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Efficacy of different media additives in basic maturation medium (TCM-199) on *in-vitro* maturation and cleavage rate of pubertal goat oocytes

K P SINGH[1], ATUL SAXENA[2], S D KHARCHE[3] and PRANEETA SINGH[4]

U.P.Pt. Deen Dayal Upadhyaya Pashu-Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura, Uttar Pradesh 281122 India

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Caprine *in-vitro* embryo production can be expected to play prominently in future goat breeding strategies in the development of biotechnologies especially gene transfer by zygote, microinjection, micromanipulation, sexing, cloning and transgenic animal production and genetic engineering (Robl *et al*. 2007 and Keefer 2008). The use of ovaries collected from slaughterhouse as source of oocytes for *in-vitro* maturation and fertilization allows large scale economical embryos production for development of cloning and transgenesis (Brackett 1992). The oocytes collected from ovaries from both prepubertal and pubertal goats yield similar *in-vitro* nuclear maturation and fertilization (Martino *et al*. 1995). Various workers added either serum of same species i.e. estrous goat serum (EGS) (Keskintepe *et al*. 1994, Yadav *et al*. 2008) or different species fetal calf serum (FCS) (Crozet *et al*. 2000, Yadav *et al*. 2008), fetal bovine serum (FBS) (Yadav *et al*. 2008) in tissue culture medium along with different hormones and bovine albumin as protein source for *in-vitro* maturation of goat oocytes (Kharche *et al*. 2005).

Keeping in view the above facts, present study was aimed to evaluate the effects of different supplementation in the basic media (TCM-199) on *in-vitro* maturation and cleavage rate of pubertal goat oocytes.

A total 733 oocytes (grade A and B) were collected from 686 pubertal goat ovaries and these oocyte were subjected for *in-vitro* maturation in different replicate in a basic maturation media of TCM-199 which was supplemented either with 20% estrous goat serum (EGS), 20% new calf serum (NCS), or 10% new calf serum (NCS) plus hormone (FSH + LH + E_2) or 20% caprine follicular fluid (CFF). Collection and processing of abattoir ovaries was done as per Singh *et al*. (2010) and Singh *et al*. (2009). Blood from jugular vein was collected aseptically from estrus does on the second day of their estrus phase, the 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 (small and medium sized follicle) present on the surface of ovaries after aspirating with a sterile 20 gauge needle attached to a 5 ml disposable plastic syringe. Follicles, which looked hemorrhagic or turbid, were eliminated. Pooled follicular content was then centrifuged at 3000 rpm for 10 min. Supernatant was carefully removed and again centrifuged as stated above to remove any remaining cells. Finally supernatant was filtered through 0.22µm sterilized syringe filter and stored in one ml aliquots at –20°C till used. 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 maturation was done as per Kharche *et al*. (2008). The *in-vitro* capacitation was done as per Singh *et al*. (2010) and the *in-vitro* fertilization of *in-vitro* matured oocytes was done as per the procedure described by Singh *et al.* (2009).

In-vitro *culture of* in-vitro *fertilized oocytes*

The *in-vitro* culture of *in-vitro* fertilized oocytes was done as per Singh *et al*. (2010)

Assessment of cleavage rate

Forty-eight hours post insemination, cleavage rate was

Present address: [1]Veterinary Officer, Anand Nagar Bhitari, Faizabad, UP (drkpsvet@rediffmail.com). [2]Professor and Head, Department of Obstetrics and Gynaecology (atulsaxena @rediffmail.com). [3]Principal Scientist, Division of PR and SM, CIRG, Makhdoom, Farah, Mathura.[4]Assistant Professor, Department of Livestock Products Technology, College of Veterinary and Animal Sciences, G.B.Pant University of Agriculture and Technology, Pantnagar 263145 (vet_praneeta12 @rediffmail.com).

Table 1. Effect of different media additives in basic maturation medium (TCM-199) on *in-vitro* maturation and cleavage rate of pubertal goat oocytes

Media additives	No. of replicates	No. of oocyte used for maturation	No. of matured oocytes	Maturation rate (%)	No. of oocytes used for fertilization	No. of cleaved oocytes	Cleavage rate (%)
20% EGS	6	136	122	89.75	122	35	28.68[a]
20% NCS	6	167	147	88.02	143	60	41.95[b]
10% NCS+ hormones	6	133	123	92.48	118	52	44.06[b]
20% CFF	6	297	266	89.56	265	107	40.37[b]

Percentages bearing different superscript in a column differed significantly (P<0.05).

evaluated by counting the number of cleaved <2 cell embryos/ number of oocytes inseminated as per Singh *et al.* (2009).

