SEASONAL VARIATION IN SEMINAL BIOCHEMICAL CONSTITUENTS IN SURTI BUFFALO BULLS

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ABSTRACT

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This investigation was carried out using 60 semen ejaculates (10 ejaculates/bull/season) obtained from 2 sexually mature healthy Surti buffalo bulls over one year period. The bulls were managed identically and were under weekly twice semen collection schedule in AV. The year was divided in to three seasons (monsoon, winter and summer).Each ejaculate was divided into two aliquots; one was assessed for physico-morphological and functional attributes, while other was used for evaluation of seminal biochemical and enzymatic profile. The overall mean initial seminal fructose content was 586.61±65.77 mg/dl. The average seminal plasma contents of total protein, total cholesterol and enzyme ALP, ACP, AST andALT activity recorded were2.54 ± 0.51 g/dl, 53.71±10.05 mg/dl, 53.71±10.05 KAU/dl, 246.78 ± 27.92 KAU/dl, 57.30 ± 10.73 IU/L and 15.38 ± 5.75 IU/L, respectively. Seasonal influence was highly significant (P<0.01) for initial fructose and all four enzymes. The lowest values for fructose, protein, ALP and ALT were observed during summer and for cholesterol, ACP and ALT during monsoon. The bulls varied significantly (P<0.01) only in their seminal fructose, protein and ALP. Among the seminal profile, significant correlations were observed for fructose with AST (-0.43), ALT (0.36) and ACP (-0.38); total protein with AST (-0.76); cholesterol with ACP (0.27); AST with ALT (-0.43), ALP (-0.44) and ACP (0.46); ALT with ALP (0.42), and ALP with ACP (-0.41). Further, significant correlations were found for ejaculate volume with seminal fructose (0.46) and ACP (-0.35);sperm concentration with seminal ALP (-0.54);mass activity with seminal fructose (-0.29), AST (0.44), ALT (-0.28), ALP (-0.39) and ACP (0.26);seminal pH with cholesterol (0.26);live sperm with seminal ALP (-0.41); abnormal sperm with seminal fructose (0.33), cholesterol (-0.41), AST (-0.32), ALP (0.27) and ACP (-0.51); intact acrosome with seminal ALT (-0.33); HOS reactive sperm with seminal AST (0.32); cold shock resistant sperm with seminal fructose (0.34), AST(-0.36), ALT (0.29), ALP (0.59) and ACP (-0.37) and MBRT with seminal fructose (0.32).

Key word: Buffalo semen, Biochemical profile, Enzymatic activity, Seasonal variation, Correlations.

INTRODUCTION

The seminal plasma is a highly complex biological fluid and contains various biochemical constituents including cholesterol and a large number of proteins of heterogenous antigenicity (Kulkarni*et al.,* 1996). The physiological role of seminal plasma proteins in acquisition of motility, capacitation, acrosome reaction and fertilizing capacity of spermatozoa has been explored recently (Kulkarni*et al.*, 1996). Amongst domesticated animals, sufficient literature is available on seminal enzymes and biochemistry of bulls of different breeds (Dhami and Sahni, 1994), but the information on seasonal variation is meagre (Kapoor, 1982; Dhami and Kodagali, 1988).This study was

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attempted to know the seasonal variation in certain seminal plasma biochemical and enzymatic constituents and their interrelationships with physico-morphological attributes of semen in Surti buffalo bulls.

MATERIALS AND METHODS

The present study was carried out at the Semen Station, Veterinary College, AAU, Anand, Gujarat during the year 2011-12.The year was divided into three seasons, viz., rainy (July-Oct), winter (Nov-Feb) and summer (Mar-Jun) according to the prevailing agro-climatic conditions of the region.Two sexually mature healthy buffalo bulls of Surti breed were included in the study. The bulls were maintained in nearly identical nutritional and managerial condition throughout the period of study.They were vaccinated regularly against Foot and Mouth disease and Haemorrhagic Septicaemia.The bulls were in regular twice a week semen collection schedulein the morning hours using artificial vagina. A total of 60 ejaculates(10 per bull/ season) were utilized for the purpose.

Each ejaculate was divided into two aliquots; one was assessed for physico-morphological and functional attributes, while the other was used for evaluation of seminal biochemical and enzymatic profile. Initial seminal fructose was estimated by using resorcinol technique (Mann, 1948). Semen samples were then centrifuged at 3000 rpm for 15 minutes and plasma samples were stored at -20°C until analyzed. The seminal plasma profiles of total protein, total cholesterol and enzymes ALP, ACP, AST andALT were estimated by using standard techniques and assay kits procured from Coral Clinical Systems, Goa, India with the help of auto-analyzer(BS-120, Mindray).

