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# Effect of maturation media on early embryonic development of goat immature oocytes

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**Abstract**
In the present study effect of maturation media on early embryonic development of goat immature oocytes was conducted. The investigations were aimed to standardize the culture conditions for *in vitro* maturation, fertilization and *in vitro* embryo development of goat immature oocytes. The mean total number of oocytes recovered per ovary by slicing method was 10.79±1.00. All the collected oocytes were washed in oocytes washing media and transfer into two different maturation media TCM 199 (supplemented with 10% FCS, FSH and estradiol (1mg/ml) and commercial available maturation medium BO-IVM for 24hrs. After maturation the oocytes were fertilized in BO-IVF medium with $2\times10^6$ sperm/ ml. After 24 hr of fertilization the presumptive zygote were transferred to *in vitro* culture medium BO-IVC. The embryo development rate was observed every 48 hr of incubation. The development rate was observed higher oocytes matured in commercial available BO-IVM medium than TCM 199 but there was not any significant difference between TCM 199 maturation medium and commercial available BO-IVM medium. From the present study it is concluded that the commercial available maturation medium i.e. BO-IVM showed higher per cent cleavage and embryonic development than TCM 199 for the culture condition in the embryonic development of the goat oocytes.

**Keywords:** Goat, oocyte maturation, *in vitro* fertilization, embryo development

## Introduction
Assisted reproductive technologies (ART) are considered a major tool for accelerating the genetic improvement in many animal species. *In vitro* techniques for oocyte maturation and fertilization are receiving more attention in small ruminant species such as sheep and goat than in large species due to their short interval, prolificacy and economical rearing (Kharche *et al.*,2009) [1]. The environment supplied for *in vitro* maturation is critical for subsequent fertilization and embryonic development. Selection of suitable oocyte culture medium with a supplementation of serum and hormones is imperative for obtaining optimal results in the *in vitro* maturation and fertilization studies in goat. This is important in improving the overall efficacy of embryo production. Maturation is the first important step which involves i) expansion of cumulus mass ii) nuclear maturation iii) cytoplasmic and membrane maturation (Pawshe *et al.*, 1996) [2]. To enhance the complete functional maturation of isolated mammalian oocyte *in vitro* various serum preparation have been added to the culture media serum mainly contains protein components including hormones trace nutrients and growth factors such as epidermal growth factor (EGF) (Hsu *et al.*, 1987) [3]. The possibility of achieving complete maturation of extra follicular oocyte in domestic animal has been investigated by using combination of 17 β estradiol $E_2$ and gonadotropins in the culture medium (Moor and Trounson 1977) [4]. The addition of $E_2$, FSH or LH either alone or in combination improved the maturation of oocytes in the medium supplemented with GES or FCS. However, the preparation of media is a hard and time consuming process, it requires weekly or biweekly attention to avoid risk of degradation of certain components and precipitation of chemicals. So now a days many researchers are using readymade media for IVM, IVF and embryo development but their standardization and utility in goat is not available in literature i.e. Brackett and Oliphant medium. (Nielson *et al.*, 2015; Pryor *et al.*, 2016) [5, 6]. The aim of the present study to evaluate the effect of different maturation media on early embryonic development of goat immature oocytes.

## Materials and Method

All chemicals and media were purchased from sigma chemical co USA. For maturation (BO-IVM), fertilization (BO-IVF) and embryo development (BO-IVC) the commercial available media were purchased from Bickland Industrial Park, Falmouth, Cornwell, TR 4TA, United Kingdom.

Goat ovaries were collected from the local slaughterhouse at an unidentified stage of reproductive cycle and carried to the laboratory within 2 to 3 hr. of harvest in normal saline maintained at 30°C supplemented with 50 µg/ml Gentamycin. Oocytes were collected from ovaries by slicing method. The oocyte containing media was collected in 50 ml sterilized tube and then centrifuged for 3 min at 500 rpm and the pellet were looked over for COCs under a zoom stereomicroscope (20x). The isolated oocytes on the base of cumulus oocyte complex layers and cytoplasm were classified as Good, Fair and Poor quality oocytes.

