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LEAKAGE OF PHOSPHATASES AND FERTILITY OF BUCK SEMEN CRYOPRESERVED UNDER DIFFERENT FREEZING MODES

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Abstract
Present study was conducted on 160 ejaculates collected at weekly interval by artificial vagina method from 13 adult Sirohi bucks. Pooled ejaculates were diluted with Tris-egg yolk-citric acid-fructose-glycerol extender (1:4), filled and sealed in French mini straws. Few straws of diluted semen were thawed (40°C/15 seconds) and assessed for acid and alkaline phosphatases (ACP and AKP) in seminal plasma of diluted semen (control group). Remaining semen straws were randomly grouped to constitute freezing mode groups (M1, M2, M3, and M4) and processed further for cryo-preservation of semen. Accordingly diluted semen straws were cooled @ -4°C/minute from 25°C up to 5°C thereafter equilibrated at this temperature for 2 hours and then frozen up to -160°C @ 15, 20, 25 and 30°C/minute for M1, M2, M3 and M4 groups respectively. These frozen straws were held at this temperature for 2 minutes then stored separately in LN2. After 7 days of storage, straws from each freezing mode group were thawed and assessed for ACP and AKP in seminal plasma. In-vivo fertility trials were also conducted with straws from control (fresh diluted semen) as well as freezing mode groups (frozen at different freezing rates). Least square analysis of variance for the data obtained revealed highly significant (P ≤ 0.01) rise in the seminal plasma ACP and AKP enzyme levels in frozen thawed semen as compared to that in fresh diluted semen. The Values of ACP and AKP also differed significantly (P ≤ 0.05) among all the freezing mode groups wherein lowest values of ACP were observed in M4 group followed by M5, M2 and M1 groups in increasing order whereas, lowest values of AKP were observed in M3 followed by M2, M4 and M1 groups in increasing order. Highest fertility rates were observed with semen from M3 followed by M2, M4 and M1 groups. On the basis of enzyme leakage and in-vivo fertility trials, the optimum freezing rate for cryopreservation protocol was arrived at 25°C/minute.

Keywords: Buck semen, Freezing rates, Seminal Plasma phosphatases, ACP, AKP

Introduction
Sirohi goat is well recognized dual purpose breed having better performance for average daily gain as compared to Kutchi and Marwari goats; hence it could be employed as an improver breed for increasing meat and milk production in medium and small sized goats (Acharya, 1992 and Groot et al., 1992). Genetic potential for production traits in goats could favorably be augmented by breeding strategies covering a large numbers of doe with germplasm of genetically superior bucks. Artificial insemination (A.I.) with frozen semen is a desirable tool for genetic improvement in animals (Nuti, 1997) but frozen semen is not utilized on a wide spread basis for A.I. in goats because available cryopreservation protocols do not provide an acceptable level of fertility (Parks and Graham, 1992). Success of artificial insemination programme requires a suitable deep-freezing methodology for cryopreservation of diluted male germplasm without or with least compromised fertilizing ability. Review of available literature reveals that freezing protocols for cryopreserving buck semen have extensively been studied for variables like semen extenders, dilution rates, equilibration period, pellet versus straw freezing, thawing temperatures, thawing rates and semen additives etc., but there is meager any report about effect of freezing rates on post thaw semen quality (Daskin et al., 2011 Naing et al. 2011 and Nor-Ashikin, Abdullah, 2011, Ansari et al., 2012 and Beltran et al., 2013). Phosphatases are found in seminal plasma, acrosomes, sub-acrososomal space, post nuclear cap, cytoplasmic droplets and tail specially the mid piece of spermatozoa (Guraya, 1987). Seminal plasma phosphatases are derived from the secretions of accessory sex glands (Abdou et al., 1974) and are involved in transport of calcium ions from external fluids of female genital tract (Restall and Wales, 1968).
The calcium ions in turn play critical and multifaceted role in the process of fertilization (Yanagimachi 1981, Metz and Manroy 1985 and Guraya 1987). Acrosomal phosphatases play important role in sperm capacitation and acrosomal reaction whereas, intracellular phosphatases play important role in cell metabolism (Guraya 1987). Semen freezing has been reported to alter cell membrane permeability and damage to acrosome causing leakage of intra-cellular enzymes (Zanveld et al. 1971 and McRorie and Williams 1974). Present study was therefore conducted to investigate suitable rate of freezing that is least detrimental to the spermatozoa with comparative low leakage of phosphatases into seminal plasma so that the freezing protocol for buck semen could further be improved.

