Preservation of Washed Spermatozoa of Mehsana Buck at Refrigeration Temperature

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Abstract

The study was done to understand the preservation of Washed Spermatozoa of Mehsana Buck at Refrigeration temperature. The washed spermatozoa from 78 semen ejaculates, 26 each from 3 Mehsana bucks, were preserved at 4 ± 1^{0}C temperature in SM and TCFEY diluents up to 72 hours and the physical characteristics of spermatozoa were studied to assess the suitability of diluents. The individual sperm motility and live spermatozoa decreased significantly whereas abnormal spermatozoa increased significantly (P<0.05) at each 12 hrs interval of preservation in two diluents. The effect of diluents on spermatozoan characteristics was less pronounced in SM than TCFEY diluent. Among the interaction studies, stage of preservation affected the live spermatozoa and their morphology. The findings suggested superiority of SM diluent over TCFEY for preservation of Mehsana buck semen.

Key Words: Buck ,Diluents, Ejeculates, Semen Preservation, Spermatozoa.

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Introduction

Artificial Insemination is a major concern in progeny testing programme of small animals which are generally reared in small geographical areas. The semen cooled to low or ultra low temperature has to be utilized for this purpose, however, the resultant fertility remains higher in Norwegian dairy goats by using semen stored at 4-5^{0}C in comparison to frozen (Leboeuf *et al.* 2000 and Paulenz *et al,* 2005). These workers have reported the Skimmed milk and Tris-egg yolk diluents for preservation of buck semen. The beneficial effect of washing on viability of spermatozoa of crossbred bucks (Beetal x Assam local) and its storage ability up to 72 hrs of preservation at refrigeration temperature has been reported by Islam *et al.* (2006) while; Ranjan *et al.* (2009) documented the similar benefits in Marwari buck semen extended in diluents containing 10% egg yolk. However, there does not seem any information on Mehsana Buck.

Therefore, the present investigation on preservation of Mehsana buck semen at refrigeration temperature vis-à-vis physical characteristics of spermatozoa was undertaken to evaluate the suitability of SM and TCFEY diluents.

Materials and Methods

The research protocol was conducted with a prior permission of Institutional Animal Ethics Committee, College of Veterinary Science, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. Three Mehsana bucks of breeding age, 12 to 18 months, were trained on teaser of same breed for two months to ejaculate the semen in Artificial Vagina; and there after utilized to obtain 26 semen ejaculates from each buck twice weekly during the period of three months.

A total of seventy eight ejaculates of acceptable quality were divided in two equal aliquots immediately after collection and each of them was washed to remove the coagulating enzyme using Krebs-Ringer-Phosphate-Glucose solution @ of 1: 9 followed by centrifugation at 3000 rpm for 5 min (Chemineau *et al.,* 1991). The supernatant was removed to obtain the sperm pack which was diluted in two different dilutors: Tris – Citric acid – Fructose – Egg yolk (TCFEY) and Skim Milk (SM) at the dilution ratio of 1:10 (Deka and Rao, 1986, Corteel, 1974). The samples were preserved at 5^{0}C temperature and were evaluated after 12, 24, 36, 48, 60 and 72 hrs of preservation. The data were

Table-1: Spermatozoan characteristics during refrigeration preservation of buck semen in TCFEY and SM diluents.

Dilutor/Stage	12 h	24 h	36 h	48 h	60 h	72 h
Individual sperm motility						
TCFEY	$80.82 + 0.34^{fa}$	$75.58 + 0.31^{ea}$	$70.26 + 0.36^{da}$	$64.75 + 0.27^{ca}$	$59.01 + 0.24^{ba}$	$52.55 + 0.46^{aa}$
SM	$81.80 + 0.33^{fb}$	$76.95 + 0.30^{eb}$	$71.68 + 0.36^{db}$	$66.38 + 0.31^{cb}$	$60.59 + 0.32^{bb}$	$53.99 + 0.37^{ab}$
Live sperm count						
TCFEY	$83.60 + 0.15^{f}$	$78.92 + 0.23^{ea}$	$73.87 + 0.22^{da}$	$68.57 + 0.24^{ca}$	$62.44 + 0.30^{ba}$	$55.19 + 0.44^{aa}$
SM	$84.19 + 0.13^{f}$	$80.02 + 0.22^{eb}$	$75.32 + 0.35^{db}$	$70.61 + 0.24^{cb}$	$65.23 + 0.46^{bb}$	$59.32 + 0.52^{ab}$
Abnormal sperm count						
TCFEY	$7.06 + 0.73^{ab}$	$9.25 + 0.77^{bb}$	$12.11 + 0.68^{cb}$	$15.11 + 0.51^{db}$	$18.44 + 0.49^{eb}$	$22.41 + 0.57^{fb}$
SM	$6.43 + 0.79^{aa}$	$7.96 + 0.76^{ba}$	$9.98 + 0.82^{ca}$	$12.18 + 0.78^{da}$	$15.05 + 0.66^{ea}$	$18.38 + 0.63^{fa}$

Means bearing different superscripts in columns and subscripts in rows differ significantly ($p < 0.05$).

