Research Article

Biochemical Profiles in Freezable and Non-Freezable Ejaculates of Mithun (*Bos frontalis*) Semen

Ponraj Perumal[1], Suresh Kumar Srivastava[2], Kezhavituo Vupru[3], Kobu Khate[3], Muthusamy Veeraselvam[4], Chandan Rajkhowa[5]

1Animal Reproduction Laboratory, ICAR- National Research Centre on Mithun, Jharnapani, Nagaland – 797 106 (India); 2 Animal Reproduction Division, ICAR- Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh – 243 122; 3 Farm Manager, ICAR- National Research Centre on Mithun, Jharnapani, Nagaland – 797 106, India; 4Veterinary University Training and Research Centre, TANUVAS, Salem, India; 5 ICAR- National Research Centre on Mithun, Jharnapani, Nagaland – 797 106 (India).

Abstract | The present study was undertaken to measure the biochemical profiles of freezable and non- freezable ejaculates of Mithun. Fifty ejaculates (twenty five ejaculates each from freezable and non- freezable ones) were collected from matured Mithun bulls. Biochemical profiles viz., alkaline phosphatase (ALP), acid phosphatase (ACP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactic acid dehydrogenase (LDH), calcium (Ca), magnesium (Mg), zinc (Zn), chloride (Cl), citric acid (CA), total seminal plasma protein (TSPP), inorganic phosphorous (IP), fructose and cholesterol concentration in sperm were estimated. The result revealed that these parameters varied significantly (P< 0.05) between the freezable and non-freezable ejaculates. Freezable ejaculates has significantly (P< 0.05) higher ALP, ACP, LDH, Ca, Mg, Zn, citric acid, TSPP, IP, fructose and cholesterol concentration and significantly (P< 0.05) lower Cl concentration than the non- freezable ejaculates. There was a correlation among the biochemicals such as ALP, ACP, LDH, Ca, Mg, Zn, CA, TSPP, IP, fructose, cholesterol and these biochemicals were negatively correlated with AST, ALT and Cl in both freezable and non-freezable ejaculates. It was concluded that most of the biochemical parameters were higher in freezable ejaculates than the non-freezable ejaculates, indicates that freezable ejaculates have has structural stability than the non-freezable ones that leads to freezable sperm has higher functional structures to move faster and in forward direction.

Keywords | Mithun, Biochemical profiles, Freezable and non-freezable ejaculates

Editor | Kuldeep Dhama, Indian Veterinary Research Institute, Uttar Pradesh, India.
Received | October 31, 2014; **Revised** | November 29, 2014; **Accepted** | November 30, 2014; **Published** | December 15, 2014
***Correspondence** | Ponraj Perumal, National Research Centre on Mithun, Jharnapani, Nagaland, India; **Email:** perumalponraj@gmail.com
Citation | Perumal P, Srivastava SK, Vupru K, Khate K, Veeraselvam M, Rajkhowa C (2015). Biochemical profiles in freezable and non-freezable ejaculates of Mithun (*Bos frontalis*) semen. Adv. Anim. Vet. Sci. 3(1): 19-26.
DOI | http://dx.doi.org/10.14737/journal.aavs/2015/3.1.19.26
ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

INTRODUCTION

Mithun (*Bos frontalis*) is a semi-wild, free–range bovine species present in the North-Eastern Hill (NEH) region of India and is believed to have originated more than 8000 years ago from wild Indian gaur (*Bos gaurus)* (Simoons, 1984). It occupies an important place in the socio-cultural and economic life of the tribal population especially in Arunachal Pradesh, Nagaland, Manipur and Mizoram states of India. Mithun population is decreasing (Livestock census of India, 2007) due to various reasons such as

insufficient number of breeding bulls, intensive inbreeding and lack of proper breeding management practices. Dwindling population of Mithun is a cause of concern which requires greater effort from all quarters to conserve the species bearing much effect on the socio-economic status of tribes. In Mithun natural breeding is being followed with accompanied limitations such as cost and disease transmission. Under such circumstances, wider application of artificial breeding of Mithun appears a viable option for conserving the species.

