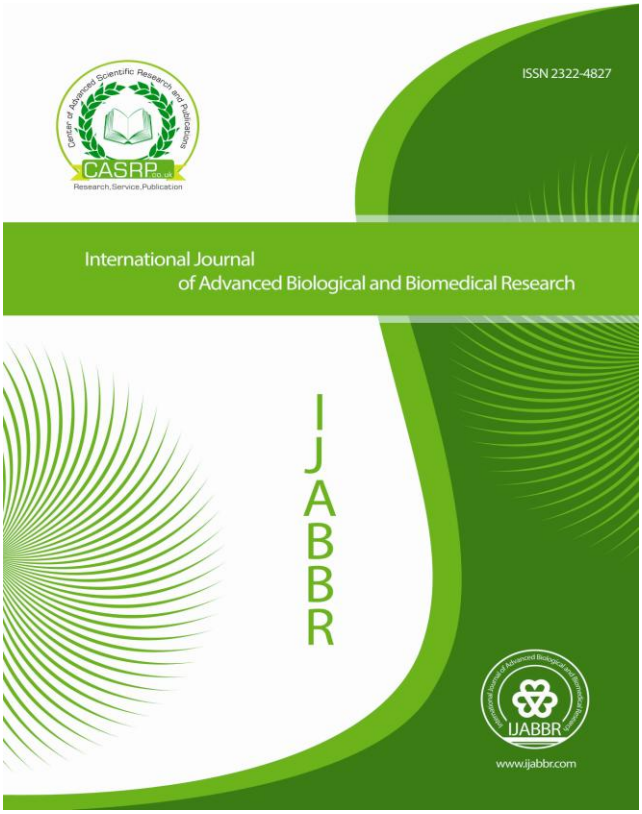

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CASRP PUBLISHER

International journal of Advanced Biological and Biomedical Research 3(4) (2015) 361–369

doi: 10.18869/IJABBR.2015.361

Original Article Open Access

Biochemical analysis of bovine (BosIndicus) seminal plasma

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Abstract

The aim of this study was to investigate the comparison between some seminal plasma parameters and age of the bull. Concentrations of various biochemical parameters like fructose, glucose, total proteins, albumin and inorganic ions including Na, K and Cl ions of seminal plasma of breeding Sahiwal bulls of different ageswere assessed. Methods: 40Sahiwal bullsdivided into 4 age groups.Semen (2ml pooled) from each bull was collected via artificial vagina. Seminal fructose was determined by Resorcinol method, while seminal glucose, totalprotein and albumin were estimated by automated clinical chemistry analyzer and inorganic ions were determined by MedicaEasylyte Na/K/Cl analyzer. Highest values of fructose, glucose, potassium and albumin were recorded in A Group bulls, On the other hand lowest values of fructose, glucose and potassium were recorded in D group, while albumin concentrations were similar in B and C groups bulls and lower in D group.Highest values of sodium and chloride were recorded in D group bulls, and lowest values of seminal sodium and chloride were recorded in group A bulls. Total proteins concentrations were similar in bulls of four age groups. Concentrations of these parameters showed high variability with respect to age in Sahiwal bulls which tends to clarify the variation of these seminal parameters with respect to age of bulls. This study suggested that seminal plasma of semen should be evaluated

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Received 12 November 2015 | Accepted 13 December 2015 | Available online 19 December 2015

iThenticate screening 16 November 2015 | English editing 11 December 2015 | Quality control 15 December 2015

for these seminal parameters before the packing of semen for cryopreservation irrespective of the age and breed of the bull.

Keywords: Biochemical components, Seminal plasma, Sahiwal bull, Age.

1. Introduction

Much have been quoted in the literature regarding the effects of seasonal and environmental variations on the seminal plasma parameters in bulls and other animals in order to assure optimal reproductive efficiency for livestock and artificial insemination (AI) industry, but there is paucity of information regarding the comparison between age of bull and its accompanying effect on the concentrations of seminal constituents in Sahiwal bulls. Therefore concentrations of various seminal parameters in different age groups of Sahiwal bulls were determined in order to elucidate the effect of age on seminal parameters. According to (Elliot, 1978; Correa et al., 1997; Rodriguez-Martinez and Larsson, 1998; Zhang et al., 1998; Brahmkshtri et al., 1999; Morrell et al., 2014) evaluation of sperm concentration and motility is frequently used to assess semen quality, but provides limited information about the potential fertility of sires.

