal goats were collected from local abattoir in normal saline solution (NSS) having 100 IU/ml penicillin and 100 microgram/ml streptomycin sulphate at 37 °C and were washed with physiological normal saline solution and DPBS (Dulbecco's phosphate buffer saline) before subjecting to the oocytes recovery.

Preparation of estrus goat serum: Blood from jugular vein was collected aseptically from estrus does on the second day of their estrus phase. Blood serum was separated, filtered, heat inactivated and stored at –20°.

Collection and preparation of caprine follicular fluid: The follicular fluid (CFF) was collected from transparent follicles centrifuged and filtered before freezing it at –20°. On the day of use, the follicular fluid was heated at 56 °C for 30 min in a water bath to fix the complement. Before use the

heat-treated follicular fluid was again filtered.

Oocytes maturation: The oocytes were recovered by puncturing the visible surface of atretic follicle (1–5 mm diameter) with an 18–gauge needle into a 35 mm sterile glass petri-dish containing oocytes collection media (OCM). The oocytes were graded as excellent, good, fair and poor depending upon the feature of cumulus layers and cytoplasm as per Kharche *et al.* (2008). Only excellent and good quality oocytes (grade A and B) were subjected to the process of *in-vitro* maturation after washing 10–20 times in 100 µl drops of oocyte handling media (OHM) and 8–10 washing in 100 µl drops of maturation medium (MM). 10–15 cumulus oocyte complexes (COCs) were placed in micro drops of 50 µl of the maturation medium containing either 20% concentration of EGS, or 20% NCS, or 10% NCS plus hormone (FSH @ 1 microgram/ml + LH @ 100 IU/ml + E₂ @ 1 microgram/ml) or 20% CFF which had been equilibrated at 38.5 °C in a CO₂ incubator. The oocytes in the 50 µl droplets were covered with sterile mineral oil and cultured in humidified atmosphere of 5% CO₂ in air at 38.5±1 °C for 27 h in a CO₂ incubator. The maturation of oocytes was confirmed by cumulus expansion and presence of first polar body after 27 h of incubation with maturation media.

The *in-vitro* capacitation, *in-vitro* fertilization and *in-vitro* culture of *in-vitro* matured and fertilized oocytes were done as per Singh *et al.* (2009).

Assessment of cleavage rate: Cleavage rate was evaluated 48 h post insemination by counting the number of oocytes cleaved (≥ 2 cell embryos) and number of oocytes inseminated.

The difference between maturation rates and cleavage rates were analyzed by the Normal Deviate test (Sendecor and Cochran 1989).

Statistically no significant differences were observed in the maturation rates and cleavage rate of prepubertal goat oocytes, which have matured in basic maturation media (TCM–199) with different media additives (Table 1).

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Table 1. Maturation and cleavage rate of *in-vitro* matured and fertilized prepubertal goat oocytes

Media additives	Number of oocytes subjected to maturation	Number (%) of oocytes matured	Number of oocytes subjected to fertilization	Number (%) of oocytes cleaved
20% EGS	146	127 (86.98%)	127	55 (43.30%)
20% NCS	146	129 (88.35%)	129	53 (41.08%)
10% NCS + hormones	176	166 (94.31%)	164	69 (42.07%)
20% CFF	218	194 (88.99%)	192	85 (44.27%)

The processes of maturation of oocytes are affected by layer of cumulus cells around the oocytes (Teotia 1996) and cytoplasmic maturation of oocytes (Crozet *et al.* 1995). Also, energy substrate and combination of amino acids (Lu *et al.* 1988), hormones (Zulke and Brackett *et al.* 1993) etc. are useful for the process of maturation. Knowing the facts grade A and B oocytes which consist of 3 to 4 layer of cumulus investment around the oocytes were used in this study. In the basic media (TCM-199) different concentration and source of serum and hormones were incorporated as a source of energy substrate, amino acids and hormones.

