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Influence of different Culture Media on embryo development following *in vitro* fertilization of caprine oocytes

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ABSTRACT

The present study was designed to compare the developmental competence of in vitro fertilized caprine embryos at different cell stages in three different culture medium(TCM-199, mSOF, KSOM). Oocytes were collected from ovaries and matured in TCM-199 medium containing FSH (5 µg/ml), LH (5 µg/ml) and estradiol-17β (1 µg/ml), supplemented with 20% fetal bovine serum at 38.5 °C and 5% CO_2 in an incubator under humidified air for 27 h. After 27 h of in vitro maturation (IVM), oocytes were denuded, washed and co-incubated with 50 µl neat semen diluted in 5 ml of sperm TALP containing 20% EGS and 10µg/ml heparin. Oocytes-sperm after 18h of co-incubation were randomly divided into three groups: Group1(TCM-199 containing 10% NCS and 4mg/ml bovine serum albumin), Group 2(Synthetic oviductal fluid containing 10% NCS and 4mg/ml BSA) and Group 3(Potassium simplex optimization medium (KSOM) containing 10% NCS and 4mg/ml BSA) and found the cleavage rate, morulae and blastocyst percentage for groups 1, 2, and 3 were 13.6, 10.14 and 2.41%, 11.7, 7.6 and 3.84% and 31.4, 12.73 and 6.05%, respectively. Statistical analysis revealed that the cleavage rate and embryo development up to morula and blastocyst stage in group 3(KSOM) was significantly higher (P<0.01%) as compare to group1 and group2.

Key words: Caprine oocytes, Cleavage, *In vitro maturation, In vitro* fertilization, Morula

Major developmentally important events take place during development of embryo from post fertilization to the blastocyst stage. Embryo yield and survival usually differ between the different culture systems and culture media (Camargo *et al*. 2006). In vitro, the pre-implantation development of the embryo occurs in close relation with the culture medium. This demands specific balanced conditions in the medium to allow embryonic metabolism(Garcia *et al*. 2016). A number of different defined culture media have been extensively evaluated in an effort to improve the quality of embryos produced in vitro and to mimic the physiological conditions that embryos would have in vivo. The aim of preparing defined media is to minimize the variation in unknown components that occurs naturally in serum. This has the advantage of providing a simplified technique that enhances cryoresistance of embryos. This is due to avoiding the supplementation of biological components such as FCS and bovine serum albumin (BSA) that may increase the potential risks of contamination (Neira *et al*. 2010). The most common defined media used in vitro culture systems are SOF (synthetic oviductal fluid), KSOM, and tissue culture medium (TCM199)(Licola *et al.* 2015).

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Keeping this in mind, the objectives of the present study is to compare three different defined culture medium to evaluate the developmental competence of embryos. Ovaries were collected from the local abattoir and transported within 4 h to the laboratory in warm saline (37 °C) containing 100 IU penicillin-G and 100 µg streptomycin sulfate per ml. The oocytes were collected from each goat ovary and graded as per the method of (Kharche *et al*. 2008). Selected oocytes were then washed three times with Oocyte Maturation Medium (OMM) containing (TCM-199 supplemented with 10% FBS, hormones (5µgmL"1 FSH, 5µgmL"1 LH and 1µgmL"1 estradiol-17β), 10 ngmL"1 EGF, Sodium Pyruvate 0.25 mM, Glutamine 100 µg/ml, LH 10 µg/ml, FSH 5µg/ml, BSA 3mg/ml & Gentamycin 50µg/ml). Washed Cumulus Oocyte Complexes (COC's) were then allowed for *in vitro* maturation at 38.5æ%C in 5% CO_2 in air for 27 h.

After the maturation, oocytes were separated from cumulus and corona cells by treatment with 0.1% hyaluronidase. The Oocytes were washed 8–10 times in Fertilization medium (TALP solution containing 20% oestrous goat serum and 10 µg/ml heparin) and kept in 50 µl drop in culture petriplate.

Fresh semen was collected by artificial vagina (AV), from a fertile purebred Sirohi buck. Semen was ejaculates for volume, colour, and consistency, gross and progressive motility. An amount of 50 µl neat semen was diluted with 5 ml of sperm TALP containing 20% EGS and 10 µg/ml

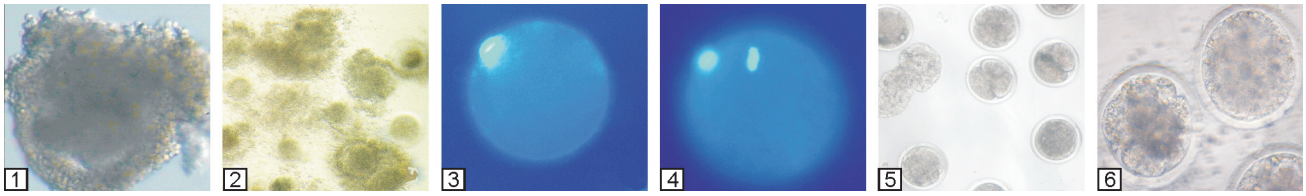

Fig. 1. 1. Immature oocyte, 2. Mature oocytes, 3, MII Oocyte with polar body 4. MII Oocyte with two shiny chromatin spots, 5. 2–8 Cell stage embryos, 6. orulae.

