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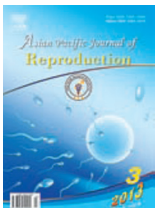

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Effect of foot and mouth disease vaccination on seminal and biochemical profiles of mithun (*Bos frontalis*) semen

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ABSTRACT

Objective: To assess the effect of foot and mouth disease (FMD) vaccination on seminal and biochemical profiles of semen of mithun at pre and post vaccinated stage. **Methods:** The breeding bulls were maintained at Semen Collection Centre, National Research Centre on Mithun, Jharnapani, Nagaland. A total of 160 ejaculates were collected from eight mithun bulls twice a week at about 4 weeks in pre vaccinated stage and 12 weeks post vaccinated stage to know the effect of vaccine stress on seminal and biochemical profiles of mithun semen. The vaccine was given at the end of 4th week of experimental period and semen was collected and evaluated upto the 16th weeks of experimental period. **Results:** It revealed that FMD vaccination affected the sperm functional and biochemical parameters significantly ($P<0.05$) upto 10th weeks of vaccination. But the animal recovered slowly in both physical health and spermiogram. **Conclusions:** The adverse effect of vaccination on seminal parameters suggest that the semen collection and preservation should be suspended till 10th weeks of vaccination to get normal fertility of sperm to avoid the failure of conception from artificial insemination using such semen in this precious species.

1. Introduction

Mithun (*Bos frontalis*), a unique free-range bovine species available in the North Eastern Hilly (NEH) region of India, is considered to be a descendents from wild Indian gaur [1]. This animal is well adopted at an altitude ranged from 300-3 000 mean sea level. Mithun is not yet endangered but are subject to severe non-cyclical population fluctuations on a local/regional basis. It also plays an important role in the socio-economic life of its rearers [2]. The recent initiatives to popularize this species as an economic beef animal demand its rearing under semi-intensive system and adoption of controlled breeding programme. In this context, it is necessary to standardize an effective semen preservation protocol for this species to adopt artificial insemination (AI) for breed improvement programme.

In India, like other bovines species mithun reared in semi-intensive system suffers from various infectious diseases like tuberculosis, para-tuberculosis, brucellosis, foot and mouth disease (FMD), infectious bovine rhinotracheitis (IBR) and bovine viral diarrhoea (BVD) [3], which has tremendous detrimental effect on profitable mithun husbandry practices. But mithun was affected severely with foot and mouth disease [4, 5] with strain of Pan Asia I [6], O strain [7] and type Asia 1 strain [5] than cattle in this region of India. Frequent outbreak of FMD is very common in this region [5.8].

The mithun breeding bulls are vaccinated against foot and mouth disease as a regular prophylactic measure. There are various reports on the effect of vaccination on the semen quality in cattle and buffalo [9-14]. But no proper report in mithun species. However, the available reports are conflicting about the quality of semen due to vaccination in cattle. Some of them reported, it does not significant effect on semen quality [12], whereas others found increased incidence in sperm abnormalities [11]. The semen quality may be affected by vaccination due to vaccine stress and anaphylactic shock [13] resulting increased temperature of the body as well as testes. As the spermatogonial stem cells [15] and fully formed spermatozoa [16] are temperature sensitive, the motility and livability of spermatozoa are proportionally decreased and total sperm abnormality is increased especially head abnormality [17]. But the recovery of quality of the semen is depending upon the nature and

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duration of the thermal insult.

All stages of spermatogenesis are susceptible, the severity of damage being related to the degree and duration of the increased temperature [18]. The semen quality and sperm morphology has returned to normal after some time but their utilization may result in decreased fertilization rates and an increased incidence of embryonic death [19] and repeat breeding. The main reason behind this poor quality of semen is due to the vaccine stress, affects the function of the epididymis as the epididymis is an organ of sperm maturity and reservoir. This leads to increased number of abnormal sperm and reduced fertility rate [20, 21]. There is subsequent decline in epididymal sperm reserves [22], thus concentration decreases as the resorption of abnormal sperm increase. But there was no report of vaccination on semen quality and biochemical profiles in mithun species. Therefore, the present study was designed to assess the effects of FMD vaccination on seminal parameters and biochemical profiles in mithun at pre vaccinated and post vaccinated stages.

2. Materials and methods

2.1. Animals and semen collection

Eight apparently healthy mithun bulls, approximately 4 to 6 yr of age, were selected from the herd derived from various hilly tracts of the NEH region of India. The average body weight of the bulls was 501 kg (493 to 507 kg) at 4 yr, which increased to 530 kg (523 to 538 kg) at 6 yr of age with good body condition (score 5-6) maintained under uniform feeding, housing and lighting conditions. Each experimental animal was daily offered *ad libitum* drinking water, 30 kg mixed jungle forages (18.4% dry matter and 10.2% crude protein) and 4 kg concentrates (87.1% dry matter and 14.5% crude protein) fortified with mineral mixture and salt. Semen was collected through per rectal massage method twice a week before and after vaccination. The vaccine was given at the end of 4th week and the semen was collected and evaluated before and after vaccination upto 16th week of experimental period to study the effect of vaccination stress, if any. Briefly, seminal vesicles were massaged centrally and backwardly resulted into erection and ejaculation. FMD vaccine-Raksha-Ovac (trivalent, containing virus types O, A, and Asia 1- strains, Indian Immunological Ltd., India) was administered at 2.0 mL by deep intramuscular injection route. These ejaculates were subjected to evaluation for volume, colour, mass activity, initial motility, concentration by haemocytometer method [23], livability by eosine-nigrosine stain [24], acrosomal integrity by giemsa stain [25] and plasma membrane integrity by hypo-osmotic swelling test (HOST) [26] as per the standard procedure with Nikon, Eclipse 80i microscope and the biochemical profiles such as aspartate amino transaminase (AST), alanine amino transaminase (ALT), alkaline phosphatase (AKP), acid phosphatase (ACP), fructose, total protein and total cholesterol were estimated with commercially available diagnostic kits. During the study, all the experimental protocols met the Institute Animal Care and Use Committee regulations.