In the present study different sources of serum, hormone, that support the process of maturation of oocytes naturally and caprine follicular fluids, which is believed to contain substances required for maturation, were used, in the maturation medium and assessed the maturation of the oocytes on the basis of morphological changes as per the criteria followed by Younis *et al.* (1991). The *in-vitro* matured oocytes had expanded cumulus cell and/or first polar bodies, on the other hand immature oocytes were characterized by the presence of compact and unexpanded cumulus cell and homogeneously dark ooplasm.

Using 20% EGS (estrous goat serum) with the basic media (TCM-199) various workers have reported a maturation rate ranging between 40 to 94% (maturation rate 40%, Younis *et al.* 1993; 88.1%, Keskinetepe *et al.* 1994; 54.79%, Agarwal *et al.* 1995; 57.58%, Mogas *et al.* 1995., 71.60%, Sharma *et al.* 1996 and 94%, Tajik and Shams Esfandabadi 2003). The result (86.98%) for this treatment group is in close conformity with Tajik and Shams Esfandabadi (2003).

With 20% NCS (new calf serum) we obtained a maturation rate of 88.35% in the prepubertal animals collected oocytes. There are certain reports where the maturation rates were near to present findings (88%, Keskinetepe *et al.* 1994), however, others have observed a lower (51.6%, Sun *et al.* 1994) or higher maturation rate compared to present results (74 to 94%, Tajik and Shams Esfandabadi 2003).

Using gonadotrophins and steroid hormones various workers have reported a maturation rate ranging from 54.8 to 100% (90 to 100%, Younis *et al.* 1991; 88 to 100%, Keskinetepe *et al.* 1994; 76.2%, Sun *et al.* 1994; 54.8%, Mogas *et al.* 1995 and 59.2%; Rao *et al.* 2002), however, the present result (94.31%) is in close approximation with the reports of Younis *et al.* (1991).

Follicular fluid was incorporated in the maturation media

for observing the effect on maturation rate. In present study, with the use of 20% CFF (caprine follicular fluid) we got a maturation rate of 88.99% in prepubertal animal collected oocytes. The present finding for this treatment group is some what close with the work of other worker too (82.60%, Sun *et al.* 1994), however, others have reported a lower (59.20%; Rao *et al.* 2002) or higher results compare to our findings (98.37%; Devaraju 1996).

In the present study we further studied the *in-vitro* fertilization (in Fert-TALP media) and cleavage rate of *in-vitro* matured oocytes matured in maturation media with different supplementation, viz. 20% EGS, 20% NCS, 10% NCS + hormone (FSH+LH+E₂ or 20% CFF).

Various workers have used Fert-TALP as the fertilization media and obtained variable cleavage results ranging between 10 and 69% (cleavage rate 10%, Younis *et al.* 1991; 46–69%, Crozet *et al.* 1995; 27.59 - 46.48%, Mogas *et al.* 1995; 17.84–55.47%, Palomo *et al.* 1995; 50.6%, Izquierdo *et al.* 1998; 51 - 58%, Koeman *et al.* 2002). In this study we have obtained the cleavage rates of 43.30, 41.08, 42.07 and 44.27%, respectively, for prepubertal goat oocytes in different treatment groups of maturation medium when the fertilization medium was kept the same. The present results for cleavage rates are in close agreement with Koeman *et al.* (2002) and significantly higher than Younis *et al.* (1991).

SUMMARY

On morphological basis the maturation rate (%) of oocytes in using 20% estrous goat serum (EGS), 20% new calf serum (NCS), or 10% new calf serum (NCS) plus hormone (FSH + LH + E₂) or 20% caprine follicular fluid (CFF) for prepubertal animals were 86.98, 88.35, 94.31 and 88.99% respectively. The mature oocytes were denuded with 0.1% hyaluronidase and transferred in Fert-TALP media (fertilization media). Oocytes were co-incubated with capacitated spermatozoa (concentration of 1×10⁶ live sperm/ml) for 18 h at 38.5±1 °C in an atmosphere of 5% CO₂ in humidified air. After 48 hr of post insemination the cleavage rates of 43.30, 41.08, 42.07 and 44.27 were obtained for prepubertal goat oocytes, respectively, in different treatment group of maturation medium when the fertilization medium was kept the same.