Materials and Methods

One Hundred Sixty ejaculates from 13 adult Sirohi bucks were collected at weekly interval by A-V method. Pooled ejaculates were diluted @ 1:4 with tris-egg yolk-citric acid-fructose-glycerol extender at room temperature (25°C) and filled in french mini straws. Few straws of diluted semen were thawed (40°C/15 sec.) and used for assessing phosphatases (ACP and AKP) in seminal plasma from fresh diluted semen (control group) of sirohi bucks, whereas, remaining straws were further processed for cryopreservation under different freezing rates. Accordingly diluted semen straws were cooled from 25°C to 5°C under controlled rate of cooling (0.4°C/minute) in a programmable biofreezer (Kryo-440-1.7;Model 10/1.7; Series III; Planer Product Ltd., Middlesex), thereafter equilibrated at 5°C for 2 hours. These equilibrated straws were frozen from 5°C to -160°C under controlled rates of freezing (15, 20, 25 and 30°C/minute for M1, M2, M3 and M4 group respectively). These frozen straws were then immersed and stored in LN₂ (-196°C). Semen straws from each group were thawed at 40°C for 15 seconds then centrifuged @ 3000 r.p.m. for 15 minutes, supernatant seminal plasma was collected thereafter alkaline phosphatase was estimated as per the method described by Kind and King (1954), whereas, acid phosphatase was assessed by King’s Method (King and Jagatheesan, 1959) using the diagnostic reagent kits supplied by Span diagnostics, Ltd, Surat, India. A minimum of 10 observations were recorded for each enzyme from semen straws of each group. The results obtained were subjected to mixed model least square and maximum likelihood computer programme PC-1 for studying the analysis of variance (Harvey, 1987). The mean value of enzymes among all the groups were compared as per Duncun’s multiple range test (Snedecor and Cochran, 1980). Fertility trials were conducted in 53 doe wherein natural estrus was detected by parading an appronded buck every morning and evening. Doe in natural heat was inseminated twice (at an interval of 8-10 hours) by deep cervical trans-vaginal insemination with diluted semen from control group as well as frozen thawed semen from each freezing mode group. A minimum of 10 doe were inseminated with semen from each group. The freezing rate with least compromised damage to sperms was arrived at on the basis of comparatively low enzyme leakage and high fertility rate.

Results and Discussion

The values of acid and alkaline phosphatases in fresh diluted as well as frozen thawed semen from different groups as well as mean ± S.E. and coefficient of variations for respective values have been depicted in Table-1. Least Squares analysis of variance showing effect of freezing rates on leakage of seminal Plasma phosphatases have been presented in Table-2 showing comparisons between the groups.

Fresh Diluted Semen

Acid and alkaline phosphatases (ACP and AKP) in fresh diluted semen from control group ranged from 22.5 to 35.625 and 45.833 to 64.1667 with respective Mean ± S.E. (CV%) 28.4180 ± 0.9086 (12.7893) and 56.375 ± 2.166 (12.1499) KA unit per 0.9221 x 10³ spermatozoa (Table-1). The ACP: AKP ratio observed in present study was 1:1.98.

In present study values of acid and alkaline phosphatases in seminal plasma of Sirohi bucks were in accordance with those reported by Varshney et al. (1978), Kapila (1992) and Kale and Tomer (2000). The ratio of AKP: ACP observed in present study (1:98:1) was in close approximation to that reported by Kale and Tomer (2000), whereas higher ratios have been reported in semen of barbari (Varshney et al. 1978) and Jamunapari (Kapila, 1992) breeds of bucks.

