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# *In vitro* production of goat (*Capra hircus*) embryo in different culture media

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**Abstract**
Considerable effort has been devoted to the development of *in vitro* fertilization and *in vitro* maturation of oocytes from farm animals. *In vivo* embryo production in goats has been studied foryears. The variability of the hormonal treatment, fertilization failure and the premature regression of *corpora luteum* were still needed to be improved. The *in vitro* production (IVP) of embryos offers the possibility of overcoming multiple ovulation embryo transfer limitations. The method of IVP of embryos involves three main steps: *in vitro* maturation of oocytes (IVM), *in vitro* fertilization of oocytes (IVF) with capacitated sperm and *in vitro* culture (IVC) of embryos up to blastocyst stage. *In vitro* embryo production technology also presents the following advantages: (i) a significant increase of embryos from high genetic value females, because oocytes can be recovered from prepubertal, pregnant and even dead or slaughtered goats, (ii) provides an excellent source of low cost embryos for basic research, embryo biotechnology studies (nuclear transfer, transgenesis, embryo sexing and stem cells) and all kinds of embryo research which require high number of embryos for manipulation and (iii) used as a strategy for the rescue of some endangered animal species by inter specific embryo transfer. Pre-implanted growth and development of embryo is deficient in many aspects and was evidenced by the great difficulty in growing embryos *in vitro*, while maintaining viability as shown by development of early embryos *in vitro* generally delayed or completely blocked at 8–16 cell stage embryo and hardly small percentage of the *In vitro* fertilized embryo develop to morula and blastocyst, which is probably due to minor physio-chemical variation and lack of certain factors in the culture system. *In vitro* development of mammalian embryos usually remains inferior to development than *in vivo* produced embryos.

**Keywords:** *in vitro* fertilization, *in vitro* maturation of oocytes, embryo, morula, blastocyst

## Introduction
Goats are the most fertile animals among the domestic species with the conception rates in the range of 90 per cent. Assisted reproduction plays an increasingly important role in highly organized breeding programmes or utilizing goats as bioreactors in the context of gene pharming. Remarkable progress has been made in the field of assisted reproduction, including collection and handling of gametes, *in vitro* fertilization, oestrus control, embryo transfer, conservation and manipulation of gametes and embryos, transgenesis and pregnancy detection. The advances in assisted reproductive technology and the utilization of embryonic stem cell. *In vitro* production of embryos by combining ovum pick-up, *in vitro* fertilization, and ET, embryo culture medium enabled embryo manipulation techniques to develop (Urdaneta *et al.*, 2003) [8]. It is now possible to freeze embryos, store them for future use or transport them internationally, bypass certain disease situations, initiate production of kids from reproductively immature doelings, and evaluate embryos before transfer. The dairy goat production has several advantages, such as small investment, short reproductive cycle, easy feeding, and quick economic returns. These advantages demonstrated the promising prospects of dairy goat industry. However, the dairy goat industry also faces several challenges, such as lesser number of large-scale intensive dairy farms, limited consumer market of milk products, and fewer high producing breeds of dairy goats etc. Therefore, it is necessary to formulate relevant breeding regulations to form a supporting breeding technique, and it is also very important to accelerate the development of key technologies of reproduction, breeding, and feeding management in the dairy goat industry. These measures could improve the production performance of dairy goats to provide more excellent goat products for human consumption. Jun Luo *et al.* (2019) [5].

## Materials and Methods

### Methods

Goat oocytes were used for *in vitro* embryo production (Galyna *et al.,* 2015) [2]. The ovaries were collected from slaughter house. The process of *in vitro* embryo production consisted of *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* culture (IVC). The matured oocytes were inseminated with *in vitro* capacitated sperm. (Gordon, 2003) [3].

### Goat ovary collection

Ovaries of goat were collected from corporation slaughter house, Chennai. Ovaries were washed three times by normal sterile containing 10µg/ml gentamicin sulphate and immersed in the same solution at 37ºC during transportation until used. All processes were performed under aseptic conditions.

### *In vitro* maturation of oocytes

Cumulus oocyte complexes (COCs) were collected from ovaries by slicing. The cumulus oocyte complexes (COCs) were morphologically assessed and only oocytes that were fully surrounded with three to five layers of compact cumulus cells and homogenous cytoplasm were selected for IVM. All selected COCs (18 – 20 numbers) were cultured in 100µl drops of maturation medium (TCM 199) supplemented with 10 per cent fetal calf serum, 0.2mM pyruvate, 5µg/ml LH and 1µg/ml $E_2$ at 38.0 ºC, 5 per cent $CO_2$ and 95 per cent relative humidity (RH) for 24 hrs. The droplets were covered with sterile mineral oil.

