EFFECT OF POLYVINYL PYRROLIDONE (PVP) ON *IN VITRO* MATURATION AND CLEAVAGE RATE OF CAPRINE OOCYTES

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ABSTRACT

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The exact role of action of serum and BSA in culture medium for oocyte maturation is still unknown. The replacement of these proteins with synthetic macromolecules such as polyvinyl pyrrolidone (PVP) need to be evaluated and compared with protein supplement in maturation medium. Therefore the present study was undertaken with two objectives vizTo study the effect of PVP on *in vitro* maturation of caprine oocytes and to evaluate the cleavage rate of oocytes matured in a medium supplemented with PVP. A total of 612 healthy cumulus oocyte complexes (COCs) were collected from 390 goat ovaries through puncture technique and were subjected to the following maturation treatments. Group-1: Maturation medium TCM-199 supplemented with 10% FBS, 3mg/ml BSA, LH (10 ±g/ml), FSH (5 ±g/ml), L-glutamine (100 ±g/ml), sodium pyruvate (0.25 mM) and gentamycin (50±g/ml) and Group-2: Maturation medium TCM-199 supplemented with 3mg/ml PVP, LH (10 ±g/ml), FSH (5 ±g/ml), L-glutamine (100 ±g/ml), sodium pyruvate (0.25 mM) and gentamycin (50±g/ml)for 27 hr in humidified atmosphere of 5% CO_2 at 38.5Ø1°C in CO_2 incubator.*In vitro* maturation rate was assessed on the basis of cumulus expansion. Expanded oocytes were denuded by treating with 0.1% hyaluronidase and used for further *in vitro* fertilization. Matured oocytes and 2-5 millions sperm/ml were co-incubated in 50 ±l drop of Fertilization medium (TALP medium+ 8 mg/ml) fatty acid free BSA and 100 ±g/ml heparin) for 18 hr. Fertilized oocytes of both groups were cultured in embryo development medium comprising of potassium simplex optimized Medium + 10 % FBS+ 3 mg/ml BSA) for cleavage and further embryo development.The Maturation and cleavage rate in group 1 and group 2 were 89.6% and 60.2% and 35.67% and 14.34%, respectively. Statistical analysis revealed that maturation rate and cleavage rate in maturation medium supplemented with serum is significantly higher as compared to PVP supplemented medium.

Key words: PVP, BSA, Maturation, *Caprine* oocytes

INTRODUCTION

Various attempts have been made to improve genetic potential of goats through artificial insemination or through embryo transfer technique. Modern husbandry has two reproductive goals: improving the efficiency of extensive production with favorable economic considerations and controlling the reproductive process for more intensive production of milk and meat (Lindsay, 1991). Zygote production via IVM and IVF of goat oocytes should enhance the efforts to make transgenic animals via microinjection of appropriate DNA into male pronuclei.

Future expansion of animal breeding programmes depends largely on the availability of large number of embryos produced economically. Biotechnological tools such as embryo splitting, sexing, cloning, and gene injection and also require a large number of pre-implantation embryos. *Caprine in vitro* embryo production can be expected to play

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prominently in future goat breeding strategies. Successful *in vitro* embryo production and transfer to recipients can decrease the generation interval; increase the number of offspring for selected does and thus optimizes exploitation of the female genetic material for propagation of valuable goats (Mogas*et al.*, 1997).

Physiology and biochemistry of zygote from the initial stage of preimplantation period to final stage of blastocyst is different along with the morphological differences. Although the various researchers has developed various culture systems but still the improvement of *Caprine* culture systems is highly desirable in terms of the production of pre-implantation stage embryos, both for biotechnological studies and for embryo transfer industry(Gordon, 1991). The predominant approach for producing the IVM/IVF embryos involves co-culture with somatic cells, using a complex culture medium containing culture blood serum. Elucidation of these factors is essential for the formulation of optimal culture media.