The observed values of acid phosphatase in seminal plasma of sirohi bucks were lower than those reported in diluted semen of Barbari bucks (Sinha et al., 1999-2000 and Tiwari 2000). Alkaline phosphatase contents of seminal plasma observed in present study were lower than those reported in barbari bucks (Varshney et al., 1978) and black-bengal x beetal bucks (Patil and Raja 1978). Mean ± S.E. values of AKP in present study were higher than those reported in black-bengal x beetal bucks by Singh et al. (1996).

These variations in ACP and AKP contents of seminal plasma could be due to differences in breeds (Sinha et al., 1985; Sinha et al., 1988 and Sinha et al., 1999-
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2000), age of bucks (Tiwari, 2000), seasons of semen collection (Baruah et al., 1992 and Kale and Tomer, 2000), sperm concentration (Kakar and Anand, 1984); rates of dilution and composition of diluents, (Singh et al., 1993). Individual variations between the bucks of same breed have also been reported by Tuli et al. (1991) and Kale and Tomer (2000).

Table-1: Phosphatases in fresh diluted semen (control group) and frozen thawed semen (freezing mode groups)

| S. N. | Phosphatases          | GroupWise Seminal Plasma Enzyme | (KA/0.9221 x 10^9 spermatozoa) |
|------|----------------------|---------------------------------|--------------------------------|
|      |                      | M1 (15° C/mt)                  | M2 (15° C/mt)                  | M3 (15° C/mt)                  | M4 (15° C/mt)                  | Control (15° C/mt)              |
| 1    | Acid Phosphatase     | 56.2500                         | 42.1875                       | 37.5000                       | 42.1875                       | 29.0625                        |
|      |                      | 55.3125                         | 43.1250                       | 37.5000                       | 44.0625                       | 22.5000                        |
|      |                      | 57.1875                         | 44.0625                       | 37.5000                       | 45.0000                       | 24.3750                        |
|      |                      | 59.0625                         | 45.0000                       | 38.4375                       | 46.8750                       | 35.6250                        |
|      |                      | 60.0000                         | 43.1250                       | 40.3125                       | 41.2500                       | 31.8750                        |
|      |                      | 60.9375                         | 44.0625                       | 40.3125                       | 43.1250                       | 26.2500                        |
|      |                      | 56.2500                         | 43.1250                       | 40.3125                       | 41.2500                       | 30.0000                        |
|      |                      | 54.3750                         | 45.9375                       | 41.2500                       | 45.9375                       | 28.1250                        |
|      |                      | 55.3125                         | 46.8750                       | 38.4375                       | 40.3125                       | 23.4375                        |
|      |                      | 58.1250                         | 47.8125                       | 38.4375                       | 42.1875                       | 33.7500                        |
|      |                      | 59.0625                         | 46.8750                       | 38.4375                       | 43.1250                       | 27.1875                        |
|      |                      | 60.0000                         | 47.8125                       | 39.3750                       | 45.0000                       | 30.0000                        |
|      |                      | 56.2500                         | 41.2500                       | 41.2500                       | 39.3750                       | 25.3125                        |
|      |                      | 57.1875                         | 48.7500                       | 41.2500                       | 41.2500                       | 29.0625                        |
|      |                      | 58.1250                         | 44.0625                       | 41.2500                       | 42.1875                       | 30.9375                        |
|      |                      | 60.9375                         | 45.9375                       | 42.1875                       | 44.0625                       | 27.1875                        |
|      |                      | Mean ± S. E.                    | 57.7734                       | 45.1172                       | 39.6094                       | 43.1250                       | 28.4180                        |
|      |                      | (C. V. %) (3.5981)              | ± 0.5197 (4.7913)             | ± 0.3968 (4.0074)             | ± 0.5135 (4.7628)             | ± 0.9086 (12.7893)              |
| 2    | Alkaline Phosphatase | 114.5833                        | 110.0000                      | 100.8333                      | 105.4167                      | 64.1667                        |
|      |                      | 142.0833                        | 100.8333                      | 82.5000                       | 123.7500                      | 59.5833                        |
|      |                      | 132.9167                        | 105.4167                      | 100.8333                      | 142.0833                      | 64.1667                        |
|      |                      | 128.3333                        | 110.0000                      | 110.0000                      | 128.3333                      | 59.5833                        |
|      |                      | 151.2500                        | 100.8333                      | 100.8333                      | 110.0000                      | 50.4167                        |
|      |                      | 105.2500                        | 96.2500                       | 96.2500                       | 123.7500                      | 55.0000                        |
|      |                      | 114.5833                        | 100.8333                      | 100.8333                      | 105.4167                      | 64.1667                        |
|      |                      | 128.3333                        | 114.5833                      | 114.5833                      | 114.5833                      | 50.4167                        |
|      |                      | 146.6667                        | 100.8333                      | 100.8333                      | 119.1667                      | 45.8333                        |
|      |                      | 128.3333                        | 96.2500                       | 91.6667                       | 123.7500                      | 50.4167                        |
|      |                      | Mean ± S. E.                    | 129.250                       | 104.9583                      | 99.9166                       | 119.6250                       | 56.3750                        |
|      |                      | (C. V. %) (11.4358)             | ± 4.6741 (5.9839)             | ± 1.9861 (8.8632)             | ± 2.8005 (9.4629)             | ± 3.5797 (12.1499)             |