The seminal plasma is a highly complex biological fluid, secreted from various accessory sex glands and testes and contains various biochemical constituents such as cholesterol, sugars, proteins, metabolic, intra cellular and antioxidant enzymes, mineral elements (Kulkarni et al., 1996). The constituents of seminal plasma have various functions such acquisition of motility, capacitation, acrosome reaction and fertilizing capacity of spermatozoa (Kulkarni et al., 1996). Several studies were conducted on composition of seminal plasma in different domestic animal species (Mann, 1964; Dabas et al., 1984; Dhami and Sahni, 1994). Further, perusal of literatures revealed meagre information on the composition of seminal plasma in Mithun Bulls. Therefore, the study was designed to measure the biochemical composition of seminal plasma in Mithun to aid in for future semen preservation.

MATERIALS AND METHODS

Ten apparently healthy Mithun Bulls of 3 to 5yr of age were selected from the herd in Mithun farm, NRC on Mithun, Jharnapani, Nagaland. The average body weight was 501 kg (493 to 507 kg) with good body condition (score 5-6) maintained under uniform feeding (farm schedule) and managemental conditions. A total of 50 ejaculates were collected through rectal massage method. These ejaculates were splited into freezable and non-freezable ejaculates based on the post thaw motility (Rao and Rao, 1996; Prasad et al., 2000). Ejaculates having post thaw motility 40% and above were considered as freezable ejaculates whereas non-freezable ejaculates were considered with a post thaw motility less than 40%.

The biochemical profiles *viz*., ALP, ACP, AST, ALT, LDH activity, Ca, Mg, Zn, Na, K, inorganic phosphorous, chloride, fructose, total seminal plasma protein and total cholesterol of the semen were estimated by commercial available kits (Cayman chemical company, USA).

The results were analysed statistically between the freezable and non-freezable ejaculates with the student 't' test using the SPSS (version 15.0; SPSS, Chicago, IL) and expressed as the mean ± S.E.M. Significant difference values of ($p<0.05$) were considered to be statistically significant after arcsine transformation of percentage data. Correlation among the biochemical parameters was estimated with Pearson's correlation coefficient. Differences at ($p < 0.05$) were considered to be statistically significant.

RESULTS AND DISCUSSION

In the present study, the result revealed that estimated different biochemical profiles varied significantly ($P<0.05$) between the freezable and non-freezable ejaculates (Table 1). Freezable ejaculates has significantly ($P< 0.05$) higher ALP, ACP, LDH, Ca, Mg, Zn, citric acid, TSPP, IP, fructose and cholesterol and significantly lower Cl content than non- freezable ejaculates. There was positive correlation among the biochemicals such as ALP, ACP, LDH, Ca, Mg, Zn, CA, TSPP, IP, fructose, cholesterol and these biochemicals were negatively correlated with AST, ALT and Cl in both freezable (Table 2) and non-freezable (Table 3) ejaculates. The present information may aid in getting good quality semen for artificial insemination by selecting freezable ejaculates based on biochemical constituents of seminal plasma.

Phosphatase enzymes such as ALP and ACP levels in seminal plasma are very important for sperm metabolism as well as sperm functions (Brooks, 1990). Therefore, estimates of these enzymes have been recommended as biomarkers for assessment of semen quality (Pesch et al., 2006). ALP in seminal plasma is primarily of testicular and epididymal origin and can be used as a clinical ejaculatory marker to differentiate azoospermia or oligospermia from ejaculatory failure (Turner and McDonnell, 2003). In the present study, the ALP was significantly decreased in the non- freezable ejaculates than the freezable ejaculates (Pesch et al., 2006). ACP is especially localized in corpus epididymidis, ductus epididymidis and vas deferens, but it is thought to be

Table 1: Mean (±S.E.) biochemical attributes of freezable and non- freezable Mithun semen