### *In vitro* fertilization (IVF) of goat oocytes

### Sperm preparation

Buck frozen semen straws were used for preparation of motile sperms. Two semen straws (0.25ml French mini) were thawed at 37 ºC for 30 sec in a water bath. Straws were then wiped with 70 per cent alcohol. 0.5 ml of semen was added to 4 ml of sperm Tyrode's albumin lactate pyruvate (TALP) medium in 15 ml centrifuge tube and centrifuged at 500g for 15 min. Supernatant was discarded and sperm pellet was collected. The sperm pellet was then layered slowly in the bottom of one ml fresh sperm TALP medium in three sterile 15 ml centrifuge tubes. Tubes were held at 45º angle and kept inside a $CO_2$ incubator for 30 min at 38 ºC under 5 per cent $CO_2$ concentration and 95 per cent RH for swim up. After incubation, supernatant from all the three tubes were collected, pooled, washed along with fresh 4ml of sperm TALP medium and centrifuged at 500g for 15min. The final pellet was collected and concentration of sperm was assessed by haemocytometer.

### Oocyte preparation

Oocytes were observed for maturation status after 24 hrs. Matured oocytes were washed in pre-equilibrated fertilization TALP medium thrice in 35mm Petri dishes. Matured oocytes were transferred to pre-equilibrated 50µl droplets (8-10 oocytes/droplet) of IVF TALP medium supplemented with heparin (10µg/ml) with sterile mineral oil. (Ambili *et al.* 2015) [1].

### Sperm oocyte co-incubation

Oocytes were inseminated with 2µl of sperm suspension which contained about two million actively motile sperms/ml and co-incubated for 18 hrs. at 38 ºC in a humidified atmosphere of 5 per cent $CO_2$ in air and 95 per cent RH.

### *In vitro* culture of goat embryos (IVC)

After IVF, cumulus cells and excess spermatozoa surrounding the zona pellucida were removed manually by pipetting in TCM 199 supplemented with 10per cent FCS. Embryo culture medium (ECM) consisted of TCM 9 ml, 1 ml FCS, 20µl Gentamicin, 2 µl ITS, 10µl of EGF and 10µl of IGF-I. After 24 hrs, presumptive zygotes were washed in embryo culture medium and transferred to the ECM droplets. Presumptive zygotes were co-cultured with goat oviductal epithelial cells (GOEC) obtained from a metestural stage of oviduct in 100µl IVC droplets of embryo culture medium for 7 – 9 days under a humidified atmosphere of 5 per cent $CO_2$ at 38 ºC. The presumptive zygotes were observed for development at the 2 to 4 cell stage, 8 to 16 cell stage, morula and blastocyst stages.

## Results and Discussion

### Comparison of different culture media on *in vitro* development of goat embryos

The comparison of different culture media on *in vitro* development of goat embryos. The percent of morula was significantly higher in TCM-199+ FBS with GOEC culture medium and without GOEC. Statistical analysis revealed that there was no significant difference ($P$>0.05) between media like TCM-199 with GOEC and without GOEC.

Goat population in India plays a major role in our meat, milk and leather production, there is much less risk in goat farming especially in drought prone areas. Genetic manipulation of goat genome and knowledge about its embryonic development would help our country to retain its supremacy. Goat embryonic stem cell research warrants great interest in various spheres of science. Goat ES cells have their use in animal models and gene knock-out experiments. These cells are closely related to human embryonic stem cells which could be a valid alternative in research. Establishment of gES cell lines would not only greatly improve animal production but also help in understanding developmental biology and disease process in this elite species. (Shiue *et al.,* 2006) [7]. Embryonic stem cells are derived from the inner cell mass of blastocyst. (Lorthongpanich *et al.,* 2011) [4].