The data generated were analyzed statistically using 2 factors factorial CRD on SAS system, and the interrelationships between various physico-morphological and functional attributes with seminal biochemical constituents were worked out statistically (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

The mean initial seminal fructose content of Surti bulls observed was 586.61±65.77 mg/dl. It differed highly significantly ($P<0.01$) between bulls and between seasons. The mean value was the highest during monsoon followed by winter and summer seasons. The concentration of initial seminal fructose obtained compared with the reports of Dhami*et al.* (1990), Dhami and Kodagali (1987) and Porwal and Karandikar (1981), while Sharma *et al.* (1994) and Kapoor (1982) reported lower value. The bull and seasonal variation in seminal fructose noted is in line with the report of Kapoor (1982) on Murrah bulls. However, Dhami and Kodagali (1987) did not find such seasonal variation in fructose content of Surti buffalo semen.The reducing sugar present in the seminal plasma of bulls is fructose and it is the main sperm nutrient in mammalian semen. The seminal fructose gives an useful indication of the fertilizing ability of bulls. The health of seminal vesicles is directly reflected by fructose content in semen ejaculate, because seminal vesicles chiefly contribute the same in the ejaculate (Mann, 1964; Ibrahim *et al.* 1985).

The average total protein concentration in the seminal plasma of Surti buffalo bulls under study was 2.54 ± 0.51 g/dl. The difference between bulls was significant; however, the influence of seasons was not significant, although an increasing trend in the values was observed from summer, monsoon to winter season. The values of proteinobtained corroborated with those reported by Kulkarni*et al.* (1996), Dhami and Kodagali (1987) and Yaqub*et al.*(1983). However, Dhami*et al.* (2003) reported comparatively higher seminal plasma total protein. Nandre (2007) observed significant difference in seminal protein content of Surti bulls between seasons, being higher in winter than in summer.According to Crabo and Jayendran (1979) the seminal plasma proteins were more like milk proteins and many of them were glycoproteins. They further opined that protein-salt interaction may play a major role during physiological events of sperm maturation, ejaculation and maturation in the female genital tract. Hence its role in the semen is vital.

The average total cholesterol concentration in the seminal plasma of Surti buffalo bulls was 53.71±10.05 mg/dl. It was neither influenced by the bulls nor by seasons.However, an increasing trend in the mean values was observed from monsoon, winter to summer season. The values of total cholesterol obtained were comparable with those reported by Shukla *et al.* (2009). However, Nema (1982) reported comparatively higher total cholesterol content, while Dhami and Shelke (2005) reported lower value. According to Komarek *et al.* (1964) 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. Dabas *et al.* (1984) reported that like phospholipids, cholesterol also acts as cryoprotective and an insulating agent for sperm membrane and thereby causing decrease in its permeability and increase in sperm survival during storage.

The mean alkaline and acid phosphatase (ALP-ACP)activities obtained in the seminal plasma of Surti buffalo bulls under study were 53.71±10.05 and 246.78 ± 27.92KAU/dl, respectively. The ALP-ACP activity was highly significantly (P<0.01) influenced by the seasons. There was an increasing trend in the mean values of ALP from summer, winter to monsoon season, while the ACP value was the highest during summer and the lowest during monsoon. The bulls varied significantly in their seminal ALP activity.The values of ALP obtained corroborated with those reported by Dhami and Sahni (1994) and Dhami and Kodagali (1988). However, Mohan *et al.* (1992), Dhami *et al.* (1990) and Reddy and Raja (1980) reported comparatively higher activity of ALP, while Ibrahim *et al.* (1985) reported lower value. Similarly, the ACP values compared with those reported by Dhami *et al.* (1990), Dhami and Kodagali (1988) and Ibrahim *et al.* (1985). However, Dhami and Sahni (1994) and Reddy and Raja (1980) reported comparatively higher ACP, while Kumar *et al.* (1984) reported lower value.Present significant seasonal variation observed in seminal ALP-ACP is in line with the report of Dhami and Kodagali (1988) and Khokhar *et al.* (1987) in cattle and buffalo bulls. Lots of variation observed in the values of ALP-ACP reported by above researchers could be due to use of different assay technique and unit of expression of values, apart from many known factors like breed, age, health and nutritional status of bulls, season/ climate etc. Seminal phosphatases play an important dephosphorylating role in sperm metabolism. Phosphatases in semen reflect the functional state of accessory sex glands and metabolic activity of spermatozoa, and thus estimation of enzyme activities in seminal plasma reflect sperm membrane integrity and are helpful in differentiating the reproductive biology of bulls of different breeds/ species (Ibrahim *et al.*, 1985).