i. Comparisons were made between the groups
ii. Values with common superscripts do not differ significantly (p ≤ 0.05)
Table-2: Least Squares Analysis of Variance Showing Effect of Freezing Rates on Leakage of Seminal Plasma Phosphatases in Semen

| S. N. | Seminal Plasma Phosphatase | Source of variance | Degree of freedom | MSS Values  |
|-------|-----------------------------|--------------------|------------------|-------------|
| 1.    | Acid Phosphatase            | Between groups     | 4                | 1786.8054 **|
|       |                             | Remainder          | 75               | 5.7883      |
| 2.    | Alkaline Phosphatase        | Between groups     | 4                | 7869.8281 **|
|       |                             | Remainder          | 75               | 102.2804    |

Note: Superscript ** denotes Significant Differences at 1% level (P ≤ 0.01)

Frozen Thawed Semen

**Acid Phosphatase**

Acid phosphatase in seminal plasma of semen from M1; M2; M3 and control groups were in the range from 54.375 to 60.9375; 41.25 to 48.75; 37.5 to 42.1875; 39.375 to 46.875 and 22.5 to 35.625 KA units per 0.9221 x 10^9 spermatozoa respectively. The Mean ± S. E. (CV %) values of ACP in respective groups were 57.7734 ± 0.5197 (3.5981); 45.1172 ± 0.5404 (4.7913); 36.0949 ± 0.3968 (4.0074); 43.125 ± 0.5135 (4.7628) and 28.418 ± 0.9086 (12.7893) KA unit per 0.9221 x 10^9 spermatozoa respectively (Table-1).

Values of acid phosphatase in seminal plasma of fresh diluted semen (control group) were lower than those from frozen thawed semen from freezing mode groups, the differences were highly significant (P ≤ 0.01). The percent rise in ACP contents from dilution to frozen thaw stage in M1; M2; M3 and M4 groups were 129.27; 86.18; 77.24 and 112.20 percent respectively. It indicates that freezing inflicts cryo-injury to spermatozoa with resultant extracellular leakage of phosphatases. Similar findings were reported by Glogowski and Strezezek (1979) and Szasz et al. (2000).

Among freezing mode groups lowest value of seminal plasma ACP was observed in M1 followed by M4, M2 and M1 groups in increasing order. Significant (p ≤ 0.05) differences were observed among all freezing mode groups. It reveals significant effects of freezing rates on leakage of ACP. It is in agreement with the opinions of Graham and Pace (1967), Mohan (1982) and Szasz et al. (2000) who stated that freezing rates significantly influence the release of enzymes from spermatozoa.

**Alkaline Phosphatase**

Alkaline phosphatase contents in seminal plasma of semen from M1; M2; M3 and control groups were in the range from 105.42 to 151.25; 96.25 to 114.5833; 91.6667 to 114.5833; 105.4167 to 142.0833 and 45.8333 to 64.1667 KA units per 0.9221 x 10^9 spermatozoa respectively. The Mean ± S. E. (CV %) values in respective groups were 129.25 ± 4.6741 (11.4358); 104.9583 ± 1.9861 (5.9839); 99.9166 ± 2.8005 (8.8632); 119.625 ± 3.5797 (9.4629) and 56.3750 ± 2.1660 (12.1499) KA units per 0.9221 x 10^9 spermatozoa (Table-1).