### Comparison of different *in vitro* culture media on *in vitro* development of goat embryos

Percentage of morula formed could be directly correlated with quality of the media used. On comparison and correlation it was found that performance of TCM 199+ 10 per cent FBS culture medium and TCM 199 + 10 per cent FBS culture medium with GOE Chad no significant difference, but the per cent of blastocyst formation in TCM – 199 + FBS with GOEC medium was higher than the medium without GOEC. The results are on par with the study reported by Pawar *et al.* (2009) [6] that the enhancement of embryo developmental potential in GOEC co-culture system was attributed to several unknown growth factors secreted by GOEC which promote embryo development. Moreover GOEC exhibited ciliary movement which ensures the continuous rolling movement of the embryos avoiding the attachment to the bottom of the culture dish.

## Conclusion

This study was designed to derive *in vitro* embryo production in different culture media. There was no significant difference in the percentage of morula formation between goat oviductal epithelial co-culture medium and the medium without goat

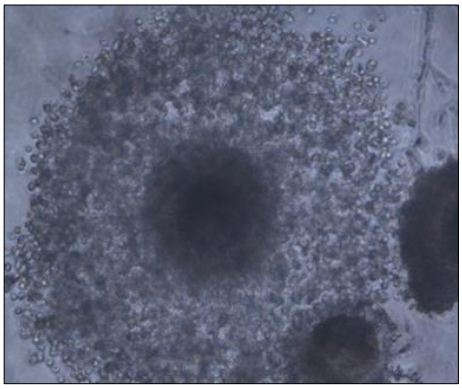
oviductal epithelial culture but the per cent of blastocyst formation in TCM – 199 + FBS with Goat oviductal Epithelial Culture medium was higher than the medium without Goat oviductal Epithelial Culture.

**Table 1:** Comparison of different *in vitro* culture media on *in vitro* development of goat embryos

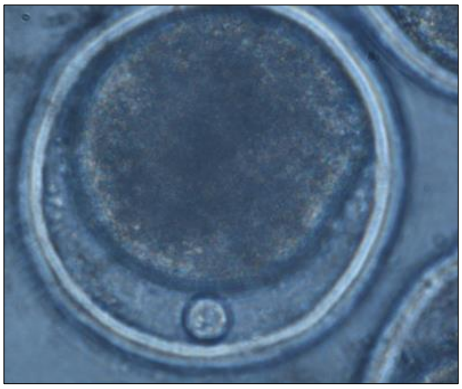
| Media | Presumptive Zygotes | Embryo developmental stage | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2 cell | 4 cell | 8 cell | 16 cell | Morula | Blastocyst |
| TCM – 199 + 10 percent FBS n** = 15 | 253 | 192 (75.89)* | 182 (71.93)* | 138 (54.54)* | 120 (47.43)* | 82 (32.01)* | 4 (1.58)* |
| TCM -199 + 10 percent FBS + Co – Culture with GOEC n**=15 | 264 | 201 (79.44)* | 196 (74.24)* | 184 (69.69)* | 172 (65.15)* | 154 (58.3)* | 17 (6.4)* |
| Total no of ovaries = 206 | 517 | 393 (76.01)* | 378 (73.11)* | 322 (62.28)* | 292 (56.47)* | 236 (45.64)* | 21 (4.06)* |

** (n = number of replicates) * Parentheses indicate percent of embryos cleaved

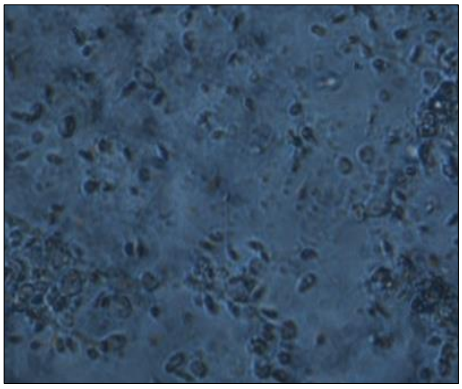
There is no significant difference between different *in vitro* culture media on *in vitro* development of goat embryos ($p$>0.05) – Chi square test



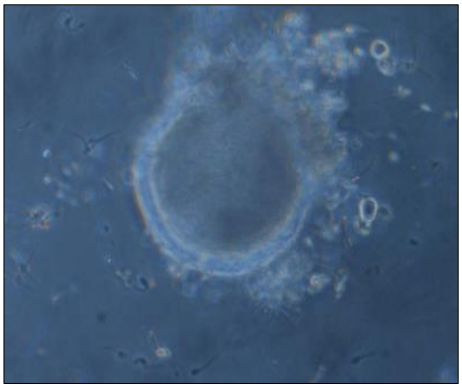
Goat oocyte – Expansion of cumulus (400x )