Biochemical attributes	Freezable semen (n=25)	Non- freezable semen (n=25)
ALP (KAU/100 mL)	230.66 ± 3.40[a]	180.70 ± 5.22[b]
ACP (KAU/100 mL)	246.75 ± 3.75[a]	189.86 ± 6.17[b]
AST (µ mole/litre)	78.62 ± 1.64[a]	89.08 ± 3.18[b]
ALT (µ mole/litre)	13.19 ± 1.30[a]	17.67 ± 1.37[b]
LDH (IU/Litre)	317.99 ± 3.58[a]	244.11 ± 6.53[b]
Ca (mg/dl)	35.13 ± 2.14[a]	25.41 ± 1.72[b]
Mg (mg/dl)	7.27 ± 0.83[a]	5.41 ± 1.14[b]
Zn (mg/dl)	3.80 ± 0.66[a]	2.46 ± 0.90[b]
Chloride (mmol/L)	320.35 ± 5.17[a]	402.94 ± 7.25[b]
Citric Acid (mg/dl)	552.18 ± 6.78[a]	443.73 ± 7.13[b]
Total Protein (g/dl)	8.22 ± 0.86[a]	6.18 ± 0.78[b]
Inorganic Phosphorous (mg/dl)	9.20 ± 1.12[a]	6.91 ± 0.83[b]
Fructose (mg/dl)	508.41 ± 6.28[a]	424.85 ± 5.89[b]
Total Cholesterol (µg/10^8sperm)	28.55 ± 1.45[a]	22.71 ± 1.44[b]

Means with different superscript within rows differ significantly ($P < 0.05$); n= Number of ejaculates

an indicator for thesecretory function of prostate (Ciereszko et al., 1992).

Like phosphatse, AST and ALT are essential for metabolic processes, which provide energy for motility, viability and fertilizing ability of spermatozoa and these transaminase activities in the ejaculates are good indicators of semen quality as these enzymes measure stability of sperm membrane (Lopez et al., 1989). Increasing abnormal sperm percentage in the ejaculate causes increased concentration of these transaminase enzymes in the extra cellular fluid which might be due to damage of sperm plasma membrane and leakage of enzymes from spermatozoa (Dogan et al., 2009). Moreover, increase in AST and ALT activities of seminal plasma of non- freezable ejaculates could be due to structural instability of the sperm or fragile nature of sperm membrane (Corteel, 1980) and less intactness of membrane of acrosome, plasma, mitochondria and flagella of the sperm.

LDH is an essential enzyme of almost universal distribution in the body which catalyses the reversible transamination of pyruvate to lactate. In semen, it is chiefly located in the mid-piece region (Dhami and Sahni, 1994). Like transaminases, LDH is also an essential enzyme responsible for metabolic processes, which provide energy for viability, motility, capacitation and fertilizing ability of spermatozoa (Sirat et al., 1996). It has been proposed that the seminal fluid LDH can be used as good indicator of sperm viability and membrane stability (Stamatiads et al., 1984). Pesch et al. (2006) reported that there was positive correlation among LDH activities, total sperm motility, progressive motility and liveability of sperm in fresh ejaculates, which may indicates that extracellular LDH ensures metabolism of spermatozoa. In the present study, extracellular LDH concentration was increased significantly ($p< 0.05$) in seminal plasma of freezable ejaculates (Table 1) than the non-freezable ejaculates. It has thus been proposed that higher LDH levels in seminal plasma of fresh semen can be used as a good indicator of higher motility, progressive motility and living sperm of ejaculates (Dube et al., 1982). The Ca, Mg, Zn and IP of ejaculate were significantly higher in freezable ejaculates than the non- freezable ejaculates, whereas chloride content was higher in non-freezable ejaculates in the present study (Table 1). Zn plays an essential role in normal testicular development, spermatogenesis and sperm motility (Wong et al., 2001). It is also a cofactor for a number of metalloenzymes in animals. Zn plays an important

Table 2: Correlation coefficient among the biochemical attributes of Freezable ejaculates in Mithun (*Bos frontalis*) bulls

No	Parameters	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	ALP	1.00	0.88*	-0.46	-0.76*	0.69*	0.77*	0.61	0.72*	-0.46	0.82*	0.86*	0.85*	0.79*	0.69*
2	ACP		1.00	-0.16	-0.86*	0.43	0.69*	0.77*	0.79*	-0.48	0.82*	0.81*	0.80*	0.90*	0.57
3	AST			1.00	0.37	-0.68*	-0.46	-0.23	-0.34	0.11	-0.90*	-0.51	-0.55	-0.10	-0.68*
4	ALT				1.00	-0.47	-0.67*	-0.79*	-0.80*	0.51	-0.91*	-0.81*	-0.76*	-0.95*	-0.57
5	LDH					1.00	0.59	0.44	0.55	-0.24	0.81*	0.74*	0.78*	0.50	0.72*
6	Ca						1.00	0.65*	0.75*	-0.33	0.69*	0.71*	0.75*	0.64*	0.69*
7	Mg							1.00	0.72*	-0.48	0.68*	0.63*	0.72*	0.80*	0.43
8	Zn								1.00	-0.53	0.87*	0.83*	0.87*	0.86*	0.58
9	Cl									1.00	-0.96*	-0.41	-0.42	-0.62*	-0.16
10	CA										1.00	0.95*	0.97*	0.96*	0.93*
11	TP											1.00	0.91*	0.83*	0.79*
12	IP												1.00	0.80*	0.83*
13	FR													1.00	0.51
14	CH														1.00

