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Assessment of transaminases and effect of freezing rates on their leakage into seminal plasma of sirohi bucks

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Abstract: Seminal plasma transaminases (GOT and GPT) were assessed in diluted (Tris-egg yolk-citric acid-fructose-glycerol extender 1:4) and thawed (40^0C for 15 seconds) semen of Sirohi bucks (control group). Diluted, cooled (from 25^0C up to 5^0C) and equilibrated (at 5^0C for 2 hours) semen straws were frozen @ 15, 20, 25 and 30^0C/minute for M_1, M_2, M_3 and M_4 groups respectively. These frozen straws were stored separately in LN_2. Effect of freezing rates on leakage of transferases was studied by assessing GOT and GPT in seminal plasma of thawed semen from M_1, M_2, M_3 and M_4 groups. The Mean $\pm$ S.E. (C.V.%) values of transferases (GOT : GPT) in M_1, M_2, M_3, M_4 and control group were 313.00 $\pm$ 8.40 (6.58) : 20.5 $\pm$ 0.43 (5.12), 272.5 $\pm$ 6.68 (6.01) : 16.33 $\pm$ 1.02 (15.33), 255.00 $\pm$ 10.1 (9.7) : 14.5 $\pm$ 0.67 (11.33), 292.0 $\pm$ 9.67 (8.09) : 19.67 $\pm$ 0.70 (8.99) and 147.67 $\pm$ 10.96 (18.17) ; 9.5 $\pm$ 0.62 (15.96) units per 0.92 x 10^9 spermatozoa respectively. Least square analysis of variance revealed highly significant ($P < 0.01$) rise in the seminal plasma GOT enzyme levels in frozen thawed semen as compared to that in fresh diluted, cooled and equilibrated semen. Among the freezing mode groups lowest GOT and GPT values in seminal plasma was observed in M_3 followed by M_2, M_4 and M_1 groups in increasing order.

Keywords: Sirohi Buck, Frozen semen, Seminal Plasma, Transferases, GOT, GPT

1. 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 favourably be augmented by breeding strategies covering a large numbers of doe with germplasm of genetically superior bucks. Artificial insemination 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 the artificial insemination in goats, in part 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. Enzyme leakage is one of reliable parameter for evaluating integrity of sperm plasma membrane; present study was therefore conducted to investigate the effect of different freezing rates on leakage of transaminases in seminal plasma.

2. Materials and Methods

160 ejaculates from 13 adult Sirohi bucks were collected at weekly interval by artificial vagina method. Pooled eja-

culates were diluted @ 1:4 with tris-egg yolk-citric acid-fructose-glycerol extender at room temperature (25^0C) and filled in french mini straws. Few straws of diluted semen were used for assessing transferases 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 straws were cooled from 25^0C to 5^0C under controlled rate of cooling (0.4^0C/minute) in programmable biofreezer thereafter equilibrated at 5^0C for 2 hours. These equilibrated straws were subjected to controlled freezing @ 15, 20, 25 and 30^0C/minute for M_1, M_2, M_3 and M_4 group respectively and frozen from 5^0C to -160^0C. These frozen straws were then immerged and stored in LN_2. Semen (-196^0C). Semen straws from each group were centrifuged @ 3000 r.p.m. for 15 minutes and supernatant seminal plasma was collected for estimating transaminases in fresh diluted semen (control group) as well as in frozen thawed semen from freezing mode groups (M_1, M_2, M_3 and M_4). Transaminases (GOT and GPT) were estimated in seminal plasma of thawed (40^0C for 15 seconds) diluted semen (control group) as well as in frozen thawed semen from M_1, M_2, M_3 and M_4 groups. Reagent kits supplied by Span diagnostics, Ltd, Surat, India were used for estimating GOT and GPT as per method described by Reitman and Frankel (1957). A minimum of 6 observations were recorded for each enzyme in each group. The results obtained were statistically analyzed for arriving at Mean $\pm$ S.E. values of enzymes for each group. The data obtained were subjected to mixed model least square and maximum likelihood computer programme PC-1 for studying the analysis of variance (Harvey, 1987). The means were compared as per Duncun's multiple range test (Snedecor and Cochran, 1980).

