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Correlation of Certain Biochemical Constituents of Seminal Plasma with Semen Characteristics in Pantja Buck

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ABSTRACT

Background: Pantja is a dual purpose indigenous goat breed, reared in *Tarai* region of Uttarakhand and Uttar Pradesh. There is a lack of thorough study about the seminal characteristics of this goat breed. Hence, the present study was conducted to evaluate the level of certain biochemical constituents and their relationship with macroscopical and microscopical semen characteristics in Pantja buck.

Methods: A total thirty two ejaculates from four sexually mature Pantja buck were analysed. The ejaculates were evaluated for volume (0.36±0.06 ml), mass activity (4.47±0.22), concentration (3.54±0.13 × 10^9/ml), individual motility (85.28±1.17%), viability (88.28±0.78%), morphological abnormalities (4.70±0.17%), plasma membrane integrity (86.31±0.62%) and acrosome integrity (94.31±1.05%) by standard semen evaluation protocol. Seminal plasma ALT (18.10±0.45 U/L), AST (124.04±0.86 U/L), ALP (49.98±0.38 U/L), LDH (216.36±1.78 U/L), GSH-Px (10.90±0.21 U/ml), MDA (2.14±0.05 nmol/ml), albumin (2.29±0.03 gm/dl), globulin (2.52±0.02 gm/dl), total proteins (4.81±0.05 gm/dl), calcium (11.07±0.18 mg/dl) and phosphorus (12.34±0.20 mg/dl) were determined spectrophotometrically. Two tailed Pearson's correlation was applied to check the relationship among the above attributes.

Result: A significant correlation was observed among the biochemical constituents of seminal plasma and seminal attributes of Pantja bucks. The selected biochemical constituents also showed significant correlation with each other.

Key words: Biochemical attributes, Indigenous, Pantja, Seminal characterics.

INTRODUCTION

Semen contains two major fractions *viz*. spermatozoa and seminal plasma. Seminal plasma is the major fraction of the semen. This is a complex mixture of secretions of testis, epididymis and the accessory sex glands of the male genital system (Mann and Lutwak-Mann, 1981). The biochemical composition of seminal plasma is irons (Na, Ca, P, K, Cl, Mg and Zn), proteins, amino acids, enzymes, vitamins, cytokines, antioxidants, lipids, hormones *etc*. (Juyena and Stelletta, 2012). Glutamic oxaloacetate transaminase (GOT) or Aspartate transaminase (AST) and Glutamic pyruvate transaminase (GPT) or Alanine transaminase (ALT) activity in the seminal plasma are important indicators of plasma membrane stability of the spermatozoa (Juyena and Stelletta. 2012). A lactate dehydrogense (LDH) enzyme is responsible for glycolysis when O_2 is limited by the NADPH mediated reduction of pyruvate lactate (Jones, 1997). Seminal plasma LDH and ALP indicates the metabolic status of the spermatozoa (Juyena and Stelletta, 2012). Transaminase enzymes are present in mid piece of the spermatozoa (Mann and Lutwak-Mann, 1981), but the source of LDH is spermatozoal cytosol and mitochondria (Burgos *et al*., 1995). The presence of ALP was observed at mid-piece, head and tail portion of the spermatozoa (Juyena and Stelletta, 2012). Sperms are very susceptible to lipid peroxidation due to oxidative stress. To protect the spermatozoa from oxidative stress related damages seminal plasma contains some endogenous oxidants like glutathione peroxidise (GSH-Px), superoxide dismutase (SOD), glutathione reductase (GR) and catalase (CAT) etc. (Juyena and Stelletta, 2012).

Pantja is a medium sized, dual purpose goat breed, has morphological similarities with deer, commonly found in *Tarai* region of Uttarakhand and adjoining areas of U.P. (Dhara *et al*., 2022). These contribute about 21% of goat population in Uttarakhand and are resistant to many of the diseases compared to other breeds (Nidhi, 2014). However, there is a lack of complete study on seminal attributes of Pantja bucks.

Hence, the present study was conducted to evaluate the level of certain biochemical constituents and their

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relationship with macroscopical and microscopical semen characteristics in Pantja buck.