### *In vitro* maturation, fertilization and embryo culture-

All the collected oocytes were washed in washing media and transfer into two different maturation media TCM 199 (Sigma Chemical) supplemented with 10% FCS, FSH (10 ug/ml) and estradiol (1mg/ml) and commercial available medium BO-IVM for 24hrs under humidified atmosphere of 6% $CO_2$ in air at 39 °C. After 24 hours of *in vitro* maturation the matured oocytes were fertilized by frozen thawed semen from the same ejaculate of the same bull at $2\times10^6$ sperm/ml in BO-IVF medium. After 20 hrs of fertilization medium, presumptive zygotes were denuded and transferred to embryo development medium (BO-IVC) covered with mineral oil under humidified atmosphere of 6% $CO_2$ in air at 39 °C. During culture period (7days) embryos were evaluated every 48 hour for rates of cleavage and embryo development for four cell and blastocyst stages.

### Statistical analysis

Effect of maturation media on early embryonic development of goat immature oocytes was analyzed by t-test.

## Results and Discussion

### Recovery of oocytes by slicing method

The effect of slicing method of oocyte recovery and quality of oocyte recovered from goat ovaries are summarized in (Table1).

**Table 1:** Collection of oocyte by slicing method and their number and quality of goat immature oocytes

| Method of collection | Total no. of ovaries | Total no. of oocytes | Mean oocytes/ovary (±SEM) | | | Mean total no of oocytes /ovary (±SEM) |
|---|---|---|---|---|---|---|
| | | | Good | Fair | Poor | |
| Slicing | 51 | 545 | 122 2.49±0.38[a] | 136 2.54±0.34[a] | 287 5.76±0.78[b] | 10.79±1.00 |

Mean bearing with different superscript (a, b) in a row show significant difference ($P<0.05$)

The total number of oocytes recovered per ovary by slicing method was 10.79±1.00. There was significantly higher number of poor quality oocyte without cumulus cell layer but having normal cytoplasm than that of good and fair quality oocyte.

For the *in vitro* embryo production the more number of good quality oocytes recovered per ovary is an important concern. The main aim of recovery method is to maximize number of oocytes per ovary which is important for *in vitro* maturation. In present study, total number of goat oocytes recovered per ovary was more; though recovery of good quality oocytes per ovary is low.

The similar observation was noted by Martino *et al.* (1994) [7] reported the higher number of poor quality oocytes was collected than good and fair quality oocytes collection in goat this may be due to addition of the small diameter follicles. Pawshe *et al*. (1994) [8] reported mean oocytes per ovary in good, fair and poor quality was 0.91±0.61, 0.80±0.07 and 0.69±0.04, respectively which was lower than the present study. Das *et al*. (1996) [9] observed lower number of good (0.7±0.09), fair (1.9±0.19) and poor (3.2±0.24) quality oocytes in slicing technique as in comparison with present results. In the present experiment the mean oocytes recovery of good quality oocytes per ovary was 2.49±0.38, lower than the results observed by Wani *et al*. (2000) [10] i.e. 5.2±0.23. They observed lower mean oocytes recovery for fair quality oocytes (2.2± 1.07) in comparison with the present experiment (2.54±0.34). Wang *et al*. (2007) [11] found higher number of good quality oocytes recovered per ovary by the slicing method (3.9) than fair (1.3) and poor (1.1) quality oocytes In accordance with present experiment Hoque *et al*. (2011) [12] observed higher number of abnormal COCs i.e. grade C and grade D (2.22±0.13) per ovary than the number of normal COCs i.e. grade A and grade B (1.91±0.10).

