Seminal Plasma Proteins as Potential Markers of Semen Characteristics in Buffalo and Bovine Bulls  
Kadoom, A.K.; F. E. El-Hkeraby; A.F. Ashour and E. M. El-Siefy  

ABSTRACT

This study aimed to find a link between seminal plasma protein profile and semen characteristics, freezing ability and subsequent field fertility of bovine and buffalo bulls. Pre- and post-thawed semen of five Holstein and four buffalo bulls was used in this study. Total pattern of proteins precipitated from seminal plasma of bovine and buffalo bulls showed four different proteins of apparent molecular masses, 14 kDa, 15 kDa and 30 kDa with informative polymorphism in number and intensity of bands in both buffalo and cattle seminal plasma proteins. Seminal proteins bands (BSA-A1, A2, A3 and BSA-30) were positively correlated with percentages of motility and livability of spermatozoa, while negatively correlated with percentage of sperm abnormalities and acrosomal integrity in pre- and post-thawed semen of buffalo and bovine bulls. It could be concluded that seminal plasma proteins can be used as a predicting measure for quality of bovine and buffalo semen. Further studies might be needed for quantitative determination of seminal plasma proteins which might give a better indicator for bull fertility when compared to the descriptive methods.  
Keywords: Bovin, buffalo, semen, freezing, seminal proteins, bands, fertility prediction.

INTRODUCTION

Physical semen characteristics and sperm morphology measurements are not always indicative of bull fertility. Accurate and predictive protein markers are still needed for evaluation of inherent bull fertility (Foxcroft et al., 2008). Seminal plasma and sperm proteins have been reported as markers of fertility in different species. High fertile bulls have higher expression of seminal plasma proteins which might be involved in energy metabolism, cell communication, spermatogenesis, and cell motility (Peddinti, 2008). These proteins are products secreted from the seminal vesicles and ampullae, and their biochemical characteristics have been well-described (Moura et al., 2006a and b).

Potential markers of fertility with several molecules are present in the seminal plasma milieu (Arlindo and Moura, 2005). In vitro testing of proteins purified from male and female reproductive fluids demonstrated that they facilitated sperm capacitation, fertilization and early embryo development (Killian, 2012) by stabilizing the sperm against premature capacitation and spontaneous acrosome reaction in addition to protection of the sperm from freezing-induced damage (Moura et al., 2006a).

Certain bovine seminal proteins are reported to induce an important cholesterol efflux from epididymal spermatozoa during capacitation (Therien et al., 1998), while others (oviduct binder sperm proteins) have been considered as a key in establishing the sperm population in the oviduct isthmus (Gwathmey et al., 2006). The prevalent protein in seminal plasma of higher-fertile bovine bulls is the 55 kDa proteins.

The biological properties of bovine seminal plasma (BSP) proteins have been extensively studied (Manjunath and Therien, 2002). It has been reported that BSP reverts the cold-shock damage on ram sperm membrane (Jobim et al., 2004). BSP contains a family of major proteins, designated BSP-A1/-A2 and BSP-A3, with apparent molecular masses, ranging from 15 to 17 kDa, and the BSP-30 kDa protein with molecular mass of 28–30 kDa, BSP make up 40–50% of the total proteins in domestic bulls.

Aim of the current study was to found a link between seminal proteins and sperm characteristics, freezing ability and field fertility of bovine and buffalo bulls as prediction tool for fertility of bovine and buffalo bulls.

MATERIALS AND METHODS:

This study was carried out at the International Livestock Management Training Center (ILMTC), Sakha, belonging to the Animal Production Research Institute, Ministry of Agriculture.

Animals:

Five Holstein and four buffalo bulls were used in this study. All bulls were healthy and clinically free of external and internal parasites. Palpation of the external genitalia of these bulls showed that they were also typically normal. The testicular tone was glandular, almost similar in size and moved freely up and down within the scrotal pouches.

Semen collection and evaluation:

Bulls were sexually prepared by teaser bull and semen was collected using artificial vagina. Two frequent ejaculates at the same day were collected twice weekly from each bull. Immediately after collection, ejaculates were measured (semen volume) and held in a water bath at 35-37°C. Ten and eight ejaculates were collected from each buffalo and bovine bull, respectively. Semen was evaluated pre- and post-freezing for motility, livability, abnormality and acrosome status.