3. Results and Discussion

3.1. GOT and GPT in diluted semen of Sirohi Bucks

GOT in fresh diluted semen was in the range from 114 to 174 with Mean $\pm$ S.E. values 147.67 $\pm$ 10.96 units per 0.922 x 10^9 spermatozoa. These values are within the range (74.10 $\pm$ 6.86 to 219.00 units per ml) reported by Kapila (1992), Singh *et al.* (1993), Tuli and Holtz (1994), Singh *et al.* (1996), Antoine and Pattabiraman (1999), Shakeel (1999), Sinha *et al.* (1999-2000) and Tiwari (2000). The mean value of GOT observed in present study is in close approximation to that reported in diluted pre-freeze semen from Barbari (Sinha *et al.,* 1999-2000 and Tiwari, 2000) and Tellicherry bucks (Sivaselvam *et al.,* 2000); however, level of GOT found in seminal plasma of Sirohi bucks is lower than that reported in diluted semen from Barbari (Varshney *et al.,* 1978), Kutchi and Sirohi bucks (Sinha *et al.,* 1999-2000). Present values of GOT are higher than those reported in diluted semen from Barbari (Shakeel, 1999), Boer (Tuli and Holtz, 1994), Beetal x Black Bengal (Singh *et al.,* 1996) and Tellicherry bucks (Antoine and Pattabiraman, 1999).

GPT in seminal plasma from fresh diluted semen (control group) ranged from 7.00 to 11.00 with Mean $\pm$ S.E. 9.5 $\pm$ 0.62 units per 0.922 x 10^9 spermatozoa. These values are within the range (9.90 + 1.52 to 56.83 + 2.121 units per ml.) as reported in different breeds of bucks by Varshney *et al.* (1978), Singh *et al.* (1996), Antoine and Pattabiraman (1999), Shakeel (1999), Sinha *et al.* (1999-2000), Sivaselvam *et al.* (2000) and Tiwari (2000). The mean value of GPT observed in present study is in close approximation to that reported by Sinha *et al.* (1999-2000) in seminal plasma of Sirohi bucks. Higher mean values of GOT than that observed in present study were reported in diluted semen from Barbari (Varshney *et al.,* 1978; Shakeel, 1999; Sinha *et al.,* 1999-2000 and Tiwari, 2000), Black Bengal x Beetal (Singh *et al.,* 1996), Kutchi (Sinha *et al.,* 1999-2000) and Tellicherry bucks (Antoine and Pattabiraman, 1999 and Sivaselvam *et al.,* 2000).