### Microscopic evaluation of cumulus cell expansion during *in vitro* maturation of oocyte

The morphology of the cumulus surrounding an oocyte is usually used a selection criteria prior to IVM and the degree of cumulus cell expansion can be used as a morphological indicator of oocyte quality following IVM (Shioya *et al*., 1988; Lonengran *et al*., 1994) [13, 14]. It has been proposed that an expanded cumulus cell indicates mature and good quality oocytes, while a compact. After 24 hours of maturation, the cumulus cell expansion was studied and presented in Table 2

**Table 2:** Microscopic evaluation of cumulus cell expansion during *in vitro* maturation of oocytes

| Media | Grade of COCs | No of Oocytes | Cumulus cell expansion level | | |
|---|---|---|---|---|---|
| | | | Upto 40% | 40-60% | Above 60% |
| TCM 199 | Good | 52 | 7 (13.46)[a] | 16 (30.77) | 29 (55.77) |
| | Fair | 49 | 7 (14.29)[a] | 14 (28.57) | 28 (57.14) |
| BO-IVM | Good | 66 | 34 (51.51)[b] | 19 (28.79) | 13 (19.70) |
| | Fair | 82 | 49 (59.76)[b] | 19 (23.17) | 14 (17.07) |

Mean bearing with different superscript (a, b) in a column differ significantly ($P<0.05$).

During the comparison of maturation media i.e. TCM 199 and BO-IVM for the cumulus cell expansion, the result revealed that in BO medium, good and fair quality oocytes show significantly higher expansion of cumulus cell upto 40 per cent. However, cumulus cell expansion was higher in TCM 199 in good and fair quality oocytes above 60% but there was no any significant difference was observed.

In the present experiment, the oocytes matured in TCM 199 showed the cumulus cell expansion upto 40 per cent i.e. 13.46 per cent which was higher than cumulus cell expansion i.e. 1.89 as reported by Mondal *et al.* (2008) [15]. However, they reported higher cumulus cell expansion 40 to 60 per cent and above 60 per cent i.e. 44 and 71.70 per cent in fair and good quality oocytes in comparision with the present experiment. The cumulus cell expansion for good quality COCs found in present experiment above 60% level of cumulus expansion (55.76%) was lower than the results observed by Talukder *et al.* (2011) [16] i.e. 63.93% and for fair quality COCs they observed higher percentage upto 40 and above 60% level of cumulus expansion 19.8 and 53.1%, respectively in comparison with our present results.

Hoque *et al.* (2011) [12] observed more cumulus cell expansion in level 3 (above 60 per cent) i.e. indicating marked expansion of cumulus cells with a compact layer or corona radiate which was 65.73% after slicing of goat ovaries and cultured in TCM199 supplemented with 2.5% bovine serum albumin (BSA) and 10% goat follicular fluid. Present results are in agreement with these findings. In contrast with our results, Nielson *et al.* (2015) [4] observed maximum cumulus cell expansion in BO-IVM medium than TCM 199 supplemented with 0.5% Bovine serum albumin (BSA), 10 IU PMSG and 5 IU HCG aspirated from slaughter house bovine ovaries.

Trousan (1992) [17] observed that for both nuclear and cytoplasmic maturation several factors were responsible. Cumulus cell play a significant role in hormonal regulation. Downs *et al.* (1988) [18] reported that the signals induced by gonadotropins are mediated by cumulus cell and transduced into oocyte via gap junction and help in complete cytoplasmic maturation. Lawrence *et al.* (1980) [19] reported that oocytes do not have receptor for gonadotropins which are present only in adjacent follicular cell. In the present study used the follitropin-V as a source of FSH and LH in maturation media which may explain the possible effects of the cumulus cell contribution in mediating the signals of FSH and LH via cumulus cells to the oocytes that ultimately increase the level of cumulus cell expansion that leads to increase in the stickiness of cumulus cell which was observed in the medium TCM 199. In the present experiment, the medium TCM199 supplemented with fetal calf serum help in providing nutrition to the cumulus cells which might be helpful in the prevention of zona hardening and showing marked cumulus expansion. However, in the BO-IVM medium supplemented with BSA as protein source showed cumulus cell expansion but not that extent to the TCM 199 medium. Fbjan *et al.* (2016) [20] reported that the presence of cumulus cells was not essential for meiotic maturation of the oocytes removed at the time of follicular development, as they already acquired competence to development.