Semen was centrifuged directly for seminal plasma separation. Semen was diluted at a rate of 20 x 10⁶ life sperm per straw with Tris-egg yolk extender (3.025 g Tris; hydroxymethyl amino methane, 1.675 g citric acid, 0.75 g glucose, 15 ml egg yolk, 7 ml glycerol, 0.005 g streptomycin, and 0.25 mg lincomycin) and completed with double distilled water up to 100 ml. The Tris-egg yolk extender was gently mixed and warmed up to 37°C in a water bath during semen extension. The extension of ejaculates with tris-egg yolk was gradual for further processing and freezing. Vials

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containing the extended semen were placed in a water bath at 37°C and cooled gradually in a refrigerator at 5°C for 4 hours as an equilibration period.

At the end of equilibration period, the extended semen was loaded in 0.25 ml labeled French straws containing about 20 x10⁶ motile sperm using a semen filling machine fixed in cooling cabinet at 5°C. Straws were transferred into a processing canister and located horizontally on static nitrogen vapor 4 cm above the surface of liquid nitrogen for 10 minutes. Straws were then placed vertically in a metal canister and immersed completely in liquid nitrogen container for storage at –196°C.

For semen thawing, straws were dipped into a water bath at 35°C for 30 seconds. The percentage of live sperm was carried out according to Hancock (1951), while assessment of the percentage of intact acrosome was conducted as indicated by Watson (1975).

Quantitative and qualitative estimation of seminal plasma proteins:

**Extraction of seminal plasma proteins:**

Protein profile comparison was performed using Sodium Dodecyl Sulphate (SDS-PAGE). All the chemicals used for SDS-PAGE were purchased (UK Ltd. Chalfont Bucking Hampshire, UK).

Then, fresh seminal plasma samples were thawed, centrifuged at 10,000 g for 60 min at 41°C. Protein was extracted in rehydration lysis buffer [7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 20 mM DTT]. Then the seminal plasma proteins were precipitated for 2 h at -20°C with four volumes of ice-cold acetone and centrifuged for 15 min at 8000 g, washed thrice with cold acetone followed by re-centrifugation to recover the pellet. The air-dried seminal plasma protein pellet was re-suspended in lysis buffer and re-centrifuged to remove any non-dissolved sample. The aliquots were stored at -80°C until use.

**Determination of protein molecular weight (SDS-PAGE) by electrophoresis:**

Nine frozen seminal plasma samples (5 samples from Holstein bulls and 4 from buffalo bulls) were thawed at room temperature. For analytical gels, each of the seminal plasma samples was adjusted to 200 µg of total protein in 100 µl of rehydration buffer solution [7M urea, 2M thiourea, 20 mM DTT, 0.5% (v/v) IPG buffer at pH3–10]. Next, the mixture was cup-loaded on to their SDS-PAGE in 15% polyacrylamide. Samples were concentrated at 70 V for 10 min; separation was performed at 120 V for 4 h. Gels were stained with Coomassie Brilliant Blue, G-250 (Asadpour, 2012).

Density of the seminal protein bands are transformed into numerical score as follow: 0= not clear, 1= light color band, 2= medium color band, 3= high color band and 4= very high color band for statistical analysis.

**Statistical analysis:**

Statistical analysis was carried out using the Statistical Package for Social Sciences programme (SPSS), version 15.00, software for windows (SPSS Inc. Chicago, IL, USA). Statistical analysis was performed after arcsine transforming the percentage values. The significant differences were set at P<0.05 level using Duncan Multiple Range Test (Duncan, 1955).

**RESULTS AND DISCUSSION**

**Effect of freezing on post thaw semen parameters:**

Buffalo semen:

Semen characteristics of buffalo bulls before freezing are shown in Table 1. Results showed that ejaculate volume averaged 3.2±0.19 ml, while percentages of motility, livability, abnormality and intact acrosome of spermatozoa were 58.7±3.13, 60.5±2.94, 16.7±2.05 and 95.0±0.50%, respectively. Significant (P<0.05) individual differences were detected with respect to all physical sperm characteristics in pre- and post-thawed semen. Similar results due to freezing-thawing were previously reported by Mahmoud et al. (2013), who showed that variation between bulls were clear in post-thawing semen motility and live sperm percentage. They added that fresh buffalo semen volume and sperm concentration varied significantly (P<0.05) between bulls, while sperm motility and livability percentages were not affected in post-thawed semen.