2.2. Statistical analyses

The results were analysed statistically and expressed as the mean ± S.E.M in graphical form from 1st to 16th week of the experiment. Means were analyzed by student *t* test between the post vaccinated and pre-vaccination stages, followed by the Tukey's post hoc test to determine significant differences between the two stages using the SPSS/PC computer program (version 15.0; SPSS, Chicago, IL). Differences with values of $P<0.05$ were considered to be statistically significant after arcsine transformation of percentage data by using SPSS 15 (SPSS, Chicago, IL, USA).

3. Results

The effects of FMD vaccination on various seminal parameters, biochemical and enzymatic profiles were studied at weekly interval 4 weeks before vaccination and 12 weeks after vaccination in mithun bulls. Moreover the protrusion, ejaculation time and length of penis at the time ejaculation were also being studied in both pre vaccinated and post vaccinated mithun bulls at weekly intervals. Results revealed that FMD vaccination in mithun bulls lead to significantly ($P<0.05$) increased protrusion and ejaculation time (Figure 1) whereas the length of penis was decreased (Figure 2) from 2nd week to 10th week of vaccination. Similarly the volume (Figure 3), mass activity (Figure 3), individual motility (Figure 4), live sperm (Figure 4), sperm concentration (Figure 5), acrosomal integrity and plasma membrane integrity (Figure 4) were decreased significantly ($P<0.05$) in the post-vaccinated mithun bulls. But the seminal parameters were significantly reduced from 6th week to 12th week of the experimental period (2nd week to 8th week of the vaccination). In the sperm abnormalities, detached sperm head and proximal cytoplasmic droplets were more than other abnormalities and total sperm abnormalities (Figure 2) were significantly ($P<0.05$) higher in post vaccinated than pre- vaccinated mithun bulls. Colour of semen revealed that in vaccinated bull, the semen was watery due to less number of sperm in the ejaculate [27] as compared to pre-vaccinated bull as creamy white [28]. ALP and ACP concentration were decreased (Figure 5) whereas AST (Figrue 4) and ALT (Figrue 2) level were increased significantly ($P<0.05$) in semen collected from vaccinated mithun bulls. Substances such as fructose (Figure 5), total cholesterol (Figrue 1) and total protein (Figrue 2) were decreased significantly ($P<0.05$) in vaccinated animal than in the pre vaccinated animals (Figure 6). The rectal temperature revealed that it increased significantly in 1st five weeks of vaccination then it reduced gradually.

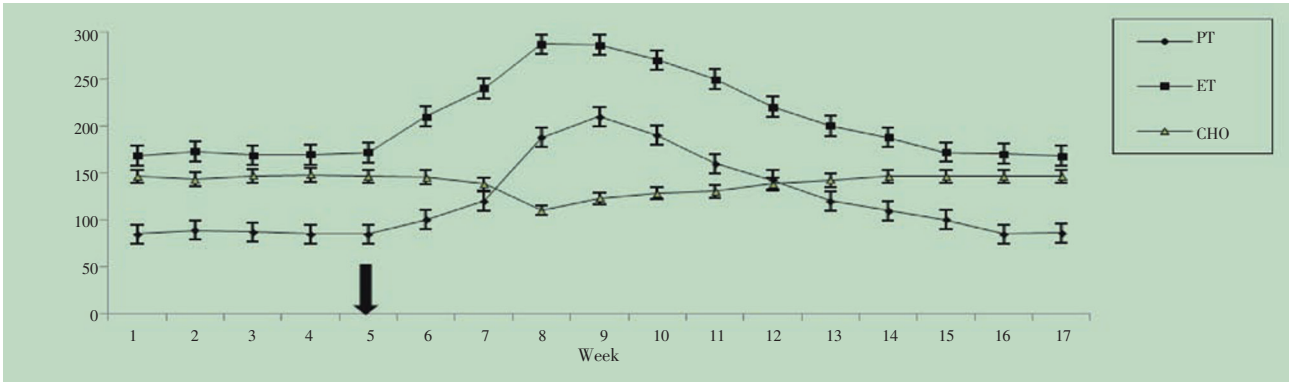

Figure 1. Effect of foot and mouth vaccination on protrusion time (PT) of penis and ejaculation time (ET) of semen and cholesterol (CHO) concentration in seminal plasma at pre and post vaccination stage in mithun bulls (Arrow indicates time of vaccination).

