EFFECT OF DIFFERENT GONADOTROPINON *IN VITRO ON DEVELOPMENT* OF IMMATURE CAPRINE OOCYTES

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ABSTRACT

The objective of the present study is to compare the embryo development from caprine oocytes matured *in vitro* in a medium supplemented with PMSG alone or in combination with hCG. Cumulus oocyte complexes (573) were matured in two treatment groups. Group 1 (n=377) COCs were matured inTCM-199 medium supplemented with 20 IU/ml PMSG. Group 2 (n=196) COCs were matured inTCM-199 medium supplemented with 20 IU/ml PMSG+20 IU/ml hCG. After 27h of maturation, oocytes were fertilized in mTALP and cultured in mCR2aa medium. The maturation rate in groups 1 and 2 were 88.63% and 91.78% respectively. Similarly, cleavage rates, morula and blastocyst production in groups 1 and 2 were 47.13, 20.00, 10.00 and 43.21, 22.85, 7.14% respectively. The results indicated that oocytes matured with 20IU/ml PMSG had a good cytoplasmic maturation that allows normal embryo development up to blastocyst stagewithout addition of hCG. Thus, PMSG at 20IU/ml in maturation medium can be used for *in vitro* embryo production.

Key words: Blastocyst,Cleavage, Goats, hCG, PMSG.

INTRODUCTION

Embryo production either *in vivo* or *in vitro* is a well-established practice to spread or conserve desirable genes of valuable individuals, in large as well as in small ruminants (Cognie *et al.*, 2004). The *in vitro* embryo production technique also has a greater potential of increasing the number of embryos produced for conducting basic research and to study the application of emerging biotechnologies, such as embryo sexing, sperm injection, nuclear transfer and transgenesis (Zhu *et al.*, 2007). This technique has successfully been used in sheep and goats. There is, however, still 60% failure of IVM/IVF oocytes reaching the blastocyst stage following *in vitro* embryo production in goats (Katska-Ksiazkiewicz *et al.*, 2007). Selection of protein supplements and hormones such as follicle stimulating hormone (FSH) and luteinizing hormone (LH) for IVM medium is important even in the subsequent *in vitro* fertilization (IVF) and embryonic development (Wang *et al.*, 1998).

Gonadotropins are commonly included in maturation media to make mature oocytes more effective in sustaining high fertilization and developmental rates. FSH generally upholds the developmental competence of oocytes for *in vitro* maturation. It also maintains follicular growth and is vital for LH receptor appearance during the final stages of follicular development (Sirard *et al.*, 2007).

Commercial PMSG has both FSH and LH like activity and appeared to be cheaper than FSH and LH. However, to our knowledge, the effects of PMSG and PMSG+hCG supplementation on *in vitro* maturation and subsequent blastocyst production of goats oocytes have not been previously investigated.

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The replacement of common hormone (FSH) with PMSG or PMSG +hCG might increase the overall embryo production efficiency and reduce the cost of technology. Thus, the present study aimed (1) To study the effect of PMSG with or without hCG on *in vitro* maturation. (2) To compare the embryonic development from oocytes matured in a medium supplemented with PMSG andPMSG+hCG.

MATERIALS AND METHODS

Goat ovaries were collected during the non breeding season between June to September from the abattoir at Agra, located about 35 km from the Central Institute for Research on Goats, Makhdoom, Farah, Mathura. Ovaries were collected just after the slaughter in 0.9% saline containing 100IU penicillin-G and 100±g streptomycin sulphate per ml and transported at 30 to 35°C within 2-3h of slaughter to avoid any detrimental effect. Upon reaching the laboratory surplus tissue on the ovaries was removed, ovaries were washed several times in sterile normal saline containing antibiotics.

Oocytes were harvested by slicing technique (Yadav *et al.*, 2007) using a surgical blade and in the oocyte collection medium consisting of Dulbecco«s phosphate-buffered saline (D-5773) with 3 mg/ml BSA. Recovered oocytes were graded as excellent (A), good (B), fair (C) and poor (D) quality depending upon the cumulus investment and cytoplasmic distribution under a stereo zoom microscope(Kharche*et al.*, 2008a).Only grades A and B oocytes were selected for maturation in this study. Group 1 (n=377) COCs were cultured with TCM-199 medium containing sodium pyruvate (0.25mM), gentamicin (50±g/ml), L-glutamine (100±g/ml), estradiol 17-□ (1±g/ml), BSA (3mg/ml), supplemented with 10% fetal bovine serum (FBS) and 20IU/ml PMSG.

Group 2 (n=196) COCs were culturd with TCM-199 medium containing sodium pyruvate (0.25mM), gentamicin (50±g/ml), L-glutamine (100±g/ml), estradiol17-□(1±g/ml), BSA (3mg/ml), supplemented with 10% fetal bovine serum (FBS) and 20IU/ml PMSG and 20IU/ml hCG.

Each group was replicated 8 times. PMSG and hCG were used as the gonadotropin source at the level of 20IU/ml (Kouamo and Kharche, 2014). Finally 10-15 COCs were transferred to a 100-±l drop of each maturation media, under mineral oil in a polystyrene culture dish (35mm x 10mm), previously incubated for 2h in a CO_2 incubator. Oocytes were cultured for 27h at 38.5°C in 5% CO_2 incubator in humidified air.