Percentage of sperm with intact acrosomes averaged 95.0±0.50 and 88.8±1.26% in fresh and frozen semen. Freezing of bad quality semen significantly decreased of both live sperm count, their advanced motility and the intactness of acrosome. These results are in agreement with Kumar et al. (2014), who mentioned that intactness of sperm acrosome of fertile bulls was significantly higher than sub-fertile bulls. Additionally, Singh et al. (2014) found that sperm motility had a high significant correlation with first service of conception.

**Table 1. Semen characteristics of pre- (fresh) and post-thawed buffalo semen.**

<table>
<thead>
<tr>
<th>Semen parameters</th>
<th>R</th>
<th>Fresh</th>
<th>PF</th>
<th>PT</th>
<th>Fresh</th>
<th>PT</th>
<th>Fresh</th>
<th>PT</th>
<th>Overall mean</th>
<th>Fresh</th>
<th>PT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume(ml)</td>
<td>10</td>
<td>2.85a</td>
<td>.25a</td>
<td>-</td>
<td>2.9a</td>
<td>.28a</td>
<td>-</td>
<td>.17a</td>
<td>-</td>
<td>.38a</td>
<td>-</td>
</tr>
<tr>
<td>Motility %</td>
<td>10</td>
<td>66.0±6.1</td>
<td>35.±5</td>
<td>-</td>
<td>74.0±6.1</td>
<td>30.±5</td>
<td>-</td>
<td>72.1±4.5</td>
<td>34.6±4.1</td>
<td>32.2±7.2</td>
<td>7.2±1.7</td>
</tr>
<tr>
<td>Life sperms %</td>
<td>10</td>
<td>68.±6.1</td>
<td>35.±1</td>
<td>-</td>
<td>69.0±3.2</td>
<td>32.±3</td>
<td>-</td>
<td>72.1±4.5</td>
<td>34.6±4.1</td>
<td>32.2±7.2</td>
<td>7.2±1.7</td>
</tr>
<tr>
<td>Abnormalities</td>
<td>10</td>
<td>1.3±8.1</td>
<td>1.3±9.1</td>
<td>-</td>
<td>15.2±14.7</td>
<td>15.6±15.2</td>
<td>-</td>
<td>29.8±25.8</td>
<td>16.2±17.5</td>
<td>25.8±28.9</td>
<td>16.2±17.5</td>
</tr>
<tr>
<td>Intact acrosome %</td>
<td>10</td>
<td>92.±5</td>
<td>95.±3</td>
<td>-</td>
<td>96.±2</td>
<td>76.±4</td>
<td>-</td>
<td>94.5±1.5</td>
<td>91.5±1.5</td>
<td>96.9±2</td>
<td>74.9±2</td>
</tr>
</tbody>
</table>

Means denoted within the same row for pre- or post-thawed semen with different superscripts are significantly different at P<0.05. * Significant difference between pre- and post-thawed semen for each bull. Number of replicates for each bull was 10 ejaculates.

**Bovine semen:**

Table (2) for bovine semen showed that semen volume averaged 198±0.19 ml, while percentages of motility, livability, abnormality and intact acrosome of spermatozoa were 66.8±2.16, 71.0±1.94, 13.8±1.39 and 92.2±0.96%, respectively. The differences among bulls
was significant (P<0.05) in fresh semen. This results agreed with that of Ray and Ghosh (2013), who noticed that bulls varied significantly (P<0.05) in all their ejaculate characteristics. Moreover, hypo-osmotic swelling (HOS) test significantly differed (P<0.01) and positively correlated with percentage of progressive motile and live sperm.

Marked reduction in all semen characteristics were observed in post-thawed as compared to fresh semen. Chowdhury et al. (2014) found that the overall values of post-thawing intact acrosome sperms were 73.7±0.31% with no significant difference among the cattle breeds, while Ray and Ghosh (2013) noticed a negative correlation between HOS test and both abnormal sperm and intact acrosome.