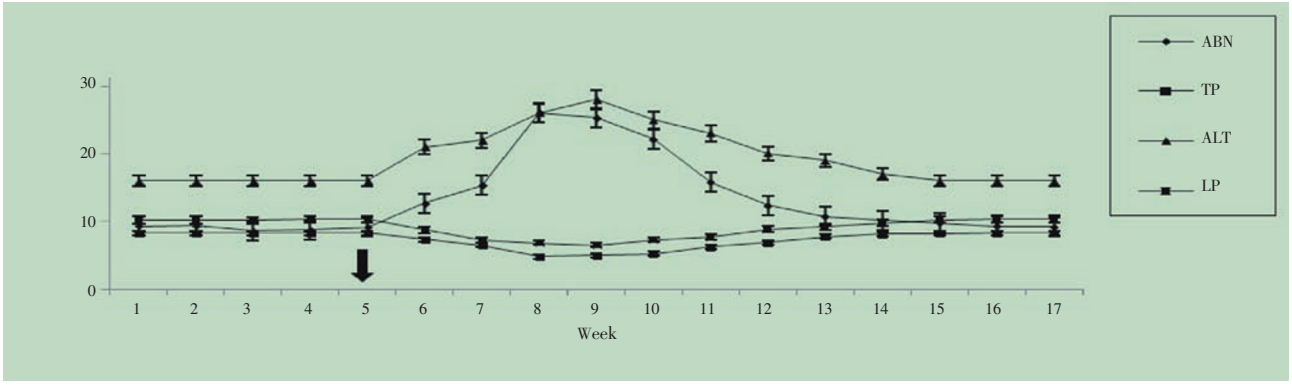

Figure 2. Effect of foot and mouth vaccination on total sperm abnormality (ABN), total protein (TP), alanine amino transaminase (ALT) concentration in seminal plasma and length of penis at the time ejaculation in mithun at pre and post vaccination stage (Arrow indicates time of vaccination).

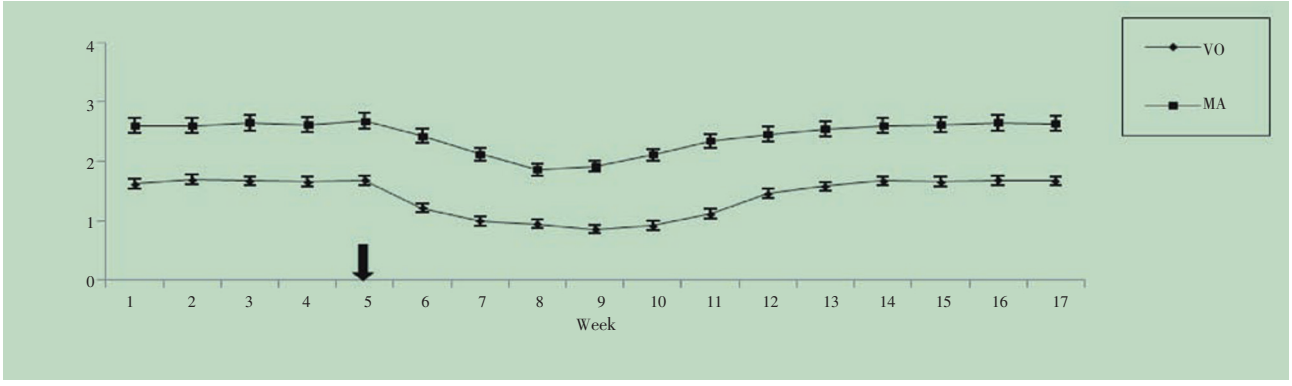

Figure 3. Effect of foot and mouth vaccination on volume (VO) and mass activity (MA) of mithun semen at pre and post vaccination stage (Arrow indicates time of vaccination).

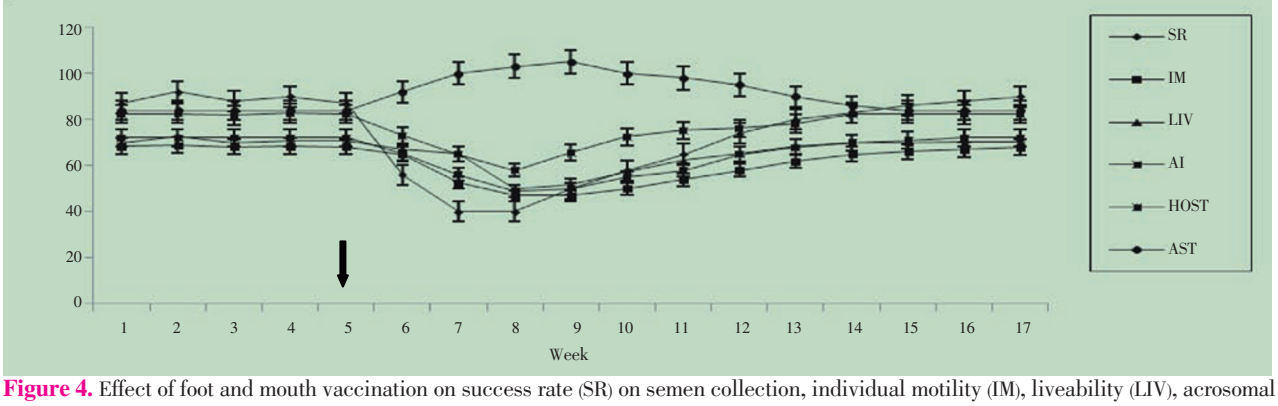

Figure 4. Effect of foot and mouth vaccination on success rate (SR) on semen collection, individual motility (IM), liveability (LIV), acrosomal integrity (AI), hypoosmotic swelling positive sperm (HOST) and aspartate amino transaminase (AST) profiles in mithun semen at pre and post vaccination stage (Arrow indicates time of vaccination).