heparin and washed 2 - 3 times by centrifugation at 1200 rpm for 5 min. The supernatant was discarded in each washing. Finally 50 µl pellet of sperm was diluted with 1 ml of fertilization media. Fertilization drop containing Oocytes were inseminated with 25 to 50 µl of finally diluted semen to obtain a sperm concentration of 1–2×10^6 sperm/ml. Gametes were co incubated for 18h in a CO_2 incubator. Oocytes-sperm after 18h of co-incubation were washed in embryo development medium for 15–20 times and randomly divided in to three groups. Group 1 (n=1511) fertilized oocytes were culture in TCM-199 containing 10% NCS and 4mg/ml bovine serum albumin (BSA) for 10 days, Group 2 (n=1333) fertilized oocytes were culture in synthetic oviductal fluid (SOF) containing 10% NCS and 4mg/ml BSA for 10 days and Group 3 (n=1000) fertilized oocytes were culture in potassium simplex optimization medium (KSOM) containing 10% NCS and 4mg/ml BSA for 10 days. Presumptive zygotes were cultured under mineral oil in 5% CO_2 in air with high humidity at 38.5ºC. Presumptive zygotes were observed for cleavage and further *in vitro* embryo development. Developing embryos were evaluated under inverted phase contrast microscope.

All data were analysed by one-way ANOVA, developmental data were arc sin transformed. The level of significance for cleavage and embryo development was recorded at 5% level of confidence (Snedecor and Cochran 1989).

The cleavage rate, morulae and blastocyst percentage for groups 1, 2, and 3 were 13.6, 10.14 and 2.41%, 11.7, 7.6 and 3.84% and 31.4, 12.73 and 6.05%, respectively. The cleavage rate was found significantly higher (P <0.01) for group 3 than for groups 1 and 2. Similarly, embryo development up to morula stage was higher (P <0.05) in KSOM compared with TCM-199 and SOF medium. In our previous studies, we obtained Cleavage rate, 2 cell, 4 cell, 8–16 cell, morulae and blastocysts were 44%, 17%, 25%, 33.11%, 16% and 2%, respectively in defined KSOM medium as compared to complex embryo development media TCM 199(Kharche *et al.* 2011). The effect of three different media RVCL, mSOF, G1.G2 for culture of intraspecies and interspecies cloned goat embryos were found no significant difference between them (Khan *et al.* 2013).

The effects of supplementation of synthetic oviduct fluid (SOF) with glucose and Minimal Essential Medium (MEM) vitamins is assessed during the first step of the in vitro culture (SOFC1) of ovine zygotes has been experimented and found vitamins must be present for optimal numbers of embryos to reach the blastocyst stage(Karami *et al.* 2012). There are some research teams using synthetic oviductal fluid (SOF) medium instead of TCM199 for sheep (Shabankareh and Akhondi 2012); (Manacha *et al.* 2016) and for goat embryos(Herrick *et al.* 2004).

The following study showed that the use of KSOM medium supplemented with 5% FBS after 3 days of culture can improve the blastocyst formation rate of SCNT(Somatic cell nuclear transfer) embryos and reduce the incidence of apoptosis compared to other two-step culture media supplemented with Bovine Serum Albumin(Aries *et al.* 2013). In another study, KSOM medium was found more appropriate than TCM 199 medium to culture the SCNT(Somatic cell nuclear transfer) embryos from the pronuclear to the cleavage stage(Wan *et al*. 2013). The most common media used in bovine in vitro culture systems are SOF (synthetic oviduct fluid), KSOM, and CR1aa(Kocyigit *et al.* 2016).

So the results indicated that the KSOM medium is batter culture medium as compared with TCM- 199 and SOF medium as far as cleavage rate and embryo development is concerned.

SUMMARY

The objectives of the present study were to compare defined and complex culture medium for their ability to support normal caprine embryonic development. Three thousand eight hundred forty four selected cumulus oocyte complexes were used for maturation in TCM-199 containing 10% Newborn calf serum (NCS), 3mg/ml BSA, 5 µg/ml FSH, 5 µg/ml LH and 1µg/ml estradiol-17β and 10 ng/ml epidermal growth factor. They were then washed in sperm TALP and then fertilized in a drop of fertilization TALP containing 1–2x10^6 spermatozoa / ml. The presumptive zygotes were randomly divided in to three groups. Group 1 (n=1511) in TCM-199, Group 2 (n=1333) fertilized oocytes in synthetic oviductal fluid (SOF) and Group 3 (n=1000) fertilized oocytes in potassium simplex optimization medium (KSOM). Statistical analysis revealed that the cleavage rate in group3 was significantly higher (P<0.01%) as compare to group1 and group2. Similarly, embryo development up to morula and blastocyst stage was also statistically significant (P<0.05) in KSOM as compare to TCM-199 and SOF. The results indicated that the KSOM medium was better among all three medium for embryo development.

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