Table 2. Semen characteristics in pre- (fresh) and post-thawed bovine semen.

<table>
<thead>
<tr>
<th>Semen parameters</th>
<th>R Fresh</th>
<th>PT Fresh</th>
<th>604 Fresh</th>
<th>PT</th>
<th>1502 Fresh</th>
<th>PT</th>
<th>1505 Fresh</th>
<th>PT</th>
<th>6690 Fresh</th>
<th>PT</th>
<th>Overall mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>5±</td>
<td>5±</td>
<td>5±</td>
<td>5±</td>
<td>5±</td>
<td>5±</td>
<td>5±</td>
<td>5±</td>
<td>5±</td>
<td>5±</td>
<td>5±</td>
</tr>
<tr>
<td>Motility %</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
</tr>
<tr>
<td>Life sperms %</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
</tr>
<tr>
<td>Abnormalities %</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
</tr>
<tr>
<td>Intact acrosome</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
<td>90±</td>
</tr>
</tbody>
</table>

Means denoted within the same row for pre- or post-thawed semen with different superscripts are significantly different at P<0.05. Significant difference between pre- and post-thawed semen for each bull. Number of replicates for each bull was 8 ejaculates.

Expression of seminal plasma proteins

The total pattern of proteins precipitated from bovine and buffalo seminal plasma was electrophoresed on SDS-PAGE fraction. The electrophoretic patterns revealed informative polymorphism in the number and intensity of bands concerning both species. Banding patterns were analyzed by SDS-PAGE (Fig. 1), which revealed the presence of four different proteins with apparent molecular masses of 14 kDa, 15 kDa, and 30 kDa for buffalo and bovine seminal plasma proteins. Two bands, BSPFA1-A2 related to 14 and 15 KDa and BASFA3 related to 30 KDa were observed, respectively (Fig.1).

Expression of seminal plasma proteins in the buffalo bull:

Seminal plasma protein bands expressed in the buffalo bull showed different proteins of apparent molecular masses, 14 kDa, 15 kDa, and 30 kDa Known collectively as BSA-A1/A2 and BSA-A3. The expression of BSA-A1/A2 and BSA-A3 were very high (very dark bands) in bull No. 198 and 219. While BSA-A1/A2, showed dark (high expression) in bull No. 90 and 85. Otherwise, BSA-A3 protein showed high expression (dark band) in bull No. 90, however, its expression was faint (low expression) in bull No. 85 as shown in Figure (1).

Fig. 1: Electrophoretic pattern of seminal plasma proteins of bovine and buffalo bulls.
Correlation between sperm parameters and seminal plasma protein expression:

Expression density of seminal plasma protein bands (BSA-A1, A2, A3 and BSA-30) was positively correlated with sperm motility and livability in both fresh and frozen-thawed semen of buffalo and bovine bulls (Killian et al., 1993; Bellin et al., 1998). In fresh semen, a highly significant negative correlation (P<0.01) between expression of BSA-A30 with sperm abnormalities and the integrity of the acrosome in buffalo bull is shown in Table 1. Based on protein expression, Chacur, (2012) noticed that bands for BSA-A1-A2 and BAS-A3 were related to low fertile bull, where bands of BSA-30 KDa was related to high fertility, which could have strong inhibitory effects on gene expression in sensitive buffalo bull genotypes. Those particular bands could be considered as a positive biochemical marker associated with fertility in the buffalo bull. While, in fresh bovine semen, negative significant correlations were found between sperm abnormalities and expression of BSA-A1 and BSA-A30 (P>0.01). These results are agreeable with the previous results of Veronica and Puttaswamy (2000), where they indicated that the BSA-A1, BSA-A2, BSA-A3 and BSA-30 KDa proteins expression were enhanced in fertile bovine seminal plasma. Moreover, post-thaw semen characteristics were significantly correlated (positively in case of motility and livability and negatively in case of sperm abnormalities and acrosomal integrity) with the expression of seminal plasma proteins (Table 3). In agreement with our results Sharma et al. (2012) found highly significant (P<0.01) correlation between various semen evaluation parameters except for the gross morphological abnormalities. However, a significant (P<0.05) negative correlation was found between the acrosome integrity and gross morphological abnormalities as reported by Chowdhury et al. (2014).