The mean aspartate aminotransferase (AST) and alanine aminotransferasee (ALT) activities in the seminal plasma of Surtibulls were 57.30 ± 10.73 and15.38 ± 5.75 IU/L, respectively. The seminal plasma AST-ALT activity varied significantly (P<0.01)due to season,the AST value being lowest and the ALT value being highest during monsoon as compared to other two seasons, which were at par. The ASTand ALT values obtained compared with those reported by Dhamiand Kodagali (1998). However, Shukla *et al.* (2009) reported comparatively higher ASTactivity, while Dhami and Shelke (2005) reported lower value. Similarly, Giri *et al.* (2011) reported comparatively lower seminal ALTactivity.Present significant seasonal variation observed in seminal AST-ALT is in line with the report of Dhami and Kodagali (1988) in Surti bulls.The AST and ALT are important transaminases present in semen, which are concerned with oxidative metabolism. The AST activity is mainly associated with the sperm cell as well as seminal plasma, and its amount in seminal plasma arises chiefly from leakage of spermatozoa. Transaminase in semen plays an important role in the catabolism of glutamate by bovine spermatozoa. There is high level of transaminase activity involving glutamate in the spermatozoa suggesting that glutamate, through its close association with citric acid cycle, may function as a reservoir of energy which is available whenever needed by the cell.

The ejaculate volume was highly significantly (P<0.01) and positively correlated with seminal fructose (0.46), and negatively with seminal ACP (-0.35). Sperm concentration per ml was significantly and negatively correlated with seminal ALP (-0.54).Mass activity was significantly and negatively correlated with seminal fructose (-0.29), ALT (-0.28) and ALP (-0.39), and positively with seminal AST (0.44) and ACP (0.26). Initial motility had significant (P<0.05) negative correlation with seminal cholesterol content (-0.23), while pH had positive correlation with cholesterol (0.26).Live sperm per cent was highly significantly (P<0.01) and negatively correlated with seminal ALP (-0.41). The abnormal sperm per cent was significantly (P<0.01) and positively correlated with seminal fructose (0.33) and ALP (0.27), and negatively with seminal cholesterol (-0.41), AST (-0.32) and ACP (-0.51).The intact acrosome showed significant negative correlation with seminal ALT (-0.33), whereas HOS reactive sperm had significant positive correlation with seminal AST (0.32). The percentage of cold shock resistant sperm was significantly (P<0.01) and positively correlated with seminal fructose (0.34), ALT (0.29) and ALP (0.59), and negatively with seminal AST (-0.36) and ACP (-0.37). The MBRT was significantly and positively correlated only with seminal fructose (0.32) (Table 2).Amongst the biochemical and enzymatic attributes, highly significant (P<0.01) correlations were observed for seminal fructose with seminal AST (-0.43,), ALT (0.36) and ACP (-0.38); seminal total protein with seminal AST (-0.76); cholesterol with ACP (0.27); seminal AST with ALT (-0.43), ALP (-0.44) and ACP (0.46); seminal ALT with ALP (0.42), ACP (-0.24), and seminal ALP with ACP (-0.41). The other interrelationships among these attributes were negligible and non-significant.

The present correlation findings among the physico-morphological and biochemical attributes are in accordance with the previous reports of Dhami and Sahni (1994), Khokhar*et al.* (1987) and Dhami and Kodagali (1987) in Surti and Murrah bulls. Abdou*et al.* (1976) observed significant positive correlation for initial fructose with ejaculate volume and live sperm per cent in both HF and Murrah bulls, while Kaker and Arora (1976) reported highly significant negative correlations ranging from 0.41 to 0.64 between seminal fructose and the motility, sperm concentration, live sperm as well as total proteins, AST, ALT, ALP and ACP activities of seminal plasma in crossbred bulls. Porwal and Karandikar (1981) found significant positive correlations of 0.78 to 0.88 between fructose content and total protein, sperm motility, concentration and live sperm per cent. Similarly, seminal plasma total protein content had highly significant correlations ranging from 0.73 to 0.89 with mass activity, sperm concentration, live sperm and initial fructose.Kumar *et al.* (1984) found total protein content to be significantly correlated with live sperm and sperm concentration in Murrah bulls.Dhami and Sahni (1994) reported significant (P<0.01) positive correlations for transaminases with sperm density, concentration and live sperm and negative correlations with ejaculate volume and pH. Since the transaminases are mainly tied-up within the sperm cells, their positive correlations with sperm concentration were conceivable. Khokhar*et al.* (1987) observed AST-ALT activities to be significantly and positively correlated with mass activity and sperm concentration, but its correlations with ejaculate volume and fertility were not significant.Yaqub*et al.* (1983) also found positive correlations of AST and ALT with sperm concentration, but not with plasma proteins, while ACP had significant negative correlations with all other traits studied. Kaker and Arora (1976) reported significant positive correlations of both ALP-ACP with transaminases and all physical attributes of semen. Thus, the present correlations findings are one or the other way corroborate with most of the above reports, suggesting that the sperm motility, morphology and viability assessment is sufficient to grade semen under routine laboratory procedures.

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