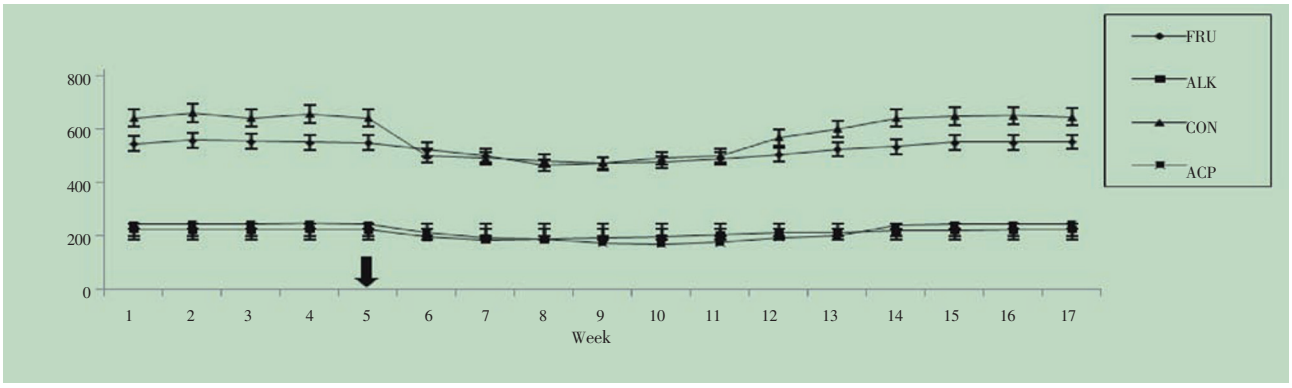

Figure 5. Effect of foot and mouth vaccination on fructose (FRU), alkaline phosphatase (ALP), acid phosphatase (ACP) and sperm concentration in mithun semen at pre and post vaccination stage (Arrow indicates time of vaccination).

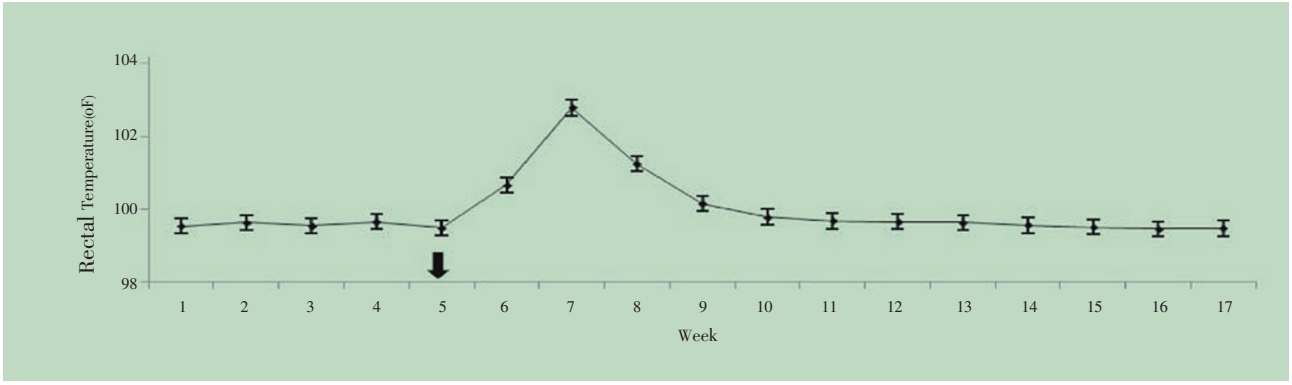

Figure 6. Effect of foot and mouth vaccination on rectal temperature of mithun at pre and post vaccination stage (Arrow indicates time of vaccination).

4. Discussion

In the present study, the results revealed that vaccination of mithun bulls has affected the seminal parameters, enzymatic and biochemical profiles of mithun semen and thus it affects the structures and functions of spermatozoa effectively. Thus, the semen from vaccinated animals is not suitable to preserve for artificial insemination.

There was no report on effect of vaccination on seminal parameters in mithun and to the best of our knowledge this is the first report of the effect of vaccination on seminal parameters, enzymatic and biochemical profiles in mithun semen. Analysis of various seminal parameters such as forward progressive motility, livability, acrosomal and plasma membrane integrity are important for extensive utilization of semen in artificial insemination. In the present study, FMD vaccination on these parameters revealed significant difference between the pre vaccinated and post vaccinated stages. The harmful effects of vaccination in semen preservation are due to it increases the body temperature in general, testes and accessory glands in specifically.