MATERIALS AND METHODS

Present study was conducted in the department of Veterinary Gynaecology and Obstetrics, G.B.P.U.A.T., Pantnagar, Uttarakhand (India). A total of four sexually matured Pantja bucks of aged between 2.0 to 3.0 years (25-30 kg body weight) were selected from Goat unit, Department of Livestock Production and Management. Semen collection was carried out by artificial vagina (AV) method twice a week for a period 10 months. Sperm motility was determined as per Singh *et al*. (2013). Spermatozoa viability and morphological abnormalities were evaluated as per Memmon *et al.* (2012). The plasma membrane integrity of the spermatozoa was assessed by hypo osmotic swelling test (HOST) as described by Zubair *et al*. (2013). Acrosome integrity of the sperm cells was assessed by Giemsa staining method as described by Watson. (1975). For biochemical evaluation semen samples were centrifuged twice at 4000 RPM for 10 minutes to separate the seminal plasma and stored at -20°C. The Seminal plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) levels were determined by using commercial kits supplied by ARKRAY Healthcare Pvt. Ltd. as per manufacturer's instructions. Seminal plasma total protein (Biuret method), albumin (BCG dye method), calcium (Arsenazo III method) and phosphorus (ammonium molybdate method) were evaluated by using commercial kits (Erba) as per manufacturer's instructions. Malondialdehyde (MDA) concentration was measured as an indicator of lipid peroxidation, by using the thiobarbituric acid (TBA) method described by Ducha *et al*. (2020). Glutathione peroxidase (GSH-Px) is an endogenous antioxidant, measured as described by Wheeler *et al*. (1990).

The data were analyzed for Pearson's correlation coefficient using SPSS 16.0 software. The values were considered statistically significant ($p \leq 0.05$). All the data are presented as Mean±SE.

RESULTS AND DISCUSSION

The mean values of macroscopical and microscopical semen parameters are depicted in Table 1. Same amount of ejaculate volume was recorded by Khandokar *et al.* (2006) in case of Black Bengal buck. However, higher ejaculate volumes were recorded by Goswami *et al*. (2020). The differences in ejaculate volume might be due to differences in breed, age, season, frequency of ejaculation and age of the male animals (Patni *et al*., 2016). In the present study, the mass activity of Pantja buck semen is in agreement with Bitto and Egbunike (2012). The individual motility in the present study is in close harmony with Patil *et al*. (2019). In contrary, higher values of individual motility was noted by Atara *et al.* (2018) in Surti goat. These differences of sperm motility might be due to variation in breed, nutrition, environment and individual variation (Umar *et al*., 2017). Similar to our study, same sperm concentration was also found by Goswami *et al*., 2020. Though, the sperm concentration in the present experiment was not in accordance with Mishra *et al.* (2010). Concentration may vary depending upon the breed, age of male animals, climate (macro and micro), season, geographical location, restraint, number of false mounting before collection and frequency of collection (Foster *et al*., 1971). The present findings related to sperm viability are on the line of Parmar *et al*. (2011). Different factors such as age, breed, nutrition of the sire, pH and osmolality of the semen, season and ambient temperature affects the viability of the spermatozoa. In case of sperm abnormalities, our results are in a close harmony with Goswami *et al*. (2020). While, Umar *et al*. (2017) documented higher values of sperm abnormalities. Ahmed *et al*. (2014) in Beetal goat found similar HOST percentage with the present findings. Although, Umar *et al*. (2017) reported higher percentage of HOST-reacted spermatozoa. The percentage of acrosome integrity in present study was in agreement with Deori *et al*. (2018).

The mean levels of seminal plasma biochemical constituents are presented in Table 2. Somewhat similar range of ALT level was reported by Sinha *et al.* (2000). However, a higher value of ALT was mentioned by Umar *et al*. (2017) and lower values by Sharma *et al*. (2013). The value of AST in present study was in the agreement with Sharma *et al*. (2013), but in contrary to Sinha *et al*. (2000). The seminal plasma LDH activity in the present study was similar the other studies conducted by Umar *et al*. (2017). A very few literature is available about seminal plasma ALP activity in goat semen. Ali and Mustafa (1986) reported higher level of seminal plasma ALP activity in Nubian goat. In the present study, seminal plasma glutathione peroxidase activity was also in the light of the findings of Anand *et al*. (2016). The obtained value of seminal plasma albumin, globulin and total proteins in Pantja buck semen in our present study were closely related to the values reported by Nidhi (2014). The calcium and phosphorus ions in the seminal plasma of Pantja bucks were in agreement with previously reported findings of Nidhi (2014).

Table 1: The value (Mean±SE) of macroscopic and microscopic semen characteristics of Pantja buck.