Alkaline phosphatase contents in seminal plasma of fresh diluted semen from control group were lower than those observed in frozen thawed semen from freezing mode groups, the differences were highly significant (P ≤ 0.01). These results are in accordance with previous reports stating that process of cryopreservation inflicts considerable damage to spermatozoa with resultant higher level of alkaline phosphatase in seminal plasma of frozen thawed semen (Singh et al., 1996 and Szasz et al., 2000).

The percent rise in AKP contents observed from dilution to frozen thaw stage in M1; M2; M3 and M4 groups were 103.3; 58.76; 39.38 and 51.75 percent respectively. In present study the percent rises in AKP contents from dilution to frozen thawed stage were lower than that reported by Singh et al. (1996).

Among freezing mode groups lowest value of AKP in seminal plasma was observed in M3 followed by M2; M4 and M1 groups in increasing order. Significant (P ≤ 0.05) differences were observed between M1 and M3; M1 and M2; M2 and M4; M3 and M4; whereas, non-significant differences were observed between M2 and M3 groups (Table-1). It revealed that freezing rates significantly affects the leakage of AKP. Similar conclusions were made by Graham and Pace (1967) and Mohan (1982).

The mean values of AKP contents in seminal plasma of frozen thawed semen from M1, M2, M3 and M4 groups were higher than those reported by Singh et al. (1996). These variations could be attributed to differences in breed (Sinha et al., 1985; Sinha et al., 1988 and Sinha et al., 1999-2000), age of bucks (Tiwari, 2000), seasons of semen collection (Baruah et al., 1992 and Kale and Tomer, 2000), individual variations between semen samples.
the bucks (Tuli et al., 1991 and Kale and Tomer, 2000), season of semen collection (Baruah et al., 1992), sperm concentration (Kakar and Anand, 1984), rates of dilution and composition of diluents (Singh et al., 1993 and Singh et al., 1996) and freezing rates (Graham and Pace, 1967; Mohan, 1982 and Szasz et al., 2000).

The enzyme release from spermatozoa has generally been viewed as cellular injury (Ingale et al., 2000), whereby membrane become inactive with altered permeability or destroyed resulting into loss of material therein (De-Reuck and Knight, 1963 and Guraya 1987). The process of cryopreservation causes diminished intracellular enzyme activity that results from leakage of enzyme into the extracellular surrounding medium. Species release differences (Roychoudhury et al., 1974) have been attributed to intrinsic differences in the cells between the species (White and Well, 1960) as well as differences in susceptibility to membrane damage between the species (Hammerstedt et al., 1978).

Increased value of phosphatases in seminal plasma a re mostly due to membrane damage. In-vivo present study. Increased value of phosphatases in seminal plasma a re Strzezek, 1979 and Szasz et al., 2000). The enzyme release from spermatozoa has generally been attributed to intrinsic differences in the cells between the species (White and Well, 1960) as well as differences in susceptibility to membrane damage between the species (Hammerstedt et al., 1978).

In-vivo fertility rates with semen from M1, M2, M3, M4 and control groups were 10.00, 27.27, 36.36, 20.00 and 45.45 percent respectively. It was higher in doe inseminated with diluted semen as compared with those inseminated with frozen thawed semen.

Among freezing mode groups highest fertility rates were observed with semen from M3 followed by M2, M4 and M1 group in decreasing order.

**Conclusion**

It was concluded that freezing inflicts cryo-injury with resultant increased leakage of phosphatase enzyme into the seminal plasma that adversely affect the fertilizing ability of semen. On the basis of phosphatase leakage and fertility rates optimum freezing rate for cryopreservation of buck semen was observed as 25°C/minute.

**References**

Abdou MSS, El - Guindi MM, Mustafa MA, El - Wishi AB and Farahat AA (1974) Comparative study of the phosphatase activity in the semen of bovine in Egypt. Zbl. Vet. Med.A-21: 759 - 69.