The protrusion and ejaculation time were increased in vaccinated animal as these depend upon the secretion of oxytocin and testosterone. The vaccination causes thermal stress to the animal and also affect the leydig cell function [10] as chronic heat treatment has to reduce testosterone concentration and spermatid concentration while the integrity of sertoli cells become compromised, which has been found to be followed by poor semen quality and a concomitant drop in motility [29]. The leydig cell function can be measured indirectly by measurement of accessory gland secretions such as fructose, ALP and ACP, etc. as these secretion depend on the testosterone. But overall vaccination stress on animals prevents proper erection of penis and ejaculation of semen. Meanwhile stress on animal in the form restraining and pressure on the reproductive tract make more stress to the animal. So that the success rate and length of penis at the time ejaculation were reduced in post vaccinated animals. Volume of semen was decreased in post vaccinated stage as compared to the pre vaccinated stage which indicates it affects the functions of accessory sex glands in mithun bulls. But some workers have reported that vaccination has increased the semen volume in cattle [15] and buffaloes [14]. Whereas other workers reported it does not affect the semen volume [9, 10, 14, 30]. The major portion in the semen is the seminal plasma, which is contributed by the accessory sex glands [31]. This may be due to the secondary activities of accessory sex glands affected following vaccination might be a possible cause for significantly decrease the ejaculate volume following vaccination.

Sperm mass activity, individual motility, livability, plasma and acrosomal integrity were decreased and total sperm

abnormalities increased significantly after vaccination was in agreement with previous report in cattle [9, 10, 11, 15, 30] and buffaloes [10, 14]. The motility of sperm cell develops during their passage through the epididymis [32]. Anaphylactic stress of vaccination, as depicted by the significant rise in body and testes temperature, causes derangement in epididymal functions and spermatogenesis [15] and could lead to vaccination-mediated declined sperm motility and with similar feature of testicular hypoplasia and degeneration [33]. Temperature could give rise to secondary abnormalities as well [34] with increase in sperm tail and mid-piece abnormalities as in testicular degeneration or partial hypoplasia of testes [35]. Rao [20] had reported low sperm motility associated with high incidence of sperm tail defects as a result of epididymal dysfunction and poor handling in the laboratory.

Adverse effect of vaccination on sperm concentration has been reported earlier in cattle [9, 15, 30], although it has not been established unanimously [11, 14]. The decreased sperm concentration might be attributed to the adverse effects of therapeutic agents on germinal cells resulting into increase in dead spermatozoa, which are subsequently phagocytosed by leucocytes [30]. In mithun bulls, FMD vaccination has deleterious effect in terms of total motile sperm per ejaculate.

The present findings in the acrosomal integrity are similar to the earlier reports by Saxena and Tripathi [37] and Gowda [38], who reported decrease in the intact acrosome percentage after vaccination. The acrosome is most sensitive, so the adverse effects were more pronounced on the acrosome after vaccination. The acrosome was either detached or broken, and this subsequently causes release of enzymes and affects the fertilizing capacity of spermatozoa. Bane and Nicander [39] reported that abnormalities develop during transformation of spermatids through uncontrolled growth of the acrosomal system. They further reported that abnormal acrosomal development could be found in case of testicular degeneration, which is temporarily caused by vaccination stress. Rathore [40] reported that in rams, following exposure to an artificially produced hot environment, acrosomal abnormalities were observed after 9 to 10 days, which suggests that the changes occurred in the epididymis during sperm maturation.

Plasma membrane integrity (HOST) has significantly ($P<0.05$) decreased after vaccination. There was a decrease 3 days post-vaccination and a return to pre-vaccination levels after 8 weeks. Similar results were reported by Singh *et al*. [41] in buffalo bull semen following vaccination. Since, HOST reflects the biochemical integrity of sperm plasma membrane, and it is involved in the process of capacitation, acrosome reaction and ultimately binding of spermatozoa to the oocyte [42]. So, this test is able to assess the fertilizing ability of spermatozoa, the ultimate aim of which is to reduce the incidence of repeat breeding. Antoine and Pattabiraman [43] reported decrease in HOS reacting spermatozoa following scrotal insulation in bucks due to rise in testicular temperature. Similarly, in a study on bulls, HOS test reacting spermatozoa were reduced after heat treatment [44]. Thus it can be proposed that vaccination affects the HOS percentage by affecting the biochemical integrity of the sperm plasma membrane.

Sperm morphology usually returns to pre-vaccination values within approximately 8 weeks of the thermal insult [45, 46]. However, a prolonged and (or) severe increase in testicular temperature will increase the interval for recovery. It appears that the decrease in semen quality associated with increased testicular temperature is ultimately related to the severity and the duration of the increased testicular temperature.

The enzyme such as AST, ALT, LDH, cholinesterases and ALP or ACP, etc [31], proteolytic enzymes, phospholipases, transaminases like AST, ALT, ACP and ALP, ATPase, glycosidase, dehydrogenases, nucleotidases, DNases, hyaluronidase [47] levels in seminal plasma are very important for sperm metabolism as well as sperm functions [48]. Therefore, estimates of these enzymes have been recommended as markers for semen quality since they indicate sperm damage [49]. AKP 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 [50]. In the present study, the AKP were significantly decreased in the semen from vaccinated than pre vaccinated mithun bulls as this enzyme have high positive correlation with semen quality, antioxidant content in the seminal plasma and negative correlation with ROS and free radical stress [51]. ACP is especially localized in corpus epididymidis, ductus epididymidis and vas deferens, but it is thought to be an indicator for the secretory function of prostate [52]. The ACP concentration was also lower in vaccinated group than pre vaccinated because it has a positive correlation with semen concentration [53].