ALP: alkaline phosphatase, ACP: acid phosphatse, AST: *aspartate aminotransferase, ALT: alanine aminotransferase, LDH:* lactic acid dehydrogenase, Ca: calcium, Mg: magnesium, Zn: zinc, Cl: chloride, CA: citric acid, TP: total protein, IP: inorganic phosphate, FR: fructose, CH: cholesterol in sperm, * Correlation coefficient were significant, $p < 0.05$

Table 3: Correlation coefficient among the biochemical attributes of non- freezable ejaculates in Mithun (*Bos frontalis*) bulls

No	Parameters	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	ALP	1.00	0.98*	-0.82*	-0.91*	0.96*	0.79*	0.95*	0.96*	-0.94*	0.84*	0.96*	0.92*	0.94*	0.82*
2	ACP		1.00	-0.75*	-0.93*	0.92*	0.69*	0.95*	0.97*	-0.96*	0.85*	0.93*	0.87*	0.94*	0.75*
3	AST			1.00	0.70*	-0.88*	-0.83*	-0.71*	-0.74*	0.67*	-0.93*	-0.75*	-0.87*	-0.67*	-0.86*
4	ALT				1.00	-0.86*	-0.63*	-0.92*	-0.95*	0.94*	-0.92*	-0.85*	-0.82*	-0.93*	-0.66*
5	LDH					1.00	0.84*	0.93*	0.91*	-0.87*	0.84*	0.93*	0.96*	0.87*	0.86*
6	Ca						1.00	0.72*	0.69*	-0.59	0.71*	0.81*	0.86*	0.65*	0.91*
7	Mg							1.00	0.96*	-0.95*	0.67*	0.94*	0.87*	0.95*	0.76*
8	Zn								1.00	-0.98*	0.86*	0.92*	0.85*	0.94*	0.75*
9	Cl									1.00	-0.94*	-0.88*	-0.82*	-0.95*	-0.67*
10	CA										1.00	0.96*	0.98*	0.93*	0.84*
11	TP											1.00	0.89*	0.91*	0.86*
12	IP												1.00	0.828	0.85*
13	FR													1.00	0.72*
14	CH														1.00

ALP: alkaline phosphatase, ACP: acid phosphatse, AST: *aspartate aminotransferase, ALT: alanine aminotransferase, LDH:* lactic acid dehydrogenase, Ca: calcium, Mg: magnesium, Zn: zinc, Cl: chloride, CA: citric acid, TP: total protein, IP: inorganic phosphate, FR: fructose, CH: cholesterol in sperm, * Correlation coefficient were significant, $p < 0.05$

role in prostate, epididymal and testicular functions (Ebisch et al., 2003). Zn has been reported to influence the process of spermatogenesis (Wong et al., 2002), controls sperm motility (Wroblewski et al., 2003), stability of sperm membrane (Kendall et al., 2000), preserves the ability of sperm nuclear chromatin to undergo de-condensation and modulates sperm functions (Suruki et al., 1995). Hypozinkemia leads to gonad dysfunction, decreased testicular weight, atrophy of seminiferous tubules and complete cessation of spermatogenesis (Martin et al., 1994).