The seminal plasma, the liquid component of semen, consists of secretions from testes, epididymis, and the accessory sex glands, contains a variety of factors (amino acids, fatty acids, asmolytes and proteins) that influence the viability and fertilizating capacity of ejaculated spermatozoa (Mann and Lutwak-Mann, 1981; 1990; Yanagimachi, 1994; Goericke et al., 2015). As seminal plasma is the immediate environment of spermatozoa, the substances in the seminal plasma could be expected to have profound effect on the quality of the sperm (Wijeranta et al., 2005). Seminal plasma was reported to be important for maintaining spermatozoa motility in bull (Baas et al., 1983). The biochemical components of bull seminal plasma, such as fructose, total protein, albumin, glucose and inorganic ions are crucial for spermatozoa survival and function (Zopfgen et al., 2000).

Seminal fructose and glucose are essential source of energy production and motility of spermatozoa (Jobim et al., 2004; Assumpcao et al., 2005; Garner et al., 2001). The potential influence of seminal proteins on male reproduction came to attention because of the studies showing that their expression is associated with breeding scores of dairy bulls (Killian et al. 1993; Cancel et al. 1997), beef bulls (Bellin et al., 1994, 1996; Parent et al., 1999) and horses (Brandon et al., 1999)

The protein composition of mammalian seminal plasma varies among species and has important effects on sperm function (Villemure et al., 2003; Mortarino et al., 1998; Cross, 1993; Miller et al., 1990; Strzezek et al., 2002). The bulk of the proteins found in seminal plasma derive from the seminal vesicles, although albumin is mainly of prostatic origin (Hirsch et al., 1991). Albumin makes up about one third of the protein content of the semen (Derek et al. 2005).

Seminal plasma also contains a complex range of organic and inorganic constituents. Sodium (Na) and potassium (K) make up most of the inorganic mineral elements in semen and so they have been referred to as bulky cation in semen. These cations along with calcium (Ca) and magnesium (Mg) in seminal plasma establish osmotic balance (Mesut et al. 2007Fernández et al. 2013). Chloride ions present in higher concentration in seminal plasma play an important role in the respiratory and motility of spermatozoa. Sperm cells in chloride diluents utilized more fructose, but less lactic acid, because chloride ions apparently favored the oxidation of lactic acid (Salisbury, 1957; David et al., 2015).

Conventional methods of semen generally measure sperm concentration, progressive motility, the percentage of viable cells and morphology irrespective of the age of bulls and quality of seminal plasma. Many countries in the world have one or more particular breed of bulls and their data regarding the analysis of their semen seminal plasma are available which shows their potency to render fertility, but unfortunately the relevant data about Sahiwal bull is scared. Therefore the present study aimed to elucidate the comparison between different ages of bulls and concentrations of various seminal parameters. Wehoped and believed that the proposed aim of the study was achieved in formulating a concise fact that bull seminal plasma of the semen should be evaluated properly in both young, elder and old age groups prior to packing of semenfor cryopreservation.

2. Materials and methods

Semen samples were collected in Semen Production Unit (SPU) Qadirabad, District Sahiwal, city of central Punjab Province, Pakistan, while biochemical analysis of seminal plasma was carried out in Zeenat laboratory, Lahore, Pakistan. A total of 40 breeding Sahiwal bulls were used, divided into 4 age groups,(ten bulls each group) of different ages designated as group A, B, C and D of 4.5-6, 6-7, 7-8 and above 8 years respectively. All the experiments were carried out with fresh ejaculated semen which were collected within one week, 10 bulls each day with prewarmed artificial vagina at (42^{o} C) (Azam et al., 1998).

All animals were kept under uniform nutritional and standard management conditions. Following semen collection, one fraction of each semen sample was used for conventional semen analysis, including visual examination, gross motility by phase contrast microscope and sperm motility by hemocytometer.