Likewise, AST and ALT are essential for metabolic processes which provide energy for survival, motility and fertility of spermatozoa and these transaminase activities in semen are good indicators of semen quality because they measure sperm membrane stability [54]. Thus, increasing the percentage of abnormal spermatozoa in ejaculate causes high concentration of transaminase enzyme in the extra cellular fluid due to sperm membrane damage and ease of leakage of enzymes from spermatozoa [55]. Moreover, increase in AST and ALT activities of seminal plasma and semen in post vaccinated stage may be due to structural instability of the sperm [56] or fragile nature of sperm membrane. In the present study, AST and ALT levels were higher in vaccinated animal as it destabilise the membrane integrity of acrosome, plasma, mitochondria and flagella of the sperm.

Fructose is reported to play important roles in sperm motility and concentration, particularly with regard to energy metabolism, through glucose utilization [57]. Fructose is one of the major energy yielding nutritive substrates present in seminal fluid [58]. Fructose is secreted from the seminal vesicles and the accessory sex glands. It is the major carbohydrate found in seminal plasma, provides over half the spermatozoa carbohydrate consumption and appears essential for normal sperm motility. In the present study, the fructose concentration was reduced significantly in vaccinated animal as indicates the vaccination affects

the accessory sex glands as seminal fructose is positively correlated with semen volume and sperm motility [59]. The determination of fructose itself is of particular significant because there is a direct relationship between the fructose level in sperm 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 [60].

Along with phospholipids, cholesterol is necessary for cell physical integrity and ensures fluidity of the cell membrane. Cholesterol plays a special role in the sperm membrane because its release from the sperm membrane initiates the key step in the process of capacitation and acrosome reaction that is crucial for fertilization [18]. These changes imply cholesterol leaving the sperm membrane and its binding to protein in seminal plasma and female reproductive organs [61]. Moreover, adding cholesterol to diluents prior to defreezing increases sperm resistance to stress caused by the freezing-defreezing procedures, preserving sperm motility and fertilization potential [62]. In the present study, the cholesterol concentration was reduced in vaccinated group as low concentration of cholesterol was present in poor quality semen [42]. According to Komarek *et al*. [63] cholesterol is the second largest lipid class present in the spermatozoa, and it is probably associated with sperm membrane and its function. Factors responsible for sperm metabolism and sperm survival have been associated with the cholesterol content in the semen. For determining the semen quality and fertility levels of the bulls, cholesterol content has been reported to serve as one of the biochemical tests.

From the above discussions, it is clear that the application of FMD vaccine has an adverse effect. Most of the seminal attributes have been affected adversely in mithun bulls. Febrile reaction due to vaccination results in testicular degeneration although of very mild nature. The adverse effect of vaccination on seminal parameters suggest that the semen collection and preservation should be suspended till 10th weeks of vaccination to get normal fertility of sperm to avoid the failure of conception from artificial insemination using such semen in this precious species.

Conflict of interest statement

The authors declare that they have no conflicts of interest

References

[1] Simoons FJ. Gayal or mithun. In: Manson IL. (ed.) *Evolution of domesticated animals*. London: Longman; 1984, p. 34–36.

[2] Mondal SK, Pal DT. Mithun: historical perspective. *Asian Agri-History* 1999; **3**: 245–260.

[3] Rajkhowa S, Rajkhowa C, Bujarbaruah KM. Diseases of mithun (*Bos frontalis*) a revised. *Vet Bulletin* 2003; **73**: IR–6R.

[4] Dutta PK, Mahanta PN, Chakrabarty AK. Foot-and-mouth disease in mithun (*Bos gaurus*). *Vet Rec* 1979; **104**: 146.

[5] Verma ND, Sarma DK. Note on foot-and-mouth disease in mithun in Arunachal Pradesh. *Indian J Virol* 1997; **13**: 75–76.

[6] OIE/FAO FMD Reference Laboratory. Serotype O virus lineages circulating in India during 2008-2010. *Ann Rep* 2010; **10**: 23.

[7] Hegde R, Gomes AR, Giridhar P, Venkatesh MD, Renukaprasad C. A note on FMD in Mithun (*Bos frontalis*) and Black buck (*Antelope cervicapra*). *Zoo's Print* 2011; **26** (6): 19.

[8] Barman NN, Sarma DK, Das S, Patgiri GP. Foot-and-mouth disease in wild and semi-domesticated animals of the north-eastern states of India. *Indian J Anim Sci* 1999; **69**: 781-783.

[9] Bhakat M, Mohanty TK, Gupta AK, Raina VS, Mondal G, Khan HM. Effect of FMD vaccination on various semen characteristics of Sahiwal bulls. *Pakistan J Agric Sci* 2008; **45**: 327-332.

[10] Bhakat M, Mohanty TK, Gupta AK, Raina VS, Brahma B, Mahapatra RK, *et al*. Effect of FMD vaccination on semen quality parameters in Karan Fries and *Murrah buffalo* bulls. *Trop Anim Health Prod* 2010; **42**: 1363–1366.