Table-2: Analysis of variance for spermatozoan characteristics in TCEFY and SM diluents during refrigeration preservation in Mehsana bucks.

Source	d.f.	Individual motility count (M.S.)	Live sperm count count (M.S.)	Abnormal sperm Count (M.S.)
Buck	2	124.181*	31.792^{NS}	434.056*
Dilutor	1	379.504*	910.181*	1352.163*
Stage	5	16800.907*	15202.515*	4094.163*
Buck × Dilutor interaction	2	2.315^{NS}	12.524^{NS}	3.282^{NS}
Buck × Stage interaction	10	1.868^{NS}	6.743^{NS}	1.429^{NS}
Dilutor × Stage interaction	5	5.599^{NS}	79.416*	65.058*
Buck × Dilutor × Stage interaction	10	2.520^{NS}	4.290^{NS}	0.619^{NS}
Error	900	36.71^{NS}	24.29	6.69

* Significant at 5% level NS = Non-significant

analyzed by factorial CRD having 3 factors (Snedecor and Cochran, 1980).

Results and Discussion

The data pertained to individual sperm motility, live spermatozoa and their abnormalities at different hours of preservation in TCFEY and SM diluents are presented in Table 1. The findings showed significant decrease in individual sperm motility and live spermatozoa at all stages of preservation in both diluents. These two traits of spermatozoa were observed to curtail more than 25 percent at 72 hrs of preservation in comparison to their initial values. Other workers have also reported a considerable decline in live spermatozoa and initial sperm motility in TCFEY and SM diluents (Das *et al..,* 1985, Mishra *et al.*1993, Takerkhede *et al.* 2005). On the other hand, the morphological abnormalities of spermatozoa increased linearly and reached beyond the acceptable limit at 72 hrs of preservation to both diluents. Simon *et al.* (2003) also documented an increased percentage of sperm abnormalities as the duration of preservation advanced.

The effect of diluents and bucks were variable on spermatozoon characteristics. The individual sperm motility, live spermatozoa and morphological abnormalities differed significantly due to these two variances whereas live spermatozoa remained unaffected among the bucks (Table-2). Almost similar findings have been reported by earlier works in bucks (Mishra *et al*., 1993; Puranik *et al.,* 1994) and Patanwadi rams (Kakdiya, 1993).

The comparative findings of two dilutors revealed the significant differences in all three spermatozoon characteristics (Table-1). The superiority of SM diluent over the TCFEY diluent was reflected at all stages of preservation in our study (Table-1), which corroborates with the earlier findings in Osmanbadi and crossbred buck semen as reported by John and Raja (1973); Das *et al.*(1985) and Puranik *et al.* (1994). However, the findings of Singh *et al.* (1980) suggested higher sperm motility in buck semen using egg yolk citrate. This variation might be attributed to the breed characters of buck used in different studies besides the holding time of washed spermatozoa as suggested by *Islam et al.* (2006).

The sperm abnormalities were found to be less in SM diluent than the TCFEY at each stage of preservation. The differences were progressive with a range of 1-4 percent during the entire period of semen

preservation in our study. The increase in these abnormalities mounted to 22.41 ± 0.57 and 18.38 ± 0.63 percent in TCFEY and SM diluents, respectively at 72hrs of preservation. In earlier study, 11.5 percent abnormalities using tris diluent at 96 hrs of refrigeration has been reported by Takakhede *et al.* (2005).

The interaction of diluents and stages of preservation was found to be significant only for the live and abnormal spermatozoa whereas other interactions with regards to buck x diluents, buck x stages of preservation were non-significant on spermatozoa characteristic. Kakadiya (1993) also reported significant interaction of stage x dilutors on initial motility, live percent and abnormality of spermatozoa in Patanwadi rams.

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Conflict of interest

Author declare that they have no conflict of interest.

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