2.1. Collection of seminal plasma

Seminal plasma was obtained by spinning 2 ml of semen at 3000 rpm for 25 minutes at room temperature, 1 ml of supernatant was recovered in sterile polystyrene labeled cuvets, and stored at deep freezer at -70^{o}C because of high temperature in the month of August in Sahiwal for further biochemical analysis.

2.2. Biochemical analysis

Seminal plasma fructose estimation:

Fructose in seminal plasma was assessed by a colorimetric, Resorcinol method (Jin-Chun Lu et al., 2006).

Seminal plasma glucose, albumin and total protein estimation:

Seminal plasma glucose, albumin and total proteins were measured by Automated clinical chemistry analyzer (Modular P-800, Roche) equipped with Calibrated for automatic systems (CFAS) (Peggy et al. 2007; Javier et al. 2005).

Seminal plasma sodium, potassium and chloride ions estimation:

Sodium, potassium and Chloride ions in seminal plasma were assessed by MedicaEasylyte Na/K/Cl analyzer selective ion electrode (Medica Corporation, USA).

2.3. Statistical analyses

Data analysis was performed using SPSS software program. All values were expressed as Mean ± Standard Deviation. Analysis of Variance (ANOVA) test was applied to study the variation between groups for seminal plasma components at significance level of ($p< 0.05$).

3. Results

Data on seminal plasma fructose, glucose, total proteins and albumin protein are presented in Table 1.

Result for seminal fructose:

Comparing the 4 groups of bulls for seminal fructose there were highly significant differences between the 4 age groups of bulls for seminal fructose ($p <0.01$). Highest seminal plasma fructose concentrations were estimated in group A bulls, while lowest values were calculated in group D bulls.

Result for seminal glucose:

Comparing the 4 age groups of bulls for seminal glucose there were no significant differences between the 4 age groups of bulls for seminal glucose ($p < 0.05$). Highest seminal plasma concentrations of glucose were recorded in Group A bulls and the lowest was recorded in group D bulls.

Result for seminal total proteins:

Comparing the 4 age groups of bulls for seminal total proteins there were no significant differences between the 4 age groups of bulls for seminal total proteins ($p < 0.05$). Thetotal protein concentrations weresimilar in the 4 age groups of bulls.

Result for sminal albumin:

Comparing the 4 age groups for seminal albumin there were no significant differences between the 4 age groups for seminal albumin ($p < 0.05$).The seminal plasma albumin concentration was highest in group Abulls followed by group D bulls and similar concentrations of albumin protein were recorded ingroup B and Cbullsrespectively have.

Table 1

Standard deviation results of seminal plasma fructose, glucose, total protein and albumin protein among different groups of Sahiwal bulls.

Groups	No. of observation	Fructose mmo1/L	Glucose mmol/L	Total protein mmol/L	Albumin mmol/L
A	10	90.66 ± 83.11*	283.62 ± 140.93**	8.52 ± 2.34**	1.18 ± 0.31**
B	10	4.56 ± 2.62*	272.77 ± 148.70**	8.50 ± 1.58**	1.08 ± 0.20**
C	10	30.66 ± 26.94*	282.02 ± 108.27**	8.42 ± 1.78**	1.08 ± 0.23**
D	10	1.12 ± 0.99*	182.91 ± 125.71**	8.77 ± 2.68**	1.16 ± 0.41**

Allvaluesare Mean ± SD, *Means within column followed by singleasterisk are significantly different ($p < 0.01$), **Means within column followed by double asterisks are significantly non different ($p < 0.05$), * $P < 0.01$, **$P < 0.05$.

The data on seminal plasma inorganic ions including Na, K and Cl are presented in Table No. 2.

Result for seminal Na:

Comparing the 4 age groups of bulls for seminal Na there were no significant differences between the 4 age groups for seminal Na ion concentration ($p < 0.05$). Highest seminal plasma Na concentration was estimatedin group D bulls and lowest concentration was determined in group A bulls.

Result for seminal K:

Comparing the4age groups of bulls for seminal K there were no significant differences between the 4 age groups of bulls for seminal K ion concentration ($p < 0.05$).Seminal plasma K concentration was significantly highest in group A bulls while lowest in group D bulls, although K concentrations in seminal plasma of group B and C bulls respectively were similar.