**Effect of maturation media on embryonic development of goat oocyte**

To determine the relative efficiency of *in vitro* maturation media for the developmental competence of *in vitro* fertilized oocytes, the oocytes were matured in two different media i.e. TCM 199 and BO- IVM maturation media, fertilized *in vitro* and transferred to BO-IVC medium for embryo development. The results of embryo development are shown in Table 3.

**Table 3:** Effect of maturation media on embryonic development of goat oocytes

| Maturation Media | No of oocytes matured | No of oocytes fertilized | Development rate (%) | | |
|---|---|---|---|---|---|
| | | | Cleavage | Morula | Blastocyst |
| TCM199 | 225 | 210 | 57 (27.14) | 8 (14.03) | 2 (3.50) |
| BO-IVM | 320 | 300 | 84 (28.00) | 18 (21.42) | 14 (16.66) |

The development of cleaved embryo upto morula and blastocyst stage was higher in BO-IVC media than the oocyte matured in TCM 199 but significant difference was not observed. The percent cleavage in present experiment 27.14 was lower than the results from the cleavage development reported by Smedt *et al.* (1992) [21] i.e. 58 percent. In the present study, the oocytes matured in TCM 199 medium shown the cleavage, morula and blastocyst development was 27.14, 14.03 and 3.50 respectively which was lower than the results observed by Pawshe *et al.* (1994) [8] i.e. 66.6, 25.0 and 21.6 percent respectively. Keshinpte *et al.* (1998) [22] observed 31.4% and 18.6% morula and blastocyst stages of goat oocyte which is in contrast with the present results. Wang *et al.* (2007) [11] observed higher cleavage and blastocyst rate i.e. 31% and 7%, respectively, when compared with our present results.

Rose and Bavister (1992) [23] reported that the use of Ham's F-12 medium for IVM of bovine oocyte produced a significant decrease in the embryonic development capacity when compared to TCM 199. Wiemer *et al.* (1991) [24] and Smedt *et al.*(1992) [21] who had found that TCM 199 supplemented with FCS and hormones is an ideal culture medium for *in vitro* maturation of goat oocytes. Hsu *et al.* (1987) [3] reported that serum mainly contains certain protein components including hormones trace nutrients and growth factor such as epidermal growth factor which enhances the union of cumulus cells to the oocytes and increase the transport of nutrients, hormones or factors involved in the controlling the rate of maturation.

Sanbuissho and Threfall (1990) [25] observed an advantageous effect of FCS when added to medium during the maturation of oocytes. In the present experiment, during maturation of oocytes in TCM 199 media supplemented with FCS and hormone showed the cleavage rate 27.14 per cent. The oocytes matured in BO medium supplemented with BSA as protein supplement showed the cleavage rate 28.00 percent which was comparatively higher than oocyte matured in TCM 199 however the morula and blastocyst development higher in oocyte matured in BO IVM media than the oocyte matured in TCM 199 media.

Similar with the present experiment, Nielson *et al.* (2015) [5] used two maturation media i.e. TCM 199 and BO-IVM medium for oocyte maturation and BO-IVF and BO-IVC for fertilization and embryo development in cattle and they reported that the oocyte matured in BO-IVM media increased the blastocyst rate, kinetic and morphology scores compared

to blastocyst produced in TCM 199 medium used for oocyte maturation. In bovine, Pryor *et al*. (2016) [6] also used BO-IVM, BO-IVF and BO-IVC media for *in vitro* maturation, fertilization and embryo development and reported that this modified ready to use media produce more higher quality embryos under varying culture condition. A commercially available medium BO-IVM which is ready to use medium does not require any additional hormone and serum supplementation also doesn't require weekly attention, pH adjustment and mainly time saving medium, as in present experiment we found higher cleavage, morula and blastocyst rate than the TCM 199 medium. However, in the view of present results.

## Conclusions

It was concluded that the commercial available maturation medium i.e. BO-IVM showed higher per cent of embryonic development than TCM 199 for the culture condition in the embryonic development of the goat oocytes.

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