Calcium and Mg are required in many physiological processes as a regulator in all living cells, including spermatozoa. The presence of Mg^{2+} and Ca^{2+} ions is necessary for the last stage of capacitation following acrosome reaction and hyperactive motility of spermatozoa. Ca flux control through the spermatozoal membrane is essential for fertilization (Bailey and Buhr, 1993) and it was noticed that the inclusion of Ca^{2+} with calcimine to isolated ram caudal spermatozoa caused stimulation of flagellar beat activity (Bradley and Forrester, 1982). But negative correlation between Ca concentration in seminal plasma and motility of bovine spermatozoa was also reported (Machal et al., 2002). In the present study, concentration of Ca and Mg were significantly higher in freezable than the non- freezable ejaculates. Calcium is positively correlated with sperm motility, viability and integrity of plasma and acrosomal membrane as Ca stabilizes the plasma membrane and influences its permeability and excitability. The higher correlation coefficient between seminal plasma Ca concentration and protein suggests that spermatozoon proteins as well as those of seminal plasma participate in extracellular Ca flow activation (Marques et al., 2000). Further, Ca is also required to stimulate the steriodogenesis in Leydig cells of the testis. Deficiency of Ca in the testes or accessory gland will leads to adverse effects on the normal function of the reproductive system and spermatogenesis (Kaplan et al., 1995).

Chloride levels were significantly ($p< 0.05$) higher in the non- freezable ejaculates than the freezable ejaculates (Table 1). The excess chloride could be toxic to bovine sperm which is neutralised by increased sodium levels (Dhami and Sahni, 1994). This could explain the reason for increased chloride in non- freezable ejaculates. In non-freezable ejaculates, seminal plasma fructose and metabolic enzyme concentrations are also low whereas chloride concentration is high (Hirsch et al., 1991). The low protein and high chloride content of the secretion is rather hostile to sperm. It is known that modulation of a variety of ion channels (like Cl) of spermatozoa is a characteristic event associated with capacitation and acrosome reaction of mammalian spermatozoa (Barrier-Battut et al., 2002). Hence, increasing the Cl level in seminal plasma may play a role in infertility.

TSPP in the seminal plasma fluid influence various functions of the sperm such as capacitation, acrosome reaction, motility, DNA integrity and fertilization of the oocyte (Moura et al., 2007). The level of TSPP in fresh semen of freezable ejaculates was significantly higher than the non- freezable ejaculates (Table 1). But some specific proteins might be responsible for the quality and freezability of semen which could not be differentiated between the two groups simply by estimating the total seminal plasma protein. Moreover, no report has so far been available on total seminal plasma protein level of freezable and non-freezable ejaculates in Mithun bull. Dhami and Sahni (1994) reported low mean protein value of static ejaculate than the motile ejaculate which supports the higher numerical value of TSPP in the highly motile samples in the present study. Similarly, Singh et al. (1989) reported a positive association of the protein values in the semen with its freezability. The reason could be the great importance of protein for motility and survival of spermatozoa during storage (Singh et al., 1989; Moura et al., 2007). Seminal plasm proteins are also known to have protective action towards sperm against lipid peroxidation (Schonek et al., 1996). But Mohanty (1999) reported higher TSPP in poor freezable bulls than the good freezable bulls. This variation could be due to different selection criteria of freezable and non- freezable ejaculates, frequency of collection, method employed for estimation, age and number of bulls studied and season (Dhami and Sahni, 1994).

Fructose and citric acid are reported to play important roles in sperm motility and concentration, particularly with regard to energy metabolism, through glucose utilization (Videla et al., 1981). Fructose is one of the major energy yielding nutritive substrates present in seminal fluid (Gonzales et al., 1997). It is secreted from the seminal vesicles and the accessory sex glands. Fructose is the major carbohydrate found in seminal plasma, provides over half the spermatozoa carbohy-

drate consumption and appears essential for normal sperm motility. In the present study, the fructose and citric acid concentration were reduced significantly in non- freezable ejaculates (Table 1) as indicates that the functions of accessory sex glands was affected as seminal fructose is positively correlated with semen volume and sperm motility (Saeed et al., 1994). The determination of fructose itself is of particular significant because there is a direct relationship between the fructose level in seminal plasma and the testosterone function of the interstitial cells of Leydig. Fructose values which fall below normal may be a consequence of inflammatory condition in the prostrate or seminal vesicles or structural abnormality of the seminal vesicles and their ducts (Schirren, 1983).

It was concluded from the study that most of the biochemical parameters were significantly higher in freezable ejaculates in comparison to the non-freezable ejaculates of Mithun. This indicates that freezable sperm has structural stability than the non-freezable sperm that leads to freezable sperm has higher functional sperm structures to move faster and forward direction.