During IVM, oocytes undergo a series of cytoplasmic changes before the resumption of nuclear maturation, leading to variable competence of the resulting embryo (Moor *et al.,* 1990). In addition, the synthesis and storage of certain forms of mRNA and protein during IVM and early embryonic development are thought to be necessary for further development (Motlik*et al.,* 1986, and Thibault*et al.,* 1987). The limited developmental competence of *Caprine* oocytes after IVM can be used to understand the factors involved in the acquisition of such ability. For this reason and to understand the requirements for development of immature oocytes through IVM, all products with undefined components should be eliminated from culture conditions.

Although serum or BSA are typically added to the medium as a protein supplement to improve culture efficiency (Leibfried-Rutledge *et al.,* 1986), different lots of this protein can produce highly variable effects during the period of culture in hamsters, ranging from highly stimulatory to highly inhibitory (McKiernan *et al.,* 1992). To elucidate the roles of protein supplements on oocyte maturation, various proteins including embryo tested BSA and fetal bovine serum (FBS) were tested for their effects on oocyte maturation and subsequent embryonic development after IVF. The replacement of these proteins with synthetic macromolecules such as polyvinyl pyrrolidone (PVP) need to be evaluated and compared with protein supplement in maturation medium i.e. More emphasis needs to be placed in the development of defined culture media for supporting oocytes and embryos *in vitro*, so that consistent results can be obtained and data from different laboratories can be compared.However, the exact role of polyvinylpyrrolidone (PVP) in culture medium for oocyte maturation is still largely unknown.

MATERIALS AND METHODS

The study was conducted at female reproduction laboratories of Physiology, Reproduction and Shelter Management (PRSM) Division, CIRG, Makhdoom.

Goat ovaries (n = 390) were collected from the local abattoir and transported within 4 hr to the laboratory in warm saline (37°C), containing100 IU penicillin-G and 100 ±g streptomycin sulfate per ml. Oocytes were retrieved by follicular puncture from the goat ovaries. Recovered oocytes were graded as excellent (A), good (B), fair (C) and poor (D) quality,depending on their cumulus investment and cytoplasmic distribution for *in vitro* maturation (Kharche*et al.,* 2008b).

Selected oocytes(n= 612) were washed 10 to 14 times in 50-100±l drops of TCM-199 medium, supplemented with L-glutamine (100 ±g/ml), sodium pyruvate (0.25 mM) and gentamycin (50 ±g/ml). The COCs were randomly divided and subjected the following two maturation treatment.

Group-1 (Control) Approximately 368 selected COCs were washed 5-6 times in TCM-199 (Sigma) containing L-glutamine (100 ±g/ml), sodium pyruvate (0.25 mM), gentamycin (50±g/ml), LH 10 ±g/ml, FSH 5 ±g/ml supplemented with 10% FBS, 3mg/ml BSA.

Group-2 (PVP) Approximately 244 selected COCs were washed 5-6 times in TCM-199 (Sigma) containing L-glutamine (100 ±g/ml), sodium pyruvate (0.25 mM), gentamycin (50±g/ml), LH 10 ±g/ml, FSH 5 ±g/ml supplemented with 3mg/ml PVP.

Both groups were matured in 50 ±l droplets of maturation medium covered with sterile mineral oil for time period of 27 hr in humidified atmosphere of 5% CO_2 at 38.5Ø1°C in CO_2 incubator.

After 27 hr of culture, oocytes were separated from the cumulus cellsby treating the complex with 0.1% hyaluronidase enzyme and passing it repeatedly through a fine pipette. Denuded oocytes were washed 10-20 times with Fert-TALP medium containing 10% FBS, 8 mg/ml fatty acid free BSA and 100 ±g/ml heparin. Approximately 15-20 oocytes were transferred in each 50 ±l drop of Fert-TALP medium covered with sterile mineral oil in a culture Petri dish in humidified atmosphere of 5% CO_2 at 38.5Ø1≥ C for 1 hr in CO_2 incubator.