3.2. Effect of Freezing Rates on Leakage of GOT

In M_1; M_2; M_3; M_4 and control group values of seminal plasma GOT were in the range between 288 to 342; 246 to 288; 210 to 276; 261 to 330 and 114 to 174 units per 0.9221 x 10^9 spermatozoa respectively. The Mean $\pm$ S. E. (C.V.%) values in respective groups were 313.00 $\pm$ 8.40 (6.58); 272.5 $\pm$ 6.68 (6.01); 255.00 $\pm$ 10.1 (9.7); 292.0 $\pm$ 9.67 (8.09) and 147.67 $\pm$ 10.96 (18.17) units per 0.9221 x 10^9 spermatozoa. Least square analysis of variance reveals highly significant ($P < 0.01$) rise in GOT enzyme levels in frozen thawed semen as compared to that in diluted semen. The percent rise in GOT from dilution to frozen thaw stage in M_1; M_2; M_3 and M_4 groups were 111.96; 84.54; 72.69 and 98.08 percent respectively. Among freezing mode groups lowest GOT enzyme was observed in seminal plasma of M_3 followed by M_2, M_4 and M_1 groups in increasing order. Highly significant ($P<0.01$) differences were observed between M_1 and M_2; M_1 and M_3; M_3 and M_4, whereas, non-significant differences were found in M_1 and M_4; M_2 and M_3; M_2 and M_4 groups. These finding are in accordance with those reported in frozen thawed semen from Barbari (Shakeel, 1999), Boer (Tuli and Holtz, 1994), Beetal x Black Bengal (Singh *et al.*, 1996), Tellicherry (Sivaselvam *et al.*, 2000) and non-descript bucks (Singh *et al*., 1993). Higher GOT values in frozen thawed semen indicate that cryopreservation inflicts injury to the spermatozoa with resultant increased permeability or breakage of plasma membrane causing release of intracellular enzymes (De Rauck and Knight, 1964 and Tuli *et al*., 1982). Lowest mean value of GOT was observed in frozen thawed semen from M_3 group followed by M_2, M_4 and M_1 groups in increasing order. Significant effects of freezing rates on leakage of transaminases observed in present study were in general agreement with findings of Graham and Pace (1967) and Mohan (1982). Contrary to these findings non-significant effects of freezing rates on GOT release as observed by Pratap *et al*. (1999) could be due to very narrow difference ($1\ ^0$C) in freezing rates (4^0C/mt and 5^0C/mt for

freezing from 4^0C to-12^0C and 4^0C to -30^0C respectively) compared by them. The mean value of GOT in seminal plasma of frozen thawed semen from M_1, M_2, M_3 and M_4 groups were in close approximation to those reported in frozen thawed semen from Tellicherry bucks (Sivaselvam *et al*., 2000). Lower values of GOT than those observed in present study were reported in frozen thawed semen from Barbari (Shakeel, 1999), Boer (Tuli and Holtz, 1994) and Black-Bengal x Beetal bucks (Singh *et al*., 1996). The variations in leakage of GOT have been attributed to differences in breeds (Sinha *et al*., 1985; Sinha *et al*., 1988 and Sinha *et al*., 1999-2000), individual variations between the bucks of same breed (Tuli *et al*., 1991), age of bucks (Tiwari, 2000), season of semen collection (Baruah *et al.,* 1992), sperm wash or sperms with intact plasma (Tuli *et al*., 1991), rate of dilution as well as composition of diluent (Singh *et al*., 1993 and Singh *et al*., 1996), glycerol levels (Bonadonna *et al*., 1974), equilibration periods (Joshi *et al*., 1990), cooling rates (Bhosrekar, 1975) and freezing rates (Graham and Pace, 1967 and Mohan, 1982).