In contrast to the present results of the current study, Veronica and Puttaswamy (2000) noticed that protein expression did not differ (P>0.05) with fertility grouping, and the majority of the functional proteins were highly expressed in seminal plasma of non-cryopreserved semen, while the cryopreserved semen contained mainly structural/extender derived proteins. In this aspect, earlier studies also showed that the seminal plasma proteins of mammals play a significant role in several essential steps such as sperm motility (Henricks et al., 1998; Kawano et al., 2004). Differences in their structures, relative abundance and patterns of expression appear to determine species-specific effects of homologous proteins (Calvete and Sanz, 2007). Seminal plasma proteins differ slightly in functionality related to their source (Rodríguez Martínez et al., 2011). Moreover, the cryopreservation extender used may have different effects on the loss of sperm surface proteins after even brief storage periods in liquid nitrogen (Lessard et al., 2000). Screening for presence of seminal plasma proteins can be included in the regular breeding soundness examination for selection of bulls (Karunakaran and Devanathan, 2016).

Table 3: Correlation between seminal proteins and different semen parameters in both buffalo and bovine bull semen

<table>
<thead>
<tr>
<th>Semen parameter</th>
<th>Buffalo bulls</th>
<th>Bovine bulls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BSA-A1</td>
<td>BSA-A02</td>
</tr>
<tr>
<td>Volume, ml</td>
<td>0.04</td>
<td>0.4</td>
</tr>
<tr>
<td>Sperm Motility, %</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Livability, %</td>
<td>0.46</td>
<td>0.46</td>
</tr>
<tr>
<td>Sperm abnormality, %</td>
<td>-0.17</td>
<td>-0.17</td>
</tr>
<tr>
<td>Intact acrosome, %</td>
<td>-0.24</td>
<td>-0.24</td>
</tr>
<tr>
<td>Post-thaw motility, %</td>
<td>0.48</td>
<td>0.48</td>
</tr>
<tr>
<td>Post-thaw livability, %</td>
<td>0.46</td>
<td>0.46</td>
</tr>
<tr>
<td>Post-thaw sperm abnormality, %</td>
<td>-0.48</td>
<td>-0.48</td>
</tr>
<tr>
<td>Post-thaw intact acrosome, %</td>
<td>-0.38</td>
<td>-0.38</td>
</tr>
</tbody>
</table>

*Correlation coefficients (r) are significantly different at P<0.05.
** Correlation coefficients (r) are significantly different at P<0.01.

CONCLUSION

It could be concluded that seminal plasma proteins can be used as potential markers of sperm freezability and fertility of bovin and buffalo bulls. Further studies are needed for quantitative determination of seminal plasma proteins and fertility of bovine and buffalo bulls which might be better than the qualitative method.

REFERENCES


"بروتينات بلازما السائل المنوي كواسمات للصلاة بالخصائص السائل المنوي في الإيقاع والجاموس "

"عبيالجواند خليقة قادمة، كركى السيد الغربي، أمين فؤاد عبد الهادي عاشور و الشناوي محمد الصيفي

معهد الاباحي الحيوانات - مركز البحوث الزراعية - وزارة الزراعة

يهدف هذا الدراسة إلى الوصول إلى رابط بين البروتينات وخصائص السائل المنوي لكل من الطائق الجاموسي والبقرى. وقد تم استخدام خمس طرق قبلي وأربع جاموسى لتجميع أصابع السائل المنوي من كل من هذه الطائقين (10 إصابة) على التوالي. قياس السائل المنوي (10 إصابة) لل待ち أن القصي الجاموسي لهذه البروتينات وحود زرم BSA-(A1, A2, A3) & BSA-(A1, A2, A3). 

من متابعة وتحليل الأحزمة المنوية بالإضافة إلى وجود ارتباط مناعي مجري مع هذه البروتينات مع الحركة والاحتلالات النهائية سواء قبل أو بعد التجربة وتشمل دوافع مع خصائص الشحوم السائل المنوي في كل من الطائق الجاموسي والبقرى. ومن هذه النتائج أن تكون فرصية للبحث في تقييم السائل المنوي لل菅_additional_text leftover