After 27h of culture, matured oocytes with expanded cumulus cells were separated from the cumulus cells by treating the complex with 0.1% hyaluronidase enzyme and passing it repeatedly through a □ne pipette. A sample of 100±l fresh semen was diluted with 5 ml of sperm-TALP medium for the respective treatment groups 1 and 2 and washed by centrifugation at 1200Ûg for 5 min. The supernatant was discarded and the pellet was diluted with 5 ml of medium and kept for capacitation in a CO_2 incubator at 38.5□C for 1hr (Kharche *et al.*, 2008b). After incubation, the sperm suspension was centrifuged and the supernatant again discarded and 100±l of the sperm pellet was diluted in 750±l Fert-TALP medium. Drops containing the oocytes were inseminated with 25¬50±l of the □nal diluted semen so as to obtain a sperm concentration of 1Û10^6 sperm/ml. After *in vitro* insemination, the oocytes and sperm were co-incubated in each group separately for 18h at 38.5□C in 5% CO_2 incubator in humidi□ed air.

After 18-24h co-incubation of oocytes with sperms, presumptive zygotes of both groups were washed 8 to 10 times in culture medium mCR2aa enriched with 10 % FBS to remove sperm cells adhered to zonapellucida and co-cultured at 38.5°C in 5 % CO_2incubator in humidified air for 9 days. Culture medium (25±l) was exchanged with 25±l of fresh medium in every 24h. The development of embryos was evaluated under inverted phase contrast microscope.

Maturation rate and cleavage rate between the different treatment groups were compared using the Chi-square test. The level of signi□cance was recorded at the 5% level of con□dence (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

The overall average oocytes recovered per ovary by slicing technique was 3.85Ø0.28. The morphological maturation rate based on cumulus cell expansion in group 1 and group 2 were 88.63% and 91.78%, respectively.Similarly, cleavage rates, morula and blastocyst production in group 1 and group 2 were 47.13, 20.00, 10.00 and 43.21, 22.85, 7.14%, respectively . No statistical difference regarding embryonic developmental stages from 2 cell to blastocyst stages between group 1 and 2 was recorded (P>0.05).

The efficiency of *in vitro* embryo production is profoundly influenced by the number and quality of oocytes which successfully complete maturation (Kharche *et al.*, 2011). Wang *et al.*(2007) who harvested oocytes from ovary of Boer goat reported 6.3 oocytes per ovary by slicing technique. Furthermore, Wani *et al.* (2000) reported that slicing yielded (9.5 Ø 0.4) significantly ($p < 0.05$) more COCs per ovary than aspiration (6.8 Ø 0.3) in sheep. Slicing technique consisted of incisions along the whole ovarian surface using a scalpel blade. The lower number of COCs recovered by the slicing method in this experiment may be attributed to follicles being embedded deeply within the cortex, the breeding season, the stage of estrus cycle, the presence of large corpus luteum and debris of ovarian tissue (HoqueMasudul *et al.*, 2011).

Based on expansion of cumulus cells, the maturation rates obtained for the two treatments were similar to those reported by Leoni *et al.* (2009). Moreover, Gupta *et al.* (2001) found that the addition of PMSG to the maturation media enhanced cumulus expansion of buffalo oocytes compared to those cultured in the media without PMSG. The presence of gonadotropins in the maturation media increases the level of intracellular cAMP, the activity of the hyaluronic acid synthesis enzyme system and induced cumulus expansion complexes (Buccione *et al.*, 1990).

The cleavage rate obtained might indicate that oocyte matured in both media had a good cytoplasmic maturation that allows normal embryo development after fertilization (Eppig, 1996). Overall cleavage rate in the present study was lower when compared to study of Natarajan *et al.*(2010), which may be due to different treatment protocols. For *in vitro* fertilization it is essential that sperm and oocyte be kept in a media that support viability of gametes, motility and assist in penetration of oocytes. Tyrode modified medium (mTALP) have been used successfully as basic media for IVF of goat oocytes (Kharche *et al.*, 2008a,b). Our result showed lower percentage of blastocyst as compared to that reported in adult goats (Wang *et al.*, 2007). Younis *et al.* (1991) using mTALP as a fertilization medium found a fertilization rate of 26.7% and cleavage rate of 10% of *in vitro* matured goat oocytes. Our data demonstrated higher cleavage rates in both groups than reported by above authors. In accordance with the present results of oocytes reaching to blastocysts stage, Mogas *et al.* (1997a,b) obtained 8.3% of blastocysts by co-culturing embryos with granulosa cells. Similarly 8% blastocysts were obtained by Koeman *et al.* (2003) with oocytes collected by LOPU from 2- to 5-month-old hormonally stimulated goats.

The results indicated that oocytes matured with 20IU/ml PMSG had a good cytoplasmic maturation that allows normal embryo development up to blastocyst stage without addition of hCG. Thus, PMSG at 20 IU/ml in maturation mediumcan be used to reduce the cost of *in vitro* embryo production.

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