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IMPROVEMENT OF THE MEDIUM AND PROCESSING PROTOCOL FOR CRYOPRESERVATION OF BOER GOAT SPERMATOZOA

By
Akeel Ahmed Memon
aeelahmedmemon@yahoo.com

Home Institute: Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam, Pakistan

Host Institute: Faculty of Veterinary Medicine, University Putra Malaysia 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

Fertility rate is lower after AI in goats with cryopreserved semen. Low fertility with frozen semen is due to the procedures related with cryopreservation that leads to sperm damage, impairs its normal function and fertilizing potential. Therefore, improvements in the freezing media and processing protocols applied to the goat semen are needed to improve the quality of goat semen for AI.

Nine Boer male goats were used in the study. Semen was collected twice a week with the aid of an artificial vagina. Ejaculates were evaluated for volume, colour, consistency, mass activity, sperm motility, sperm concentration and sperm morphology and ejaculates qualified the standard criteria were processed according to the needs of each experiment. Sperm cytological characteristics were evaluated before freezing and post thawing.

The effects of different buffers, egg yolk concentrations and sperm dilution rates were analysed to improve extending media for chilled and post thawed Boer goat spermatozoa. Tris buffer demonstrated practical and beneficial effects on sperm cytological characteristics before and after freezing. Significant ($P<0.05$) improvement was observed with Tris buffer in terms of motility and acrosome integrity of post thawed spermatozoa.

In the present study, significantly higher motility, membrane integrity and acrosome integrity were observed with the 18% egg yolk concentration compared to other egg yolk concentrations of 12% and 6%. A study was conducted to evaluate the effects of semen dilution rate on the characteristics of chilled and post thawed Boer goat semen. Results indicated that significant differences exist between low and high semen dilution rates. A significant improvement in the viability of pre and post thawed spermatozoa was observed with low dilution rate.

Four antioxidants at different concentrations were tested to determine their effectiveness in the preservation of Boer goat semen. Antioxidants (BHT, hypotaurine, cysteine and ascorbic acid) have improved the quality of chilled and frozen thawed stored Boer goat spermatozoa in terms of motility, membrane integrity, morphology, acrosome integrity and viability. Individual concentrations of each antioxidant were established in Tris fructose egg yolk glycerol extender. Significant ($P<0.05$) better results were obtained for chilled and frozen thawed Boer spermatozoa quality characteristics with 2 mM, 10 mM, 5 mM and 8.5 mg/ml concentrations of butylated hydroxytoluene, hypotaurine, cysteine and ascorbic acid, respectively.

Effects of antioxidants at the time of semen collection, and in the washing solution were subsequently evaluated to find out the best time of antioxidant addition to reduce oxidative stress, and improve chilled and post thawed quality of sperm. The results of these studies showed that the addition of antioxidants to the washing solution significantly improved the motility of chilled spermatozoa. Furthermore it was observed that motility and acrosome integrity of post thawed spermatozoa was significantly improved when washing solution was supplemented with antioxidants. However, the addition of the extender supplemented with antioxidants in collection tubes of the artificial vagina did not improve the quality of chilled and frozen thawed spermatozoa.

The results of the present study demonstrated that the rate of cooling significantly influenced the quality of chilled and frozen thawed Boer goat semen. Significantly ($P<0.05$) higher motility was observed with slow cooling rates and antioxidant supplementation.

The use of a programmable freezer for freezing spermatozoa compared to a polystyrene box method of freezing in a medium supplemented with antioxidants was investigated. In this experiment, significantly higher results were observed in motility, membrane integrity, morphology, acrosome integrity and viability of post thawed Boer goat spermatozoa extended with antioxidant in the programmable freezer compared to the polystyrene box method.

The ability of antioxidants to reduce lipid peroxidation (LPO) after freeze thawing was measured using the thiobarbituric acid method. Results showed that addition of antioxidants significantly reduced ($P<0.05$) the rate of LPO in comparison to control. Ascorbic acid exhibited significantly

lower values (1.27±0.28 nmol/2x10^8 spermatozoa), than butylated hydroxytoluene (1.32±0.42 nmol/2x10^8 spermatozoa), cysteine (2.27±0.16 nmol/2x10^8 spermatozoa) and hypotaurine (2.38±0.17 nmol/2x10^8 spermatozoa) than control (3.52±0.54 nmol/2x10^8 spermatozoa). However, differences among the supplements were non-significant. The overall pregnancy rate (%) was 34.38 %. Significantly higher ($P<0.05$) pregnancy rate was observed with ascorbic acid (42.85%) and butylated hydroxytoluene (35.71%) compared to those of hypotaurine (33.33%), cysteine (33.33%) and control (26.38%). In summary, this study pointed to the magnitude of improvement made possible when processing protocol and cryopreservation medium are optimised for Boer goat semen.