[11] Kammar NF, Gangadhar KS. Effect of foot-and-mouth vaccination on reaction time and some seminal characteristics in Surti bulls. *Indian J Anim Reprod* 1998; **19**: 149- 150.

[12] Mangurkar BR, Phadnis YP, Bhosrekar MR. Effect of foot and mouth disease vaccination on semen characteristics of exotic and cross-bred bulls. *Indian J Anim Reprod* 2000; **21**: 135-137.

[13] Murugavel K, Veerapandian C, Subramanian A. Effect of black quarter vaccination on semen quality in Murrah bulls. *Indian J Anim Sci* 1997; **67**: 597 -598.

[14] Pankaj PK, Raina VS, Roy B, Mohanty TK, Mishra A. Studies on effect of vaccination on seminal characteristics of riverine buffaloes (*Bubalus bubalis*). *Buffalo Bulletin* 2007; **26**: 91-103.

[15] Venkatareddy J, Venkatamunichetty A, Ramachandran SV, Sreeraman V. Effect of foot and mouth disease vaccination on semen quality. *Indian J Anim Reprod* 1991; **12**: 13-14.

[16] Anderson J. *The semen of animals and its use for artificial insemination*. India: Green World Publishers; 2001.

[17] Barth AD, Oko RJ. *Abnormal morphology of bovine spermatozoa*. Ames: Iowa State University Press; 1989.

[18] Witte TS, Schäfer-Somi S. Involvement of cholesterol, calcium and progesterone in the induction of capacitation and acrosome reaction of mammalian spermatozoa. *Anim Reprod Sci* 2007; **102**: 181-193.

[19] Burfening PJ, Ulberg LC. Embryonic survival subsequent to culture of rabbit spermatozoa at 38 ℃ and 40 ℃. *J Reprod Ferti* 1968; **15**: 87-92.

[20] Rao AR. Sperm akinesia in a hollikar bull. *Indian Vet J* 1976; **53**: 414-418.

[21] Rao VJ, Venkataswami V. Histo-pathology of testicular tissue of crossbred bulls in foot and mouth disease vaccination. *Indian Vet J* 1974; **48**: 1101.

[22] Rao AR, Bane A, Gustafasson BK. Changes in the morphology of spermatozoa during their passage through the genital tract in dairy bulls with normal and impaired spermatogenesis. *Theriogenology* 1980; **14**: 10-12.

[23] Tomar NS. *Artificial insemination and reproduction of cattle and buffalos*. Allahabad: Sarojprakashan; 1997.

[24] Robeck TR, O'Brien JK. Effect of cryopreservation methods and

precryopreservation storage on bottlenose dolphin (*Tursiops truncatus*) sperms. *Biol Rep* 2004; **70**: 1340–1348.

[25]Watson PF. Use of giemsa stain to detect change in acrosome of frozen ram spermatozoa. *Vet Rec* 1975; **97**: 12–15.

[26]Buckett WM, Luckas MJ, Aird IA, Farquharson RG, Kingsland CR, Lewis-Jones DI. The hypo-osmotic swelling test in recurrent miscarriage. *Fertil Steril* 1997; **68**: 506 –509.

[27]Hafez ESE. *Reproduction in farm animals*. 6th ed. Philadelphia: Lea and Febiger; 1993, p. 405-439.

[28]Karunakaran M, Dhali A, Mech A, Khate K, Rajkhowa C, Mishra DP. Preservation of mithun (*Bos frontalis*) semen at refrigeration temperature. *Anim Reprod Sci* 2007; **101**: 257–264.

[29]Egbounlike GN, Tongun VA, Agiang EA. Sperm production in ruminants in hot humid climate. *World Rev of Anim Prod* 1985; **21**: 11-17.

[30]Singh R, Verma HK, Kumar S. Effect of the foot and mouth disease vaccination on the semen quality of buffalo bulls. *Ind J Anim Sci* 2003; **73**: 1319-1323.

[31]Roberts SJ. *Veterinary obstetrics and genital diseases*. 2nd ed. New Delhi: CBS Publisher and Distributors; 1971.

[32]Moulikrishnan K, Ramamohan Rao A. Epididymal dysfunction in buffalo bulls. *Ind Vet J* 1986; **63**: 1013-1016.

[33]Arthur GH, Noakes DE, Pearson H. *Veterinary reproduction and obstetrics*. 6th ed. London: Bailliere Tindal; 1989, p. 509-584.

[34]Venkataswami V, Rao VJ. Preliminary report on the effect of foot and mouth disease vaccination on semen quality of crossbred bulls. *Ind Vet J* 1970; **47**: 23-29.

[35]Sullivan JJ. Morphology and motility of spermatozoa. In: Salisbury GW, Van Demark NL, Lodge JR. (eds.) *Physiology of reproduction and artificial insemination of cattle*. 2nd ed. San Francisco: W.H. Free man; 1978, p. 286-328.

[36]Mann T, Mann CL. *Male reproductive function and semen*. Heidelberg, New York: Springer- Verlag Berlin; 1981.

[37]Saxena VB, Tripathi SS. Effect of foot and mouth disease vaccination on semen quantity, quality and preservability in Jersey bulls. *Indian Vet J* 1977; **54**: 959-964.