Volume (ml)	Mass activity (0-5)	Sperm Concentration (billion/ml)	Individual motility (%)	Viability (%)	Sperm abnormality (%)	Host (%)	Acrosome Integrity (%)
0.36±0.06	4.47±0.22	3.54±0.13	85.28±1.17	88.28±0.78	4.70±0.17	86.31±0.62	94.31±1.05

Table 2: The value (Mean±SE) of certain biochemical constituents of Pantja buck semen.

ALT(U/L)	AST (U/L)	ALP (U/L)	LDH (U/L)	GSH-Px (U/ml)	MDA (nmol/ml)	Albumin (gm/dl)	Globulin (gm/dl)	Total protein (gm/dl)	Ca (mg/dl)	P (mg/dl)
18.10±0.45	124.04±0.86	49.98±0.38	216.36±1.78	10.90±0.21	2.14±0.05	2.29±0.03	2.52±0.02	4.81±0.05	11.07±0.18	12.34±0.20

ALT- Alanine amino transferase, AST- Aspertate amino trasferase, ALP- Alkaline phosphatase, LDH- Lactate dehydrogenase, GSH-Px- Glutathione peroxidase, MDA- Malondialdehyde, Ca- Calcium, P- Phosphorus..

The level of correlation between the seminal plasma biochemical constituents and semen characteristics is represented in Table 3. Two tailed Pearson's correlation analysis showed that seminal plasma ALT level has significant negative correlation ($p \leq 0.01$) with sperm mass activity, individual motility, plasma membrane integrity and acrosome integrity and a significant positive ($p \leq 0.01$) correlation with morphologically abnormal spermatozoa. Seminal plasma AST had a significant negative ($p \leq 0.05$) correlation with plasma membrane integrity and acrosome integrity and a significant ($p \leq 0.05$) positive correlation with morphologically abnormal spermatozoa. Glutamic oxaloacetate transaminase (GOT) or Aspartate transaminase (AST) and Glutamic pyruvate transaminase (GPT) or Alanine transaminase (ALT) activity in the seminal plasma are two very important indicator of plasma membrane stability of the spermatozoa (Juyena and Stelletta. 2012). Level of GOT and GPT are negatively correlated with the percent live spermatozoa (Taha *et al*., 2000). Umar *et al*. (2017) reported a significant positive relationship of seminal plasma ALT and AST level with morphologically abnormal spermatozoa and a significantly negative correlation of AST with plasma membrane integrity, which is in agreement with present study. On contrary the study indicated that seminal plasma ALT had significant positive correlation with HOST-reacted spermatozoa.

However, seminal plasma alkaline phosphatase (ALP) activity was exhibited significant ($p \leq 0.01$) positive correlation with ejaculate volume and spermatozoa concentration in the ejaculates. Viudes-De-Castro *et* al. (2015) observed a significant positive correlation of ALP with sperm concentration in the ejaculates, which is in the harmony with the present study. The activity of ALP in seminal plasma is a biomarker of the sperm motility, metabolism, health status of the accessory sex glands and sperm plasma membrane integrity (Gupta *et al*., 2019).

Lactate dehydogenase (LDH) showed significant negative correlation with ejaculates volume ($p \leq 0.01$), sperm concentration ($p \leq 0.01$) and positive correlation with spermatozoa viability ($p < 0.05$). Previous studies also reported same results in agreement with the present findings (Talluri *et al*., 2017).

Seminal plasma glutathione peroxidase activity exhibited significant ($p \leq 0.01$) positive correlation with mass activity, individual motility, viability, HOST-reacted spermatozoa and acrosome integrity and significant ($p \leq 0.01$) negative correlation with morphologically abnormal spermatozoa. Malondialdehyde (MDA) concentration had significant negative correlation with individual motility ($p \leq 0.01$), live sperm percentage ($p \leq 0.05$), plasma membrane integrity ($p \leq 0.05$) and acrosome integrity ($p < 0.05$) and significant positive correlation with sperm morphological abnormality ($p \leq 0.05$). Glutathione peroxidase present in the seminal plasma acts as endogenous antioxidants and neutralizes the free radicals. Similarly, Crisol *et al*. (2012) reported a significant positive correlation of seminal plasma

Table 3: Correlation between certain biochemical constituents of seminal plasma and semen characteristics of Pantja buck.

