Short Paper

Study of TALP and TRIS citrate medium on caprine sperm capacitation and subsequent in vitro embryo production

Agarwal, S.¹; Kharche, S. D.² and Bhatiya, A. K.³

¹Ph.D. Scholar in Biotechnology, Department of Biotechnology, Institute of Applied Science and Humanities, GLA University, Mathura, Uttar Pradesh, India; ²Animal Physiology and Reproduction Division, ICAR-Central Institute for Research on Goats (CIRG), Makhdooom, Farah-281122, Mathura, Uttar Pradesh, India; ³Department of Biotechnology, Institute of Applied Science and Humanities, GLA University, Mathura, Uttar Pradesh, India

Correspondence: S. D. Kharche, Animal Physiology and Reproduction Division, ICAR-Central Institute for Research on Goats (CIRG), Makhdooom, Farah-281122, Mathura, Uttar Pradesh, India. E-mail: kharche1@rediffmail.com

(Received 4 Feb 2017; revised version 27 Apr 2017; accepted 17 May 2017)

Summary

The aim of the present investigation was to compare Tyrode’s albumin lactate pyruvate (TALP) medium and TRIS citrate medium for capacitation of caprine sperm. In experiment 1 capacitation was assessed by chlortetracycline assay and in experiment 2 with in vitro fertilization and embryo development. In experiment 2, cumulus oocyte complexes (COCs) recovered by slicing the caprine ovaries were matured in maturation medium for 27 h in humidified atmosphere at 38.5°C with 5% CO₂. After 27 h of culture a total of 2480 in vitro matured oocytes were selected and randomly divided into two groups. Group 1 (n=1124) matured oocytes were fertilized by the spermatozoa capacitated in TALP medium and in group 2 (n=1356) matured oocytes were fertilized by the spermatozoa capacitated in TRIS citrate medium. The results of experiment 1 indicated a comparatively more number of sperms with capacitation and fertilization is a well-established technology with a variety of applications in basic and applied sciences (Bavister, 2002). Experimentation in in vitro fertilization (IVF) and in vitro culture (IVC) of caprine oocytes has led to marked improvements in the production of embryos in vitro and the birth of kids (Kharche et al., 2011). These advances have allowed for progress in the study of early fertilization events so that the efficiency of embryo production in vitro can be improved. One such process is sperm capacitation (Bedford, 1983), which involves modifications in membrane composition and fluidity, increases in intracellular cAMP, induction of tyrosine phosphorylation events, and the expression of hyperactivated motility (Aitken and Nixon, 2013). Sperm undergo these changes within the female reproductive tract or, in vitro, when incubated in a medium that supports capacitation.

To date, several media additives, of both synthetic and animal origin, have been successfully used to support in vitro capacitation of small ruminants spermatozoa. In caprine, the media employed for sperm capacitation and fertilization are modified Tyrode’s albumin lactate pyruvate medium (TALP, Kharche et al., 2011), modified Defined Medium (mDM, Crozet et al., 1995), TCM-199 (Slavik and Fulka, 1992), Brackett and Oliphant (BO) and modified Synthetic Oviductal Fluid (mSOF) (Kharche et al., 2011).

To the best of our knowledge, despite advances in researches regarding additives that may support mammalian sperm capacitation in vitro, the capacitation of sperm in TRIS citrate has not been reported in any species. TRIS citrate diluent at pH = 6.8 is commonly used as diluting medium for semen cryopreservation and artificial insemination (Kharche et al., 2013; Gangwar et al., 2015). Therefore the present investigation was undertaken with the objectives being to determine the optimal medium between TALP (pH = 7.2-7.4) and TRIS citrate (pH = 6.8) for sperm capacitation and subsequent embryo development of in vitro fertilized caprine oocytes.

Materials and Methods

Experiment 1. Assessment of in vitro capacitation

Fresh semen was collected using the artificial vagina from six adult Sirohi bucks. The first and second seminal ejaculates were examined for volume, color, consistency
and gross sperm and progressive motility. Each collected semen sample was divided into two groups for capacitation of spermatozoa. Washing of semen samples was done as per Kharche et al. (2016). In group 1, washing was done with sperm TALP medium while for group 2 semen washing TRIS citrate medium was used. Sperms were then kept for capacitation in a CO$_2$ incubator in humidified atmosphere of 5% CO$_2$ at 38.5°C for 1 h. The capacitation status of fresh buck semen was assessed by chlortetracycline staining as per the method of Elkhawagah et al. (2013). Semen sample from each buck was assessed and at least 180-200 spermatozoa per slide were evaluated and classified into one of these three Chlortetracycline (CTC) staining patterns described by Fraser et al. (1995).

**Experiment 2. In vitro embryo production**

Collection of ovaries, recovery of oocytes and *in vitro* maturation (IVM) was done as per Kharche et al. (2016). Briefly, a total of 2666 cumulus oocyte complexes (COCs), recovered by slicing the 721 caprine ovaries, were matured in TCM199 supplemented with 10% fetal bovine serum (FBS) + 10% follicular fluid + EGF (10 ng/ml) + BSA (3 mg/ml) + FSH (5 µg/ml) + LH (10 µg/ml) + estradiol (1 µg/ml) + 10% follicular fluid + 10% fetal bovine serum (FBS) + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% follicular fluid + 10% folli

**Results**

Because of the simplicity and reliability of CTC method, this was used to differentiate between capacitated and non-capacitated sperms. However, in group 2 a comparatively more number of sperms showed CTC pattern B than group 1 (55.32 ± 0.91% vs 47.96 ± 0.20%). Furthermore, CTC pattern F and CTC pattern acrosome reacted (AR) in group 1 (13.78 ± 0.36 and 37.24 ± 0.26, respectively) was higher than that of group 2 (10.15 ± 0.44 and 32.99 ± 0.19, respectively).