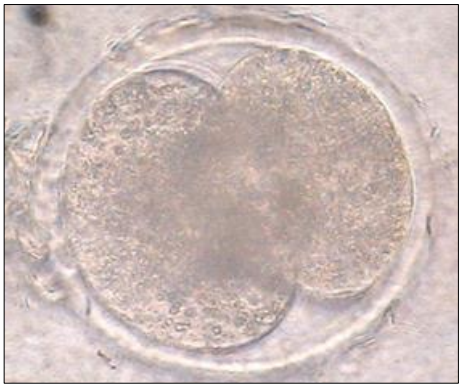
Goat oocyte- Maturation (400x)



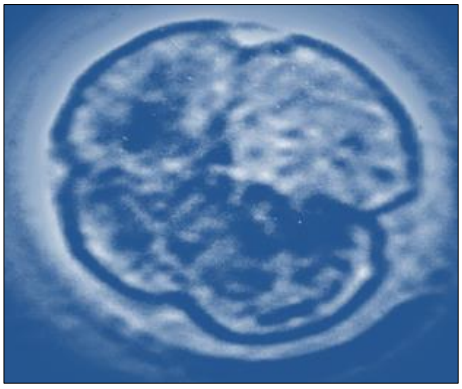
Buck sperm for *in vitro* fertilization (400x)



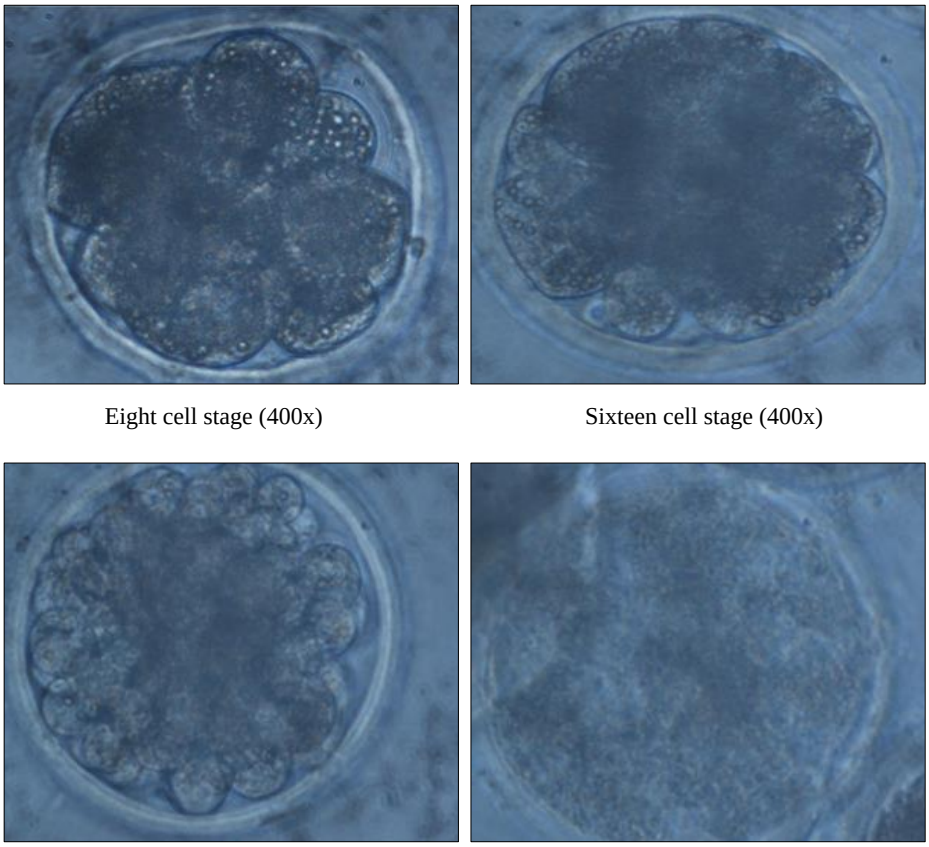
Co-incubation of sperm and oocyte (400x)



Two cell Stage (400x)



Four cell stage (400x)



Eight cell stage (400x) Sixteen cell stage (400x)

Morula stage embryo (400x) Early blastocyst (400x)

*In vitro* development of goat embryos

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