	Volume	Conc.	Mass activity	Individual motility	Viability	Host	Acrosome integrity	Abnormal sperm
ALT	-0.182	0.227	-0.458**	-0.454**	-0.347	-0.609**	-0.559**	0.708**
AST	-0.288	0.034	-0.177	-0.295	-0.204	-0.369*	-0.423*	0.352*
ALP	0.457**	0.957**	-0.064	-0.108	0.259	-0.134	0.071	0.070
LDH	-0.498**	-0.572**	0.116	0.294	0.427*	0.282	0.342	-0.313
GSH-Px	0.294	0.051	0.628**	0.749**	0.644**	0.699**	0.786**	-0.684**
MDA	-0.282	-0.138	-0.269	-0.451**	-0.382*	-0.381*	-0.433*	0.441*
Albumin	0.509**	0.230	0.628**	0.801**	0.561**	0.630**	0.673**	-0.631**
Globulin	0.418*	0.251	0.519**	0.695**	0.476**	0.492**	0.530**	-0.495**
Total protein	0.487**	0.226	0.595**	0.775**	0.531**	0.580**	0.624**	-0.571**
Ca	0.335	-0.027	0.384*	0.394*	0.168	0.259	0.310	-0.275
P	0.252	-0.047	0.380*	0.456**	0.171	0.300	0.280	-0.284

*P≤0.05 and **P≤0.01.

Table 4: Correlation between the biochemical constituents of seminal plasma in Pantja buck semen.

	ALT	AST	ALP	LDH	GSH-Px	MDA	A	G	Total Protein	Ca	P
ALT	1										
AST	0.489**	1									
ALP	0.192	0.097	1								
LDH	0.125	0.102	-0.567**	1							
GSH-Px	-0.399*	-0.350*	-0.045	-0.043	1						
MDA	0.250	-0.099	-0.050	0.441*	-0.336	1					
Albumin	-0.236	-0.136	0.147	-0.509**	0.683**	-0.631**	1				
Globulin	-0.116	-0.006	0.188	-0.451**	0.650**	-0.598**	0.931**	1			
Total protein	-0.183	-0.069	0.150	-0.494**	0.679**	-0.634**	0.989**	0.967**	1		
Ca	-0.276	-0.317	0.049	0.032	0.369*	-0.157	0.346	0.391*	0.360*	1	
P	-0.249	-0.332	-0.073	0.062	0.394*	-0.142	0.390*	0.444*	0.405*	0.891**	1

*P≤0.05 and **P≤0.01.

glutathione peroxidase activity with total sperm motility, progressive motility and normal spermatozoa percentage.

Seminal plasma albumin, globulin and total protein showed significant (p≤0.01) positive correlation with volume, mass activity, individual motility, live sperm percentage, plasma membrane and acrosome integrity and negative correlation with sperm morphological abnormalities. Seminal plasma calcium and phosphorus levels were positively correlated with mass activity and individual motility of spermatozoa. The present findings are in agreement with Umar *et al*. (2017). Seminal plasma calcium had a significant (p≤0.05) positive correlation with mass motility and individual motility. Similarly, seminal plasma phosphorous showed a significant positive correlation with mass motility (p≤0.05) and individual motility (p≤0.01). Correlation of seminal plasma calcium and phosphorus also reported in earlier study (Umar *et al*., 2017).

Table 4 represents the correlation between the biochemical constituents of seminal plasma. ALT and AST positively (p≤0.01) correlated with each other and showed negative correlation with GSH-Px (p≤0.05). ALP had significant (p≤0.01) negative correlation with LDH. However, LDH exhibited significant positive (p≤0.05) correlation with MDA concentration and significant negative (p≤0.01) correlation with seminal plasma albumin, globulin and total protein. Seminal plasma glutathione peroxidase activity had significant (p≤0.01) positive correlation with albumin, globulin and total protein. Oppositely, MDA showed significant (p≤0.01) negative correlation with above three constituents. Furthermore, seminal plasma albumin, globulin and total protein was positively correlated with each other (p≤0.01). Calcium revealed significant (p≤0.05) positive correlation with GSH-Px, globulin and total protein. Similarly, phosphorus showed significant (p≤0.05) positive correlation with GSH-Px, albumin, globulin and total protein. Calcium and phosphorus was significantly (p≤0.01) correlated with each other. Similar findings were also observed by Umar *et al*. (2017).

CONCLUSION

On the basis of the above findings, it can be concluded that the seminal plasma total proteins, albumin, globulin, calcium and phosphorus were positively correlated with semen characteristics except the sperm morphological abnormalities and membrane integrity. Although, seminal plasma AST, ALT and MDA were positively correlated with

spermatozoa abnormalities. All the biochemical constituents were also correlated with each other.

Conflict of interest: None.

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