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Effect of holding and washing on the quality of goat semen

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Abstract

A total of 18 pooled semen samples collected from 4 healthy crossbred (Beetal × Assam Local) bucks, twice weekly for a period of 12 weeks, were processed via three processing methods, viz. Method I (holding after washing: semen sample was washed twice, extended, held for 0, 1, 3 and 5 h and preserved), Method II (washing after holding: semen sample held for 0, 1, 3 and 5 h, washed twice, extended and preserved) and Method III (holding of undiluted semen: semen sample held for 0, 1, 3 and 5 h, extended and preserved). An isotonic Tris buffer was used to wash the spermatozoa with the aid of centrifugation at 3000 rotations per minute (rpm) for 10 min. A Tris–citric acid–fructose–egg yolk extender was used to extend the semen processed with all three processing methods and preserved at 5 °C up to 72 h. Holding of semen at 24 °C in each processing method caused deterioration of the semen quality during preservation, as the holding time increased from 0 (without holding), 1, 3 and 5 h. The initial sperm motility declined from 85.8 to 71.7% in Method I, from 85.8 to 21.7% in Method II and from 84.2 to 45.0% in Method III, as the holding time increased from 0 to 5 h. The corresponding values were 66.7 and 49.2% for Method I, 66.7 and 8.0% for Method II and 42.5 and 25.8% for Method III, following 72 h of preservation. The percentage of live sperm and intact acrosomes also showed a similar decreasing trend as the holding time increased from 0 to 5 h in all processing methods as revealed in percentage of sperm motility. Washing of spermatozoa without a holding period maintained significantly higher sperm motility (66.7%), live sperm counts (83.0%) and incidence of intact acrosomes (79.3%) following 72 h at preservation, compared to the corresponding values (42.5, 65.2 and 73.3% for sperm motility, live sperm counts and intact acrosomes, respectively) recorded in unwashed semen without a holding period. The values recorded for washed semen without holding were also significantly higher than for semen held for 1, 3 and 5 h in all the three processing methods following 72 h of preservation. This study revealed that the holding of semen caused deterioration of the spermatozoa, whereas, washing without holding had a beneficial effect on the viability of spermatozoa in maintaining its storage ability for up to 72 h of preservation at refrigeration temperature (5 °C).

Keywords: Goat semen; Holding time; Seminal plasma removal; Preservation quality

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1. Introduction

Goat spermatozoa are susceptible to cold shock, which causes the death of numerous spermatozoa during processing for preservation at lower temperature, although the degree of susceptibility is less than that experienced in boar spermatozoa (Sahni and Roy, 1972). Holding, a procedure of retaining or incubation of semen at different room temperatures (18–30 °C) for a few hours before or after processing in boar semen prior to preservation, is generally beneficial (Graham et al., 1971; Pursel et al., 1973; Tamuli, 1993; Nath et al., 1994). Thus, it may be expected that a similar procedure may have some beneficial effect on goat spermatozoa and help sperms to develop a possible resistance against cold shock. However, there is no information currently available on the effect of the holding on the quality of goat semen during preservation at refrigeration temperature (5 °C).

An egg yolk-coagulating enzyme is present in the seminal plasma of goat semen, which has limited the use of egg yolk containing extenders for the preservation of goat semen (Roy, 1957). Removal of the seminal plasma by washing the spermatozoa with an isotonic buffer has been reported to improve the quality of goat semen during preservation at various temperatures (Irritani et al., 1961; Deka and Rao, 1986; Misra et al., 1993). However, the effect of the removal of seminal plasma on the quality of goat semen during preservation following holding has not been reported. This study was performed to determine the effect of holding, with or without seminal plasma, on the quality of goat semen for preservation at 5 °C.

2. Materials and methods

Four healthy crossbred bucks (Beetal × Assam Local) approximately, 2.5 years of age were selected from the Goat Research Station, Assam Agricultural University, Burnihat, Assam, India. The animals were maintained under standard managemental conditions practiced on the farm. Semen samples were collected twice weekly in the morning from each buck with the aid of the artificial vagina for a period of 12 weeks. Immediately after collection, each ejaculate was evaluated for mass motility (based on the numerical scale 0–4) and initial motility as per the method described by Zemjanis (1970). Semen samples showing +3 or more mass motility and 80% or more initial motility were pooled. A total of 18 pooled semen samples were used for the study.

2.1. Washing of semen

The pooled semen sample was diluted (1:5) with warm (30 °C) Tris buffer and centrifuged at room temperature (24–27 °C) for 10 min at 3000 × *g*. The clear supernatant was aspirated with the aid of a Pasteur pipette. Tris buffer was then added into the sediment to restore the initial volume during the first washing and centrifuged again. After the second washing, the extension of the sediment was done using a Tris extender. The volume of fresh semen prior to washing was taken into consideration for extension of sediment at the rate of 1:10. The same washing method was used for processing in Methods I and II.

2.2. Holding of semen

The pooled semen sample was placed in a 5ml plastic tube and properly sealed and stored in a biochemical oxygen demand (BOD) incubator for incubation or holding of the semen at 24 °C for different periods (1, 3 and 5 h)—before processing of the undiluted semen and before preservation of the washed and extended semen.

2.3. Extension of semen

A Tris extender (Foote, 1970) modified by Deka and Rao (1986) was used for dilution of semen (Table 1).

All the components of the extender, except egg yolk were mixed and the pH adjusted to 6.8 using a 5% citric acid solution. The egg yolk was added just prior to the

Table 1
Composition of Tris extender used in the dilution of buck semen

Components	Quantity
Tris (hydroxymethylaminomethane) (g)	2.422
Citric acid (g)	1.34
Fructose (g)	1.0
Egg yolk (ml)	20
Distilled water (ml)	80
Penicillin (IU)	80000
Streptomycin (μg)	100000