Table 2

Standard deviation results of seminal plasma Na, K and Cl ions among different groups of Sahiwal bulls.

Groups	No. of Observation	Sodium mmo1/L	Potassium mmo1/L	Chloride mmo1/L
A	10	73.00 ± 21.80**	19.53 ± 8.70**	95.70 ± 10.30**
B	10	86.35 ± 11.04**	16.87 ± 5.54**	100.48 ± 6.78**
C	10	79.58 ± 18.46**	17.37 ± 7.72**	96.46 ± 13.74**
D	10	87.16 ± 7.59**	15.36 ± 5.19**	102.02 ± 4.55**

All values are Mean ±SD, **Means within columnfollowed by double asterisks are significantly non different, **$P < 0.05$.

Result for seminal Cl:

Comparing the 4 age groups for seminal chloride there were no significant differences between the 4 age groups for seminal Cl ion concentration ($p < 0.05$).Highest concentration of Cl was recorded in group D bulls and group A bulls showed lowest Cl ion concentration.

4. Discussion

The aim of this study was to compare the seminal plasma components concentrations between different ages of Sahiwal bulls of same breed. Biochemical evaluation of seminal plasma is prerequisite in assessing fertility levels and diagnosing male reproductive disorders (Barrier et al., 2002; Massanyi et al., 2004; Meizel et al., 1997). Therefore we determined biochemical constituents in terms of seminal fructose, glucose, total proteins, albumin and inorganic ions including Na, K and Cl.

Fructose represents the main sugar in seminal plasma and they are essential for ATP production and motility of the spermatozoa (Inskeep et al., 1985; Williams and Ford, 2001; Kaczmarek et al., 2013). In the present study there were significant differences between the 4 groups of Sahiwal bulls for seminal fructose ($p < 0.01$). Substantially highest concentrations were estimated in Group A bulls while lowest concentrations of fructose were calculated in group D bulls. This rise in fructose concentration in young mature bulls (Group A) is might be related with age, since group A comprises of young mature bulls as compared to old bulls in group D. Present study values are higher than those reported by (Inskeep et al., 1985) in mature bulls but they did not related them with age. The increased weight and size of bulls are other factors that may effect.

According to Martikanien et al. 1980 glucose inseminal plasma of bulls and other species is important for Adenosinetriphosphat (ATP) production, sperm capacitation and acrosome reaction. In the present study there were no significant differences between the 4 age groups of studied animals for glucose ($p < 0.05$). Glucose concentrations were also highest in group A bulls while lowest seminal glucose were estimated in group D bulls. This rise in glucose concentration in young mature bulls in the present study is likely be due to small ages of bulls. Inthe present study, seminal glucose concentrations were higher than those reported by (Assupico et al., 2005) in Nelore bulls, however they not mentioned the relative age of their studied bulls. It is reasonable that glucose concentration was affected by factors other than age, breeds and feeding conditions.

Many studies have shown that low content of seminal proteins is associated with poor semen quality (Verma et al., 1985; Dhami and Kodagali, 1989; Bergeron et al., 2004.). In the present study there were non significant differences between the 4 age groups of Sahiwal bulls for total protein ($p < 0.05$). Total protein concentrations were similar in all the 4 groups of bulls. The exact reason for this similarity of total protein in 4 age groups of bulls is beyond the scope of the presentstudy; however more refined study is required to find the logic behind this similarity. The present study values oftotal protein concentrations were higher than those reported by (Assupico et al., 2005; Tribulo et al., 2014) and lower than those reported by (Ronocoletta, 1999) in Nelor bulls this difference is perhaps due to higher genetic variability between the species of bulls. It is also assumed that total protein concentration may be effected by factors such as feeding regimes and seasonal variations other than age of bull.

In fact, the beneficial effects of seminal proteins in improving sperm motility reside in their contact of albumin, another seminal protein, since epididymalspermatozoa lack progressive motility and acquire it upon the addition of either seminal plasma (motility factor) or albumin (Lindholmer, 1974; Słowińska et al., 2014). For the 4 age groups of bulls investigated in the present studythere were no significant differences for seminal albumin ($p< 0.05$). Highest seminal plasma albumin was calculated in group A bulls followed by group D bulls, although groups Band C bulls have similar albumin values which reflect low differences in the ages of 2 group bulls.