REFERENCES

- Bailey JL, Buhr MM (1993). Cryopreservation alters the Ca^{2+} flux of bovine spermatozoa. Can. J. Anim. Sci. 74(1): 45-51.
- Barrier-Battut I, Delajarraud H, Legrand E, Buyas JF, Fieni F, Tainturier D, Thorin C, Pouliquen H (2002). Calcium, magnesium, copper and zinc in seminal plasma of fertile stallions and their relationship with semen freezability. Theriogenology. 58(2-4): 229-232.
- Bradley MP, Forrester IT (1982). Human and ram seminal plasma both contain a calcium-dependent regulator protein, calsemin. J. Androl. 3(5): 289-296.
- Brooks DE (1990). Biochemistry of the male accessory glands. In: Marshall's physiology of reproduction. (ED.) Lamming, G.E. 4th edn. Edinburgh, Churchill Livingstone. Pp. 569-690.
- Ciereszko A, Glogowski J, Strzezek J, Demianowicz W (1992). Low stability of aspartate aminotransferase activity in boars semen. Theriogenology. 37(6): 1269-1281.
- Corteel JM (1980). Effects du plasma séminal sur la survie et la fertilité des spermatozoids conservés *in vitro*. Reprod. Nutri. Develop. 20(4A): 1111-1123.
- Dabas YPS, Verma MC, Tripathi SS, Saxena VB (1984). Comparative study of biochemical constituents of bull and Murrah buffalo semen. Indian J. Anim. Res. 18(2): 121-123.
- Dhami AJ, Sahni KL (1994). Comparative appraisal of physico-morphological and enzymatic attributes of semen and their interrelationships in ox and buffalo. J. Applied Anim. Res. 5(1): 13-20.
- Dogan I, Polat U, Nur Z (2009). Correlations between seminal plasma enzyme activities and semen parameters in seminal fluid of Arabian horses. Iranian J. Vet. Res. 10(2): 119-124.
- Dube GD, Dwaraknath PK, Vyas KK (1982). Lactic dehydrogenase in relation to semen quality. Indian J. Dairy Sci. 35(1): 80-82.
- Ebisch TMW, Van Heerde WL, Thomos CMG, Vander Put N, Wong WY, Steegers Theunissen RPM (2003). C677T methylene tetrahydrofolate reductase polymorphism interferes with effect of folic acid and zinc sulphate on sperm concentration. Fertil. Steril. 80(5): 1190–1194.
- Gonzales GF, Villena A (1997). Influence of low corrected seminal fructose levels on sperm chromatin stability in semen from men attending an infertility service. Fertil. Steril. 67(4): 763-768.
- Hirsch IH, Jeyendran RS, Sedor J, Rosecrans RR, Staas WE (1991). Biochemical analysis of electroejaculates in spinal cord injured men: comparison to normal ejaculates. J. Uro. 145(1): 73-76.
- Kaplan A, Jack R, Opheim KE, Toivola B, Lyon AW (1995). Mineral and trace elements. In: Clinical chemistry. 4th edn. Baltimore, Williams and Wilkins. Pp. 351-356.
- Kendall NR, McMullan S, Green A, Rodway RG (2000). Effect of zinc, cobalt and selenium soluble glass bolus on trace element status and semen quality of ram lambs. Anim. Reprod. Sci. 62(4): 277–283.
- Kulkarni BA, Dhande SG, Patankar DD (1996). Seminal plasma protein profiles of Holstein Friesian, Jersey and crossbred bulls. Inte. J. Anim. Sci. 11(4): 133-37.
- Lopez ML, Grez P, Gribbel I, Bustos Obregón E (1989). Cytochemical and ultrastructural characteristics of the stallion epididymis (*Equus cabalus*). J. Submicros. Cytol. Pathol. 21(1): 103-120.
- Machal L, Chladek G, Strakova E (2002). Copper, phosphorus and calcium in bovine blood and seminal plasma in relation to semen quality. J. Anim. Feed Sci. 11(3): 425-435.
- Mann T (1964). The biochemistry of semen and of the male reproductive tract. John Wiley and Sons, New York.
- Marques VA, Goulart LR, Silva AEDF (2000). Variations of protein profiles and calcium and phospholipase A2 concentrations in thawed bovine semen and their relation to acrosome reaction. Genet. Mol. Biol. 23(4): 825-829.

