Chapter 10

# Seminal Plasma Proteins as Potential Markers of Relative Fertility in Zebu Bulls (*Bos taurus indicus*)

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http://dx.doi.org/10.5772/45758

## 1. Introduction

Progress has been made in developing reliable indicators of ejaculate quality that allow exclusion of low quality ejaculates for use in artificial insemination (AI). Physical semen characteristics and sperm morphology measurements are not always indicative of fertility and reproductive performance in animals, and accurate and predictive genetic and protein markers are still needed (Foxcroft et al., 2008). Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) represents a valuable tool for the separation and characterization of proteins from complex biological samples (Killian, 1992; Killian et al., 1993).

In the case of dairy sires, data sets are avaliable to assess fertility of individual males based on insemination of large numbers of cows using semen that has been frozen by standardizing procedures. This information has been used to demonstrate that fertility associated proteins exist in bull seminal plasma (Killian et al., 1993). Seminal plasma is composed of secretions from the male accessory sex glands and epididymis, which contains many organic and inorganic components that have effects on sperm quality (Foxcroft et al., 2008). The proteins secreted into seminal plasma may play an important role during sperm capacitation and fertilization (Rodriguez-Martinez et al., 1998), and may also serve to protect sperm from damage or to maintain their longevity.

Specific proteins in seminal plasma have been identified as potential markers of male fertility or infertility in the human (Martinez-Heredia et al., 2008; Yamakawa et al., 2007). Comprehensive proteomic analyses have been conducted in the bull (Moura et al., 2006a; Moura et al., 2006b; Chacur et al., 2010a; 2011a). The literature on the effects of seasons on the semen characteristics and upon seminal plasma proteins in Nellore (*Bos taurus indicus*) cattle under natural conditions in Brazil has already been recently studied in Brazil (Chacur et al., 2006b; 2007; 2010b; 2011a).

There is evidence revealing that seminal plasma prevents premature capacitation of sperm (Eng & Oliphant, 1978) and protects sperm from peroxidative damage (Jones et al., 1979; Schönech et al., 1996). Its well known that low temperatures alter the function of spermatozoa (Watson, 1995). Cold shock results in the destabilization of sperm membranes and impairment of sperm function, and it is also well known that animal spermatozoa are sensitive to cold-shock stress as the bull (Schönech et al., 1996).

Seminal plasma has also been shown to have deleterious effect on bovine sperm during semen storage at ambient temperatures and a damaging effect during semen cooling and freezing. Recent studies have shown that proteins from bovine seminal plasma (BSP) may modulate sperm properties. Two proteins (26kDa, pI 6.2; 55kDa, pI 4.5) predominate in higher-fertility bulls and two proteins (16kDa, pI 4.1; 16 kDa, pI 6.7) predominate in lower-fertility bulls. The major protein fraction of bovine seminal plasma is represented by three acidic proteins, designated as BSP-A1/-A2, BSP-A3 and BSP-30kDa (collectively called BSP proteins). These proteins are secretory products of the seminal vesicles and ampullae, and their biochemical characteristics have been well-described (Moura et al., 2006a; 2006b).

Although many autors believe that the seminal plasma proteins may function to stabilize the sperm against premature capacitation and spontaneous acrosome reaction. Significantly, these proteins may also protect the sperm from cooling-induced damage, such as cryocapacitation. The establishment of 2-D PAGE reference map could represent a usefull tool for the study of the still poorly understood nature and functions of the seminal plasma proteins, for the identification of previously unknown proteins, and for the comparison of seminal plasma protein composition between males of differing fertility. Many autors believe that the bull seminal plasma contains fertility associated protein markers. Comparison of individual 2-D PAGE maps with the reference map could provide a useful key to relate protein pattern changes to some physiopathological events influencing the reproductive sphere (Mortarino et al., 1998).

The present chapter describes the use of electrophoresis in animal reproduction: Electrophoresis and fertility in animals; Approaches to use of electrophoresis; Effect of seasonal changes in seminal plasma proteins of zebu bulls (*Bos taurus indicus*).

## 2. Electrophoresis and fertility in animals

The development of genetic markers to identify bulls of high breeding value represents one of the ways of genetic gain achievement in dairy farming. Several studies have been performed in an attempt to uncover the relationship between semen quality and fertility (Linford et al., 1976; Saacke et al., 1994). Thus, sperm morphology and motility, the number of sperm of sperm per insemination, percentage of acrosome-reacted sperm, and *in vitro* fertilization have been extensively evaluated as an indication of sperm ability to fertilize an egg. Evidence suggests that seminal plasma, which is a complex mixture of secretions originating from the testis, epididymis, and accessory glands, contains factors that modulate the fertilizing ability of sperm (Amann & Griel, 1974; Henault et al., 1995).

Recent studies have shown that proteins from bovine seminal plasma (BSP) may modulate sperm properties (Killian, 1992; Bellin et al., 1994). Two proteins (26kDa, pI 6.2; 55kDa, pI 4.5) predominate in higher-fertility bulls and two proteins (16kDa, pI 4.1; 16 kDa, pI 6.7) predominate in lower-fertility bulls (Killian et al., 1993).

### 2.1. Electrophoresis and seminal plasma

Secretions from the accessory sex glans are mixed with sperm and ejaculation and contribute to the majority of semen volume and components. Some accessory sex glands proteins are known to bind to the spermatozoa membrane and affect its function and properties (Yanagimachi, 1994).