3.3. Effect of Freezing Rates on Leakage of GPT

Glutamate pyruvate transaminases enzyme in seminal plasma of M_1; M_2; M_3; M_4 and control groups were in the range from 19 to 22; 13 to 19; 13 to 17; 16 to 20 and 7 to 11 units per 0.9221 x 10^9 spermatozoa respectively. The Mean $\pm$ S. E. (C.V.%) values in respective groups were 20.5 $\pm$ 0.43 (5.12); 16.33 $\pm$ 1.02 (15.33); 14.5 $\pm$ 0.67 (11.33); 19.67 $\pm$ 0.70 (8.99) and 9.5 $\pm$ 0.62 (15.96) units per 0.9221 x 10^9 spermatozoa. Least square analysis of variance revealed highly significant ($P < 0.01$) differences in GPT values from frozen thawed semen as compared to that in diluted thawed semen. The percent rise in GPT from dilution to frozen thaw stage in M_1; M_2; M_3 and M_4 groups were 115.79; 71.93; 52.63 and 107.02 percent respectively. Among the freezing mode groups lowest GPT value in seminal plasma was observed in M_3 followed by M_2, M_4 and M_1 groups in increasing order. Highly significant ($P< 0.01$) differences were observed between M_1 and M_2; M_1 and M_3; M_2 and M_4; M_3 and M_4 groups, whereas, non-significant difference in GPT contents were observe in frozen thawed semen from M_1 and M_4; M_2 and M_3 groups. Similar findings showing increased GOT contents in seminal plasma from frozen thawed semen attributed to process of cryopreservation have been reported by Singh *et al*. (1993), Singh *et al*. (1996), Shakeel (1999), Ingale *et al*. (2000) and Sivaselvam *et al*. (2000). Among freezing mode groups lowest mean value of GPT was observed in frozen thawed semen from M_3 group followed by M_2, M_4 and M_1 groups in increasing order. Significant effect of freezing rates on leakage of GPT release observed in present study is in agreement with the findings of Graham and Pace (1967) and Mohan (1982). Contrary to these findings non-significant effects of freezing rates on GPT release as observed by Pratap *et al*. (1999) could be due to very narrow difference (1 ^{0}C) in freezing rates (4^0C/mt and 5^0C/mt for freezing from 4^0C to-12^0C and 4^0C to -30^0C respectively) compared by them. The percent rise in GPT values from dilution to frozen thawed stage in different breeds of bucks have been reported in the range from 40.23 to 287.12 percent (Singh *et al*., 1993; Singh *et al*., 1996; Shakeel, 1999 and Sivaselvam *et al*., 2000). The percent rises in GPT values observed in present study were within this range. The mean values of GPT in frozen thawed semen from different breeds of bucks have been reported in the range from 21.96 to 220.00 units per ml. (Singh *et al.,* 1996; Shakeel, 1999 and Sivaselvam *et al*., 2000). The values observed in frozen thawed semen from M_1, M_2, M_3 and M_4 groups are within this range. The variations in GPT release have been attributed to differences in breeds (Sinha *et al*., 1999-2000), individual variations between the bucks (Tuli *et al*., 1991), age of bucks (Tiwari, 2000), season of semen collection (Baruah *et al.,* 1992), washing or non washing of sperms (Tuli *et al*., 1991), rates of dilution and composition of diluent (Singh *et al*., 1993 and Singh *et al*., 1996), glycerol levels (Bonadonna *et al*., 1974), equilibration periods (Joshi *et al*., 1990), cooling rates (Bhosrekar, 1975) and freezing rates (Graham and Pace, 1967 and Mohan, 1982).

The extra cellular activity of transaminases is due to their leakage into seminal plasma caused by damage inflicted upon spermatozoa (Salisbury *et al*., 1985 and Kapila, 1992), hence seminal plasma transaminases are evaluated as an index of measurement of injury to spermatozoa incurred during the process of cryopreservation (Graham and Pace, 1967; Jani *et al.*, 1983, Singh *et al*., 1996 and Ingale *et al*., 2000). The enzyme release from spermatozoa has generally been viewed as cellular injury (Sidhu *et al.,*1996 and Ingale *et al*., 2000), whereby membrane become inactive with altered permeability or destroyed resulting into loss of material therein (De-Rauck and Knight, 1964). 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).

4. Conclusion

Normal seminal plasma values of glutamic oxaloacetic transaminases (GOT) and glutamic pyruvic transaminase (GPT) enzymes in diluted semen from sirohi bucks ranges from 114 to 174 with Mean $\pm$ S.E. values 147.67 $\pm$ 10.96 and 7.00 to 11.00 with Mean $\pm$ S.E. 9.5 $\pm$ 0.62 units per 0.922 x 10^9 spermatozoa respectively. On comparing freezing rates (15, 20, 25 and 30^0C/minute) for cryopreservation of diluted cooled and equilibrated semen least leakage of transaminases was observed with freezing @ 25^0C/minute. It is concluded that damage to sperm plasma membrane and leakage of transaminases will be comparatively low if buck semen is frozen @ 25^0C per minute.

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