Fresh semen was collected using the artificial vagina from a fertile purebreed adult Sirohi buck. The first and second seminal ejaculates were examined for volume, color, consistency and gross sperm and progressive motility. A sample of 100±l fresh semen was diluted with 5ml ofsperm TALP medium containing 4mg/ml fatty acid free BSA and washed by centrifugation at 1200rpm for 5 min. The supernatant was discarded and semen pellet was again dissolved in 5ml sperm TALP and again centrifuged at 1200rpm for 5 min. Final washing was done with 2ml fert TALP medium and100 ±l of pellet was added in 900 ±l of fert TALP medium.Sperms were then kept for incubation in a CO_2 incubator in humidified atmosphere of 5% CO_2at 38.5Ø 1≥ C for 45 mins.

Fert TALP medium drops containing the matured oocytes were inseminated with 25¬50±l of the final 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 for 18 hr at 38.5°C with 5% CO_2 in humidified atmosphere.

After 18 hr of co-incubation, oocytes were washed 8-10 times in embryo development medium (potassium simplex optimized holding medium) in order to separate adhering sperm cells. Oocytes were finally transferred into 50±l drop of embryo development medium for 48 hr in humidified atmosphere of 5% CO_2 at 38.5Ø1≥C in CO_2 incubator.

After 48 hr of post insemination, fertilized oocytes were evaluated under phase contrast microscope for cleavage rate. Cleaved oocytes were cultured in embryo development medium (KSOM) further for 8-10 days. Development stage of cleaved oocytes was observed and 50% media was replaced every 4thday.

The maturation rate of oocytes was calculated in percentages. Cleavage rates among different treatment groups were compared using Chi-square test. The level of significance was observed at 5% level (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

A total of 612 healthy oocytes from 390 ovaries were recovered by puncture technique. The recovery rate of oocytes per ovary was recorded 1.56. In group-1, a total of 368 oocytes were selected for maturation while in group-2 a total of 244 oocytes were selected. After 27 hr of maturation, the evaluation of *in vitro* matured oocytes was made on the basis of cumulus expansion. Maturation rate in Group-1 (Control) 89.6 % was significantly higher than Group-2 (PVP) 60.2% . It clearly indicates that BSA provides favorable environment for *in vitro* maturation of oocytes as comparison to PVP. Thus maturation media containing BSA enhanced and improved maturation rate better than PVP.

Our results were comparable with Le Gal *et al.* (1992), who found that the establishment of metaphase-II (evidence by extrusion of the first polar body) was almost completed (>85%) at 27hr after the initiation of the maturation process. But Agrawal (1992) reported that 37.50% of caprine oocytes matured to metaphase II within 24 hr of culture.

The oocytes matured in group1 (n=330) and group2 (n=147) were used for *in vitro* fertilization. After 48 hr of post insemination, fertilized oocytes were evaluated under phase contrast microscope for cleavage rate. Cleaved oocytes were cultured in embryo development medium further for 8-10 days. Statistical analysis revealed that cleavage rate in group 1(control) 35.67% was found significantly higher as compare to group 2 (14.34%).Our results are favorable with the result of Kim *et al.* (1996). They determined the effects of follicular fluid and PVP in the maturation medium on bovine oocyte maturation, fertilization and subsequent development, as well as on the number of cells in blastocysts following culture.

Ali *et al.* (2002) evaluated different sources of albumin as a protein supplement and a synthetic macromolecule PVP during *in vitro* maturation (IVM) of bovine oocytes in synthetic oviduct fluid medium (SOF). They obtained a high proportion of morula and blastocysts from IVM oocytes cultured in medium containing PVP-40. Our results are contradictory with Ali *et al.* (2002). We obtained significantly lower maturation and cleavage rate in PVP supplemented group than BSA supplemented group. This may be due to PVP 0930 used in our study while Ali *et al.* (2002) cultured immature oocytes in medium containing PVP-40.

It can be concluded that the maturation rate in group 1 (control, 89.6%) was significantly higher ($P<0.05$) than that of group 2 (PVP, 60.2%). Cleavage rate observed in group 1 (35.67%) was significantly higher ($P<0.05$) than that of group 2 (14.34%). Embryo development up to morula stage did not differed significantly in both groups.

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