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REFERENCES

- Agarwal K P, Sharma G T, Saxena C and Sharma N. 1995. Chronology of first meiotic events of caprine oocytes matured *in-vitro*. *Indian Journal of Animal Sciences* **65** (3): 285–88.
- Crozet N, Ahmed-Ali M and Dubos M. 1995. Developmental competence of goat oocytes from follicles of different size categories following maturation, fertilization and culture *in vitro*. *Journal of Reproduction and Fertility* **103**: 293–98.
- Devaraju B. 1996. 'Assessment of different procedures for *in-vitro* maturation of goat oocytes.' M.V.Sc. Thesis Acharya N.G.Ranga Agricultural university, Rajendranagar Haderabad
- Izquierdo D, Villamediana P, Palomo M J, Mogas T and Paramio M T. 1998. Effect of sperm capacitation and fertilization media on IVF and early embryo development of prepubertal goat oocytes. *Theriogenology* **49**: 1501–13.
- 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.
- Kharche S D, Goel A K, Jindal S K and Sinha N K. 2008. Birth of female kid from *in vitro* matured and fertilized caprine oocytes. *Indian Journal of Animal Sciences* **78** (7): 680–85.
- Koeman J K, Keefer C L, Baldassarre H, Lane M, Gardner D K and Downey B R. 2002. Developmental competence of prepubertal and adult goat oocytes cultured in semi-defined media. *Theriogenology* **53**: 297 (Abstract).
- Lu K H, Gordon I, Gallagher M and Mgovorn H. 1988. Birth of twins after transfer of cattle embryos produced by *in vitro* techniques. *Veterinary Record* **122**: 539–40.
- Mogas T, Izquierdo M D, Palomo M J and Paramio M T. 1995. Effect of hormone, serum source and culture system on IVM and IVF of prepubertal goat oocytes and subsequent embryo development. *Theriogenology* **43**: 284.
- Mogas T, Palomo M J, Izquierdo M D and Paramio M T. 1997. Developmental capacity of *in vitro* matured and fertilized oocytes from prepubertal and adult goats. *Theriogenology* **47**: 1189–03.
- Palomo M J, Izquierdo M D and Paramio M T. 1995. Effect of heparin and sperm concentration on IVF of prepubertal goat oocytes. *Theriogenology* **43**: 292 (Abstract).
- Rao B S, Naidu D, Amarnath R, Vagdevi A S, Rao K V, Brahmaiah and Rao V H. 2002. *In vitro* maturation of sheep oocytes in different media during breeding and non breeding season. *Small ruminant Research* **43**: 31–36.
- Sharma G T, Majumdar A C and Bonde S W. 1996. Chronology of maturational events in goat oocytes cultured *in vitro*. *Small Ruminant Research* **22**: 25–30.
- Singh K P, Saxena A, Kharche S D and Singh P. 2009. Studies of *in vitro* maturation, fertilization and cleavage rate of prepubertal and pubertal goat oocytes. *Indian Journal of Animal Sciences* **79** (6): 550–53.
- Sun F J, Holm P, Irvine B and Seamark R F. 1994. Effect of sheep and human follicular fluid on the maturation of sheep oocytes *in vitro*. *Theriogenology* **41**: 981–88.
- Tajik P and Shams Esfandabadi N. 2003. *In-vitro* maturation of caprine oocytes in different culture media. *Small Ruminant Research* **47**: 155–58.
- Teotia A. 1996. 'Fertilization and developmental competence of caprine oocytes matured over granulose cell monolayer.' M.V.Sc. Thesis, submitted to deemed university IVRI, Izatnagar, Bareilly, India.
- Younis A I, Keskintepe L, Mackie K and Brackett B G. 1992. *In vitro* maturation and fertilization of Toggenburg oocytes. *Theriogenology* **37**: 330.
- Younis A I, Zuelke K A, Harper K M, Oliveira M A L and Brackett B G. 1991. *In vitro* fertilization of goat oocytes. *Biology of Reproduction* **44**: 1177–82.
- Zulke K A and Brackett B G. 1993. Increased glutamine metabolism in bovine cumulus cell enclosed oocytes after *in vitro* maturation with luteinizing hormone. *Biology of Reproduction* **48**: 815–20.