The cleavage rate of *in-vitro* matured caprine oocytes in group 1 and group 2 was 35.93 ± 3.17% and 39.96 ± 3.04%, respectively. The blastocyst production was comparatively higher in group 2 (9.81 ± 1.99%) than that of group 1 (5.82 ± 1.68%), respectively (Figs. 1A-D). The cleavage rate and blastocyst production were comparatively higher following capacitation of spermatozoa in TRIS citrate as compared to TALP medium.

![Fig. 1: Sperm, oocytes and embryos used in the experiments. A: Goat spermatozoa, B: Immature oocytes, C: Cleaved oocytes, and D: Blastocyst](image)

**Discussion**

The improvement of caprine IVF technology is highly desirable in terms of the production of preimplantation stage embryos, both for biotechnological studies and for the embryo transfer industry (Wu et al., 2015). One contributing factor to this poor understanding is that many culture systems employ the same medium for capacitation as well as for fertilization (Kharche et al., 2011; Kharche et al., 2016).

To the best of our knowledge, despite advances in knowledge regarding additives that may support mammalian sperm capacitation *in vitro*, capacitation of sperm in TRIS citrate has not been reported in any development between the different treatment groups were compared using the chi square test. The level of significance was recorded at the 5% level of confidence (Snedecor and Cochran, 1989).
species. The main purpose of the diluent is to extend fresh semen to increase total volume of the semen ejaculated from a particular buck at a specific time and its extended use over a large number of does to get more kids (Kharche et al., 2007; Kharche et al., 2008a, b; Kharche et al., 2013). However, diluents used for buck semen should have similar properties to that of fresh semen in terms of hydrogen ion concentration (pH), toxicity and energy source. TRIS citrate diluent at pH 6.8 is commonly used as diluting medium for semen cryopreservation and artificial insemination (AI) (Gangwar et al., 2015). Since, the needs and the metabolic activity of the male and female gametes are not the same (First and Parrish, 1987) thus it is necessary to use one medium for sperm capacitation and another for oocyte insemination. Because of the simplicity and reliability of CTC method, this was used to differentiate between capacitated and non-capacitated sperms in TALP and TRIS citrate medium. Our results demonstrated higher caprine capacitated spermatozoa (pattern B) in both TALP (47.96 ± 0.20%) and TRIS citrate medium (55.32 ± 0.91%) as compared to that of ram spermatozoa (13.0 ± 1.0%) in SOF medium (Perez et al., 1996). Similarly, the result of incapacitated caprine spermatozoa (pattern F) was lower in both TALP and TRIS medium than that of ram spermatozoa (78.0 ± 2.0%) whereas AR caprine spermatozoa were higher in both TALP and TRIS citrate than that of ram spermatozoa in SOF medium (Perez et al., 1996). After CTC staining, three types of fluorescent patterns were visible under the microscope and were similar to those reported for bovine (Elkhawagah et al., 2013). A high percentage of spermatozoa exhibited CTC pattern B (capacitated spermatozoa) in both groups (TALP and TRIS citrate).

Our result of cleavage rate following spermatozoa capacitated in TALP is in agreement with that reported by Younis et al. (1991) in TALP (26.7%), mDM (33.3%), and mH-M199 (35.7%) and Kharche et al. (2011) in BO medium (32.84%) and mSOF medium (36.21%) in caprine oocytes, whereas it was comparatively higher following spermatozoa capacitated in TRIS citrate medium.

Capacitation has been shown to be correlated with changes in sperm intracellular ion concentrations, plasma membrane fluidity, metabolism, and motility (Yanagimachi, 1994). Although these changes have been known for many years to accompany the process of capacitation, the reason for embryo development underlying these events is poorly understood. Since in our study, the cleavage rate and blastocyst production of in-vitro matured caprine oocytes in group 2 was comparatively higher than group 1 (39.96 ± 3.04% vs 35.93 ± 3.17%, respectively) and (9.81 ± 1.99% and 5.82 ± 1.68%, respectively). It is well documented that the first two embryonic cell divisions are primarily controlled by the maternal genes (Braude et al., 1988) and paternal effects commence at the 4-cell stage. Further, the detrimental effects of sperm DNA damage are more prominent during the later stages of embryo development (Dar et al., 2013).

In conclusion, TRIS citrate can be used as an alternative and effective media for sperm capacitation to get higher cleavage rate and blastocyst production.

Acknowledgements

The authors wish to thank the ADG, NFBSFARA, New Delhi for providing the funding and the Head, Animal Physiology and Reproduction Division and Director, CIRG, Makhdoom, Farah, Mathura, UP, India for providing the facilities needed.

Conflict of interest

On behalf of all co-authors, the corresponding author indicates that there is no conflict of interest involved in publishing this research paper.

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