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In Vitro Fertilization Of Goat Oocytes Matured In Two Different Media

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ABSTRACT

A study was undertaken to conduct *in vitro* fertilization in goats under local conditions using either Tissue Culture Medium-199 (TCM-199) or Ham's F-10 Medium. Goat ovaries procured from slaughter house were directed to slicing technique to retrieve the cumulus oocyte complexes, graded and *in vitro* fertilization (IVF) was conducted in both TCM-199 and Ham's F-10 medium. Among total oocytes harvested, A, B, C and D grades contributed 42, 29.27, 18.85 and 9.85 per cent, respectively in which D grade was considered uncultivable and discarded. The study was conducted in 2 groups *viz.*, group I and group II where TCM-199 and Ham's F-10 were used as culture media respectively. The maturation rate was the highest in A grade followed by slight decline in B grade and a significant ($p \leq 0.01$) decline from both A and B, in C grade oocytes in both media. The fertilization rate was assessed by cleavage rate of oocytes in TCM 199 and Ham's F-10. The cleavage rate in group I (27.5 ± 2.77) was significantly ($p \leq 0.01$) higher than group II (19.52 ± 1.39). In this study, TCM-199 was found to be more suitable medium for IVF of goat oocytes than Ham's F-10 medium under local conditions.

KEYWORDS: *In vitro* fertilization, cleavage rate, Oocytes, Goat, Media

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INTRODUCTION

The most critical hurdle faced by the livestock industry is the lowered reproductive efficiency[1]. Ample efforts are rendered to overcome the problem in all the farm animals to augment the production. *In vitro* fertilization (IVF) is an emerging assisted reproductive technique far superior to Artificial Insemination (AI) and Embryo Transfer (ET). The capability to produce bovine embryos from ovarian oocytes by IVF has progressed very rapidly during the past few years[2]. In goats, IVF has resulted in the birth of live offspring[3]. An economical and successful means of *in vitro* embryo production can be expected to play a prominent role in future breeding strategies. Culture media and conditions play a pivotal role for a successful IVF. The most widely used media for culturing the oocytes are Ham's F-10 and Tissue Culture Medium-199 (TCM-199) supplemented with Foetal Calf Serum (FCS) and hormones[4]. As very few studies have been reported for goats when compared to the other two domestic ruminant species[5,6]. The present study was designed using goat as the experimental model with the objective to conduct IVF, assess fertilization by the cleavage rates both in TCM-199 and Ham's F-10 medium and subsequently to compare the efficiency of both the media in IVF of goat oocytes under local conditions.

MATERIALS AND METHODS

Goat ovaries were collected from slaughter house and transported to the laboratory in an isothermic container filled with saline. The recovery and IVF of oocytes collected from the goat ovaries on a single day constituted one trial. After grading the oocytes, each trial was assigned to one of the 2 groups in which TCM-199 and Ham's F-10 were used for culture in group 1 and group 2, respectively. In the laboratory i.e. on day1 the oocytes were collected from the ovary as per the standard slicing technique described by Pawshe[7]. The oocytes were immediately screened and graded as described by Fry[8]. A, B and C grade oocytes were considered to be culturable and used for further processing. A, B and C grade oocytes were subjected to *in vitro* maturation in two trials Viz. group 1 and group 2 in which TCM-199 and Ham's F-10 were used as maturation media, respectively as described by Kothandaraman[9]. On day 2, semen was collected from bucks using artificial vagina and transported to the laboratory at 37°C immediately after collection. After assessing the gross motility and concentration, the motile fraction of sperm was separated by swim up procedure described by O'Doherty[10]. The motile fraction of sperm was washed twice by centrifugation at 200 g for 7 min and then capacitated by incubating with an equal volume of 200 µg heparin/ml before adding equal volume of modified Ca^{2+} free Tyrode's capacitation medium and kept in CO_2 incubator for 15 min.

After 27 h of maturation of oocytes, cumulus cells were removed by vortexing the oocytes for 90 secs. The oocytes were washed 3 times in fertilization medium. The fertilization medium

remained common for both the groups containing NaCl (666 mg/100 ml), $NaH_2PO_4.2H_2O$ (6.2 mg/ 100 ml) sodium lactate (112.1 mg/100ml), $MgCl_2.6H_2O$ (10 mg/100 ml), $CaCl_2.2H_2O$ (29.4 mg/100 ml), HEPES (240 mg/100 ml), phenol red (1 mg/100 ml), sodium pyruvate (5.5 mg/100 ml), BSA (600 mg/100 ml) and glucose (100 mg/100 ml). After washing 10 to 15 oocytes were placed in 100 µl droplet of the fertilization medium that has been covered with warm mineral oil.After capacitation, sperm concentration was assessed in a haemocytometer. Aliquots of sperm suspensions (10 to 20 µl) to give a final concentration of 1×10^6 live sperm/ml were added to the fertilization droplets, having matured oocytes. The oocytes were co-incubated with sperm for 18 to 20 h at 38.5°C under 5 per cent CO_2 and 95 per cent RH.

On day 3, *i.e.* After 18 h of co-incubation of sperm and oocyte, the process of cleavage and expulsion of the second polar body indicated the successful fertilization of oocytes.

RESULTS AND DISCUSSION

The number, cleavage rate of oocytes are given in Table 1. Cleavage rates of the oocytes irrespective of their grade reflect the fertilized oocytes. The mean cleavage rate of oocytes in TCM-199 was more (27.5 ± 2.77) than Ham's F-10 medium (19.52 ± 1.39). The cleavage rate of oocytes irrespective of grade was significantly ($p \geq 0.01$) higher in TCM-199 than in Ham's F-10 medium. Xu[11] compared TCM-199 and Ham's F-10 medium and obtained a cleavage rate of 33.3 ± 4.9 and 19.4 ± 3.7 per cent, respectively which was in close agreement with the present study. The observation of this study was that the TCM-199 could be a better medium than Ham's F-10 for culturing caprine oocytes during IVF concurred with the reports[12,13]. However, the cleavage rates is much lesser than results obtained in cattle *in vitro* technique using epidermal growth factor.[14]

TABLE – 1 Cleavage rates (per cent) of Goat oocytes in TCM-199 and Ham's F-10 medium (Mean ± SE)

Media	Number of trials	Number of oocytes utilized for *in vitro* fertilization	Number of cleaved oocytes	Cleavage rate (%) Mean ± S.E.
TCM-199	8	600	170	27.57^a ± 2.77
Ham's F-10	8	440	97	19.52^b ± 1.39

*Values bearing different superscripts (a,b) differ significantly (P≤0.01)

The concentrations of glucose and hypoxanthine in complex media such as TCM-199 and Ham's F-10 medium were 1000 mg/l, 0.30 mg/l and 1100 mg/l, 4 mg/l, respectively. Both glucose and hypoxanthine have been implicated in 'block' to hamster embryo[15] and mouse embryos[16], respectively. Probably the higher concentrations of hypoxanthine and glucose in Ham's F-10 than TCM-199 might be attributed to the reduced efficiency of Ham's F-10.

CONCLUSION

It was concluded from the present study that TCM-199 was preferable to Ham's F-10 medium for IVF of goat oocytes under local conditions.

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