Statistical analysis

The difference between maturation rates and cleavage rates were analyzed by the normal deviate test (Snedecor and Cochran 1989).

In this experiment, no significant differences were observed in the maturation rates of pubertal goat oocytes, which have matured in basic maturation media (TCM-199) with different media additives (Table 1). However, the cleavage rate with 20% EGS supplementation were lower (P<0.05) than with other media additives (Table 1).

Yadav *et al*. (2008) reported the maturation and cleavage rate of prepubertal goat oocytes, which were matured in TCM-199 supplemented with 20% EGS, 20% estrous sheep serum (ESS) and 20% fetal bovine serum (FBS) as 80.70, 70.62 and 83.12% and 70.89, 38.69 and 72.66%, respectively. In present study, the maturation and cleavage rate of pubertal goat oocytes, which were matured in TCM-199 supplemented with 20% EGS, 20% NCS, 10% NCS plus hormones (FSH+ LH+ E_2) or 20% CFF were 89.75, 88.02, 92.48 and 89.56% and 28.68, 41.95, 44.06 and 40.37% respectively. The present study showed comparatively higher maturation and lower cleavage rate than the above findings.

Singh *et al*. (2009) reported the maturation and cleavage rate of oocytes using 20% EGS, 20% NCS, 10% NCS plus hormones (FSH+ LH+ E_2) or 20% CFF and fertilized in fert-TALP media were 86.98, 88.35, 94.31and 88.99% and 43.30, 41.08, 42.07 and 44.27%, respectively, for prepubertal animals. In the present study, the maturation and cleavage rate for pubertal animals were almost similar except for the 20% EGS, which showed significantly (P< 0.05) lower cleavage rate.

Singh *et al*. (2010) reported the cleavage rate of pubertal goat oocytes which were matured in maturation media TCM-199 supplemented with different supplements viz 20% EGS, 20% NCS, 10% NCS plus hormones (FSH+ LH+ E_2) or 20% CFF and co-cultured with granulosa cell monolayer and oviductal epithelial cells, were 45.93 and 43.49%, respectively. In present study, the cleavage rate ranged from 28.68 – 44.06%. The present study depicted, comparatively lower cleavage rate for the pubertal goat oocytes, which were cleaved without co-culture cells. Kharche *et al*. (2011) reported 44% cleavage rate with goat oocytes, which were matured in maturation media TCM-199 supplemented with 10% fetal bovine serum (FBS) plus hormones (FSH + LH) and bovine serum albumin (BSA). In present study, the cleavage rate found was 44.06% with pubertal goat oocytes which were matured in basic maturation media TCM-199 supplemented with 10% NCS plus hormones (FSH+ LH+ E_2). Both these finding are in similarity with each other.

Our findings are contrary to others (Totey *et al*. 1992 and Pawshe *et al*.1996) who observed better maturation of oocytes with incorporation of gonadotropin and steroid hormone in *in-vitro* culture media. Although the hormonal constituents of follicular fluids is helpful in maturation of oocytes and in ovulation process (Hafez 1987) CFF incorporation in the maturation medium did not reflect any advantage over other supplementation.

SUMMARY

Good quality oocytes (733) were collected from 686 pubertal goat ovaries of abattoir origin. Collected oocytes were matured in maturation medium (TCM-199) supplemented with either of different supplements, viz. 20% estrous goat serum (EGS), 20% new calf serum (NCS), or 10% new calf serum (NCS) plus hormone (FSH + LH + E_2) or 20% caprine follicular fluid (CFF) and maturation rate (%) were found as 89.75, 88.02, 92.48 and 89.56%, 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 1×10^6 live sperm / ml) for 18 h at 38.5 ± 1°C in an atmosphere of 5% CO_2 in humidified air. After 48 h post insemination the cleavage rate of 28.68, 41.95, 44.06 and 40.37% were observed for pubertal goat oocytes, respectively, in different treatment group of maturation medium when fertilization medium was kept same. The present study concluded that different media additives in basic maturation medium (TCM-199) showed similar maturation rate. Whereas cleavage rate was found to

be significantly (P< 0.05) lower with 20% EGS compared to other media additives.

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