Acharya RM. (1992) Goat Genetic Resource and Their Management. In: Lokeshwar RR (Ed) Research in Goats, Indian experience. Chapter 1.Central Institute for Research on Goats, Makhdoom, Mathura, Uttar-Pradesh, India. 1-21.

Ansari M, Towhid A, Shahrbabak MM and Bahreini M (2012) Docosahexaenoic acid and alpha-tocopherol improve sperm cryosurvival in goat. Slovak J. Anim. Sci. 45: 7-13.

Baruah B, Joshi BC and Tripathi KC (1992) Physico-chemical characteristics of the semen of black-bengal goats under seasonally varying macro-environment. In: Proceeding of Vth International Conference on Goats, March 2-8, 1992 New Delhi, India.

Beltran MAG, Atabay EP, Edwin C, Atabay EC, Cruz EM, Aquino FP and Cruz LC (2013) Optimized Extenders for Cryopreservation of Buck Semen for Artificial Insemination. Philipp J Vet Anim Sci. 39: 1-10

Daskin A, Kulaksiz R, Akcay E and Erol H (2011). The Effect of Different Dilution Rates of Angora Buck Semen Frozen with Bioxcell Extender on the Post-thaw Quality. J Fac Vet Med Univ Erciyes 8: 23-26.

De-Reuck AVS and Knight J (1963) Cellular Injury. In: Proceedings of CIBA foundation symposium on Cellular Injury. July 2-4, 1963 London.

Glogowski and Strzezek J (1979). Some biochemical characters of alkaline phosphatase in bull semen during freezing. MedycynaVeterinarynajnr., 35: 179-182.

Graham EE and Pace MM (1967) Some biochemical changes in spermatozoa due to freezing. Cryobiology, 4: 75-84.

Groot B De, Prasad RAN, Soni RL, Nett P and Kropl W (1992) Performance of Sirohi goats under village conditions in Rajasthan, India. In: Proceedings of Vth International Conference on Goats, March 2-8, 1992 New Delhi, India.

Guraya SS (1987). Biology of Spermatogenesis and Spermatozoa in Mammals. First edition, Springer - Verlag, Berlin Heidelberg, New York.

Hammerstedt AH, Keith AD, Snipes W, Amann RP, Arruda D and Girel Jr. JC (1978). Use of spin labels to evaluate effect of cold shock and os-molarity of sperm. Biology of Reproduction. 18: 686-696.

Harvey WR (1987). Mixed Model Least Squares and Maximum Likelihood Computer Program. 1987: PC-1.

Ingale ND, Suthar BN and Sharma VK (2000) Pellet freezing of ram semen and associated alterations in enzyme activity. Indian Journal of Animal Science. 70: 839-840.

Kakar SS and Anand SR (1984) Acrosomal damage and enzyme leakage during freeze preservation of buffalo spermatozoa. Indian Journal of Experimental Biology. 22: 5-10.

Kale MM and Tomer OS (2000) Enzymatic constituents in crossbred buck semen. Indian Journal of Animal Science, 70: 30-32.

Kapila R (1992) Leakage of Enzymes during Freezing of Goat Semen, M. Sc, Dissertation, N.D.R.I. (Deemed University), Karnal, India.
S. Sharma et al. (2013). Int J Appl Sci Biotechnol, Vol. 1(4): 202-207

Kind PRM and King EJ (1954) Estimation of plasma phosphatase by determination of hydrolyzed phenol with amino-antipyrine. Journal of Clinical Pathology. 7: 322.

King EJ and Jagatheesan KA (1959) A Method for the Determination of Tartrate-Labile Prostatic Acid Phosphatase in Serum. Journal of Clinical Pathology. 12: 85.

Mc-Rorie RA and Williams WL (1974) Biochemistry of mammalian fertilization. Annual Rev. Biochem. 43: 777-802.

Medrano A, Terrazas A and Soto R (2010) Principles and Perspectives for the Conservation of Goat Buck Semen. Small Ruminant Research. 89: 140–143.

Mohan J (1982) Investigations on Effect of Different Freezing Rates on Metabolic Behaviour of Buffalo Frozen Semen. M. Sc. Dissertation, N. D. R. I. (Deemed University), Karnal, Haryana, India.