[38]Gowda BK. Effect of foot and mouth disease vaccination on pre- and post-freezing semen parameters. MVSc Thesis, University of Agricultural Science, Bangalore, India; 1993.

[39]Bane A, Nicander L. Development of sperm abnormalities. *J Reprod Fertil* 1966; **11**: 133.

[40]Rathore AK. Acrosomal abnormalities in ram spermatozoa due to heat stress. *Brit Vet J* 1970; **126**: 440-443.

[41]Singh R, Verma HK, Kumar S, Matharoo JS. Effect of foot and mouth disease vaccination on the reproductive capacity of the buffalo bulls. *Ind J Anim Reprod* 2004; **25**: 45-47.

[42]Argov N, Sklan D, Zeron Y, Roth Z. Association between seasonal changes in fatty-acid composition, expression of VLDL receptor and bovine sperm quality. *Theriogenology* 2007; **67**: 878-885.

[43]Antoine D, Pattabiraman SR. Spermatozoa response to hypo-osmotic swelling and freezability tests after testicular degeneration in bucks. *Ind J Anim Reprod* 1999; **20**: 54-56.

[44]Sivaramalingam K. Studies on hypo-osmotic swelling test (HOST) for evaluation of bull spermatozoa. MVSc Thesis, Madras Veterinary College, Madras. India. 1994.

[45]Kastelic JP, Cook RB, Coulter GH, Saacke RG. Insulating the scrotal neck affects semen quality and scrotal/testicular temperatures in the bull. *Theriogenology* 1996; **45**: 935-942.

[46]Wildeus S, Entwistle KW. Spermiogram and sperm reserves in hybrid *Bos indicus* X *Bos taurus* bulls after scrotal insulation. *J Reprod Fertil* 1983; **69**: 711-716.

[47]Bhosrekar MR. *Semen production and artificial insemination*. 1st ed. India: BAIF Development Foundation; 1990, p. 4-205.

[48]Brooks DE. Biochemistry of the male accessory glands. In: Lamming GE. (ed.) *Marshall's physiology of reproduction*. 4th ed. Edinburgh: Churchill Livingstone; 1990, p. 569-690.

[49]Pesch S, Bergmann M, Bostedt H. Determination of some enzymes and macro and microelements in stallion seminal plasma and their correlations to semen quality. *Theriogenology* 2006; **66**: 307-313.

[50]Turner RM, McDonnell SM. Alkaline phosphatase in stallion semen: characterization and clinical applications. *Theriogenology* 2003; **60**: 1-10.

[51]Ciereszko A, Glogowski J, Strzezek J, Demianowicz W. Low stability of aspartate aminotransferase activity in boars semen. *Theriogenology* 1992; **37**: 1269-1281.

[52]López ML, Grez P, Gribbel I, Bustos Obregón E. Cytochemical and ultrastructural characteristics of the stallion epididymis (*Equus cabalus*). *J Submicrosc Cytol Pathol* 1989; **21**: 103-120.

[53]Dogan I, Polat U, Nur Z. Correlations between seminal plasma enzyme activities and semen parameters in seminal fluid of Arabian horses. *Iranian J Vet Res* 2009; **10**: 119-124.

[54]Corteel JM. Effects du plasma séminal sur la survie et la fertilité des spermatozoids conservés *in vitro*. *Reprod Nutr Dev* 1980; **20**: 1111-1123.

[55]Gundogan M. Some reproductive parameters and seminal plasma constituents in relation to season in Akkaraman and Awassi Rams. *Tur J Vet Anim Sci* 2006; **30**: 95-100.

[56]Buckland RB. The activity of six enzymes of chicken seminal plasma and sperm. 1. Effect of *in vitro* storage and full sib families on enzyme activity and fertility. *Poult Sci* 1971; **50**: 1724-1734.

[57]Videla E, Blanco AM, Galli ME, Fernandez-Collazo E. Human seminal biochemistry: fructose, ascorbic acid, citric acid, acid phosphatase and their relationship with sperm count. *Andrologia* 1981; **13**: 212 -214.

[58]Gonzales GF, Villena A. Influence of low corrected seminal fructose levels on sperm chromatin stability in semen from men attending an infertility service. *Fertil Steril* 1997; **67**: 763–768.

[59]Saeed S, Khan FA, Rehman SB, Khan DA, Ahmad M. Biochemical parameters in evaluation of oligospermia. *J Pak Med Assoc* 1994; **44**: 137–140.

[60]Schirren C. Schireng AG.(ed.) *Textbook of practical andrology*. Germany: Hamburg; 1983, p. 17–31.

[61]Therien I, Moreau R, Manjunath P. Major proteins of bovine seminal plasma and high density lipoproteins induce cholesterol efflux from epididymal sperm. *Biol Reprod* 1998; **59**: 768-776.

[63]Komarek RJ, Pickett BW, Lanz RN, Jensen RG. Lipid composition of bovine spermatozoa and seminal plasma. *J Dairy Sci* 1964; **47**: 531-534.

[68]Moore AI, Squires EL, Graham JK. Adding cholesterol to the stallion sperm plasma membrane improves cryosurvival. *Cryobiology* 2005; **51**: 241-249.