The Na ion is an important element for spermatozoa functioning (Machal et al., 2002; Mosaferi et al., 2005). For the 4 age groups of studiedanimals Na concentration was highest in the group D bulls than in other groups of bulls (A, B, and C) ($P < 0.05$).Whereas group A represented the lowest Na concentration. The average seminal Na ion concentration obtained by (Mesut et al., 2007) in Brown Swiss and Holstein bulls ranged from 104.40 to 103.25 mmol/L respectively, and by (Maan, 1964; Słowińska et al., 2015.) in bull was higher than present study values. It is unlikely to be due to methodological sensitivities between researchers worldwide.

K ion is a natural metabolic inhibitor and higher K ion concentration in seminal plasma decreases the metabolic activity of spermatozoa. Therefore, there is generally a negative correlation between K and sperm motility (Gur et al., 2000; Massanyi et al., 2003; Beyersdorf, 1998; Joseph et al., 2013). For the 4 groups of investigated animals group A showed highest and group D represented lowest K ion concentration while groups B and Cshowed similar K ion concentration ($p < 0.05$). Highest K concentrations in young mature bulls and lowest in old agebulls reflect the probable reason due to age.

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 (Purohitet et al. 1999; Meizel, 1997). For the 4 groups of studied animals group D bullsshowed highest and in group A lowest Cl ion concentration were calculated ($p < 0.05$). The present study seminal Na and K ions concentration were lower than those reported by (Kwanl et al., 2000) in Nili-Ravi buffalo and Sahiwal X Friesian bulls, by (Shukla et al., 2009) in Murrahbuffalo bulls and by (Mesut et al., 2007) in Holstein bulls, that is may due to be genetic variation between those bulls reported by above referenced authors and Sahiwal bulls. Although they did not compared their estimated Na and K concentrations with particular age of the bull.while our seminal Cl ion concentration were higher than those reported by (Mesut et al., 2007) in Holstein bulls and Brown Swiss bulls. In the present study analysis of seminal Na and K ions also proved that Na ion concentration is higher than K those reported by (Salisbury, 1962). Although all

the above researchers did not compared their calculated values of Na, K andClions in their studies with respect to particular age of the bulls. These conflicting results are possibly due to genetic dissimilarities between the bulls in addition to the climatic affects.

Implications for public:

Concentrations of few seminal plasma parameters decreases with increasing age of the bull. Therefore young mature bulls should be preferred to use for fertility purposes in order to minimize the risk of infertility. Many artificial insemination (AI) centers uses the so called conventional semen extenders for semen freezability irrespective of the quality of the semen, should be formulated after the biochemical evaluation of seminal plasma. At present different protocols are used for the freezing of the semen for future use, in this way it is utmost need to select the best possible semen for cryopreservation in order to save precious and valuable breeds of animal to be extinct.

5. Conclusion

The calculation of highest concentrations of some seminal parameters in young mature bulls and their lowest concentrations in old agebulls and vice versa, gives insight into the notion that age effects the concentrations of some seminal biochemical parameters in the semen of Sahiwal bulls, while one seminal parameter concentrations were similar in 4 age groups required more refined and systematic studies to prove the exact role of age and its accompanying effect on the concentrations of seminal parameters. This periodicity in seminal plasma parameters of semen with respect to age of bull suggested that analysis of biochemical components of seminal plasma of semen in the evaluation of bull fertility and prior to semen freezability irrespective of the age and breed of the bull.

Acknowledgements

The authors are grateful to DrZeenatHussain and her staff for providing all the relevant Lab facilities and to DrSajed for sample collection and so many thanks to DrIqbalKakar for guidance and courage throughout this research work.

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How to cite this article: Khan, A., Yasinzai, M.M., Kakar, M.A., 2015. Biochemical analysis of bovine (BosIndicus) seminal plasma. International journal of Advanced Biological and Biomedical Research, 3(4), 361-369.