- Martin GB, White CL, Markey CM, Blackberry MA (1994). Effect of dietary zinc deficiency on the reproductive system of young male sheep: testicular growth and the secretion of inhibin and testosterone. J. Reprod. Fertil. 101(1): 87–96.
- Mohanty DN (1999). Studies on reproductive soundness, freezability and fertility in CB bulls. Thesis Ph.D. Deemed University, Indian Veterinary Research Institute, Izatnagar, India.
- Moura AA, Chapman DA, Koc H, Killian GC (2007). A comprehensive proteomic analysis of the accessory sex gland fluid from mature Holstein Bulls. Anim. Reprod. Sci. 98(3-4): 169-188.
- Pesch S, Bergmann M, Bostedt H (2006). Determination of some enzymes and macro and microelements in stallion seminal plasma and their correlations to semen quality. Theriogenology. 66(2): 307-313.
- Prasad JK, Kumar S, Mohan G, Shankar U, Agarwal SK (2000). Biochemical studies pertaining to freezability of cross bred bull semen. Indian J. Vet. Res. 8(1): 37-40.
- Rao AVN, Rao YVR (1996). Seminal characteristic and freezability of crossbred bulls. Indian Vet. J. 73(10): 1086-1088.
- Saeed S, Khan FA, Rehman SB, Khan DA, Ahmad M (1994). Biochemical parameters in evaluation of oligospermia. J. Pak. Med. Asso. 44(6): 137-140.
- Schirren C (1983). Textbook of practical Andrology. Schireng, A, G. Hamburg Germany. Pp. 17-31.
- Schonek C, Braun J, Einspanier R (1996). Sperm viability is influenced by the bovine seminal plasma protein aSPF: effects on motility, mitochondrial activity and lipid peroxidation. Theriogenology. 45(3): 633-642.
- Simoons FJ (1984). Gayal or Mithun. In: Evolution of Domesticated Animals. (ED.) Manson, I.L. Longman, London. Pp. 34-36.
- Singh DM, Pangawkar GR, Hundal RS, Chaudhary KC (1989). Studies on certain biochemical constituents of seminal plasma in exotic and crossbred bulls. Indian Vet. J. 66(12): 1112-1115.
- Sirat MP, Sinha AK, Singh BK, Prasad L (1996). Effect of cryoprotectants on release of various enzymes from buck spermatozoa during freezing. Theriogenology. 45(2): 405-416.
- Stamatiads K, Karayanidis A, Tsakalof P (1984). Relationship between LDH activity in seminal plasma and viability of ram spermatozoa. In: The male in farm animal reproduction. (ED.) Courot, M. Martinus- Nijhoff Publishers, Boston. Pp. 257-263.
- Suruki T, Nakajima K, Yamamoto A, Yamanaka H (1995). Metallothionein binding zinc inhibits nuclear chromatin decondensation of human spermatozoa. Andrologia. 27(3): 161-164.
- Turner RM, McDonnell SM (2003). Alkaline phosphatase in stallion semen: characterization and clinical applications. Theriogenology. 60(1): 1-10.
- Videla E, Blanco AM, Galli ME, Fernandez-Collazo E (1981). Human seminal biochemistry: fructose, ascorbic acid, citric acid, acid phosphatase and their relationship with sperm count. Andrologia. 13(3): 212 -214.
- Wong WY, Flik G, Groenen PM, Swinkels DW, Thomas CM, Copius-Peereboom JH, Merkus HM, Steegers-Theunissen RP (2001). The impact of calcium, magnesium, zinc, and copper in blood and seminal plasma on se-men parameters in men. Reprod. Toxicol. 15(2): 131-136.
- Wong WY, Merkus HM, Thomas CM, Menkveld R, Zielthuis GA, Steegers- Theunissen RP (2002). Effect of folic acid and zinc sulphate on male factor sub fertility, a double blind, randomized placed controlled trial. Fertil. Steril. 77(3): 491-498.
- Wroblewski N, Schill WB, Henkel R (2003). Metal chelators change the human sperms motility pattern. Fertil. Steril. 79(Suppl. 3): 1584–1589.