Metz CB and Manroy A (1985) Biology of Fertilization (Vol. IIA). Academic Press, London. 215-134.

Naing SW, Haron AW, Goriman MAK, Yusoff R, Bakar MZA, Sarasafi K, Bukan MM, Thein M, Kyaw T and San MM (2011) Effect of Seminal Plasma Removal, Washing Solutions, and Centrifugation Regimes on Boer Goat Semen Cryopreservation. Pertanika J. Trop. Agric. Sci. 34: 271 - 279.

Nor-Ashikin MNK and Abdullah RBA (2011) Comparison between tris-citric acid yolk, yolk albumin citrate and skimmed milk extenders on sperm motility, livability and mass movement in frozen-thawed goat sperm. Biomedical Research. 22: 285-288

Nutí L (1997) Techniques for Artificial Insemination of Goats. In: Youngquist, RS (Editor), Current Therapy in Large Animal Theriogenology. W.B. Saunders Company, Philadelphia. pp.499-504.

Parks JE and Graham JK (1992) Effects of cryopreservation procedures on sperm membranes. Theriogenology. 38: 209–222.

Patil RV and Raja C KSV (1978) Acid and alkaline phosphatase and amylase concentration in the semen of Malabari bucks. Current Science. 47:319.

Restall BJ and Wales RG (1968) The Fallopian Tube of the Sheep. V. Secretion from the Ampulla and Isthmus. Australian Journal of Biological Science. 21: 491.

Roychoudhury PN, Pareek PK and Gowda HC (1974) Effect of cold shock on glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) release from bull and ram spermatozoa. Andrologia. 6: 315-319.

Singh LP, Meur SK and Purbej LN (1993) Leakage of transaminases during preservation of buck semen. Indian Journal of Animal Science, 63: 963-965.

Singh MP, Sinha AK, Sinha BK and Prasad RL (1996) Effect of Cryoprotectants on release of various enzymes from buck spermatozoa during freezing. Theriogenology. 45: 405-416.

Sinha NK, Gupta VK, Goel AK and Nandy DK (1999-2000) Automation of semen freezing protocol for higher post thaw motility and fertility in goats. Annual Report, Central Institute for Research on Goats, Makhdoom Mathura, India. 33-36.

Sinha MP, Sinha SN, Balraj, Singh BK and Singh DK (1985) Character of semen and lactic acid profile at different hours of preservation in Jamunapari and Barbari bucks. Indian Journal of Animal Reproduction. 6: 91-94.

Sinha SN, Singh SK and Sinha AK (1988) Leakage of transaminases and lactic dehydrogenases in chilled semen of buck of different breeds. Indian Journal of Animal Reproduction. 9:131-134.

Snedecor GW and Cochran WG (1980) Statistical Methods 7th edition, Oxford and IBH Publication Company, Calcutta, India.

Szasz F, Gabor G and Solti L (2000) Comparative study of different methods for dog semen cryopreservation and testing under clinical conditions. Acta Veterinaria Hungarica. 48: 325-333.

Tiwari HA (2000)Studies on The Growth, Testicular Biometry, Semen Production and It’s Quality in Barbari Bucks, M.V.Sc. Thesis, C.S. Azad University of Agriculture and Technology, Kanpur, India, 2000.

Tuli RK, Schmidt–Bulain R and Holtz W (1991) Influence of thawing temperature on viability and release of GOT in frozen semen from Boer goats. Animal Reproduction Science. 25: 125-131.

Varshney VP, Sengupta BP and Pandey MD (1978) Enzymatic constituents of goat semen. Indian Veterinary Journal, 55: 348-349.

White IG and Wales RG (1960) The susceptibility of spermatozoa to cold shock. International Journal of Fertility, 5:195-201.

Yanagimachi R (1981) Mechanisms of fertilization in mammals.In: Fertilization and Embryonic Development. MastroianniL and Biggers J.D. (eds.), Plenum Press, New York. 81-182

Zaneveld LJD, Robertson RT, Kessler M and Williams WL (1971) Inhibition of fertilization in vitro by pancreatic and seminal plasma trypsin inhibitors. Journal of Reproduction and Fertility, 25: 287.