Mammalian seminal plasma is constituted by secretions of the male accessory glans in which the spermatozoa are suspended in semen. Seminal plasma is an extremely complex fluid containing a wide variety of both organic and inorganic chemical constituents, among which only proteins present high molecular masses. The protein composition varies from species to species but in all the cases investigated so far these components have important effects on sperm function (Killian et al., 1993). Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) represents a valuable tool for the separation and characterization of proteins from complex biological samples. Seminal plasma, a physiological secretion from multiple glands of the male reproductive tract, is the natural medium for maturation of the spermatozoa through hormonal, enzymatic, and surface-modifiyng events (Killian et al., 1993).

The establishment of a 2-D PAGE reference map could represent a useful tool for the study of the still poorly understood nature and function of the seminal plasma proteins, for the identification of previously unknown proteins, and for the comparison of seminal plasma protein composition between males of differing fertility. In particular, it seems likely that bull seminal plasma contains fertility-associated protein markers (Killian et al., 1993).

## 3. The use of the SDS-PAGE and 2-D PAGE

### 3.1. Proteins of the male accessory glands and fertility

In the case of dairy sires, data sets are avaliable to assess fertility of individual males based on insemination of large numbers of cows using semen that has been frozen by standardizing procedures. This information has been used to demonstrate that fertility associated proteins exist in bull seminal plasma (Killian et al., 1993).

The ability to analyze the components secreted exclusively from the accessory sex glands has provided unique information about proyeins they secret and that are correlated with fertility indexes. Relating expression levels of specific proteins to fertility phenotype should serve as a sound foundation to evaluate their role in sperm function and fertilization (Moura et al., 2006b).

### *3.1.1. Heparin binding proteins (HBPs)*

Heparin, a commercially available, sulfated glycosaminoglycan, induces capacitation/ acrosome reactions in sperm from bulls (Lenz et al., 1983). Sperm from high-fertility bulls have a greater frequency of acrosome reactions in response to heparin-like material (Lenz et al., 1988). Heparin-binding proteins (HBPs) are produced by the male accessory glands, secreted into seminal fluid (Nass et al., 1990). Fertility for Group 1 (HBP-B5) in sperm membranes but undetectable HBP-B5 in seminal fluid) was 82% of 1,692 cows. Group 2 (HBP-B5 detectable in seminal fluid as well as in sperm membranes) had 67% of 919 cows pregnant. Group 3 (detectable HBP-B5 in the seminal fluid and undetectable HBP-B5 in the sperm membranes) and Group 4 (undetectable HBP-B5 in the seminal fluid and sperm membrane) had 63% of 747 and 1,208 cows pregnant, respectively (Bellin et al., 1994). These trials indicated that grouping bulls according to the presence or absence of the greatest affinity herarin-binding protein (HBP-B5) on sperm membranes and in seminal fluid was an effective means of identifying fertility potential. Thus, understanding the protein composition of sperm membranes can be directly applicable to field situations and result in a more efficient production of calves (Bellin et al., 1994).

Other studies have shown that accessory glands produced and secreted HBP into seminal fluid , and HBP bound to sperm at ejaculation (Miller et al., 1990; Nass et al., 1990). Miller et al. (1990) reported that HBP constituted 28% of the protein component of seminal fluid or approximately 19.6 mg HBP/mL of ejaculate, and HBP-B5 constituted 6% of the total HBP in fluid from vasectomized bulls. The average concentration of total HBP represents 19.2 mg and 0.14 mg per mL of ejaculate in bovine seminal plasma and sperm membrane, respectively (Bellin et al., 1994). Although all HBPs may bind to sperm surfaces (Bellin et al., 1996). The concentration of the 30-kDa HBP, namely fertility associated antigen (FAA), on bovine sperm has been paired with a greater fertility potential (Bellin et al., 1998). FAA represents 0.8% of total HBP and has been identified as a deoxyribonuclease I-like protein (McCauley et al., 1999).

Studies have shown that these BSP proteins facilitate capacitation by promoting cholesterol efflux from sperm membranes (Thérien et al., 1998). Because sperm membrane cholesterol has an important role in modulating membrane bilayer fluidity and stability (Bloch, 1985; Yeagle, 1985), its efflux may perturb membrane structure and thereby lead to capacitation (Davis, 1981; Ehrenwald et al., 1988). In the context of sperm cryopreservation may lead to a decrease in sperm resistance to cold shock (Darin-Bennett & White, 1977; White, 1993). Thus, changes induced by the BSP proteins in the sperm membrane may have influence on sperm fertilizing ability and the success of the cryopreservation process (Nauc & Manjunath, 2000).

The characterization of seminal plasma proteins including osteopontin (OPN) 55 kDa and the study of their binding proteins (HBPs) will be the first step in understanding their role in the fertilization and identification of HBPs would provide information that could improve the knowledge of this aspect of reproductive physiology in Nellore bulls (Fernandes et al., 2008). In Nellore bulls (HBPs) bands with molecular weights ranging from 15 to 63 kDa were observed: 15 kDa, 17 kDa, 22 kDa, 25 kDa, 39 kDa, 53 kDa, 58 kDa and 63 kDa (Fernandes et al., 2008).

### *3.1.2. Spermadesin*

In dairy cattle the average intensity of spermadesin (14 kDa) was higher in bulls of low fertility. The most basic isoform of accessory sex gland fluid is equivalent to the one originally found as an antifertility factor in the seminal plasma (Moura et al., 2006b). Z13 is a seminal plasma protein made up of two disulfide-linked 13-kDa subunits. The data indicate that the protein is a dimer of 26 kDa in native conditions and a monomer of 13 kDa in the presence of reductants. Therefore it antifertility peptide reported by can be suggested that Z13 presents at least one intermolecular S-S bridge (Tedeschi et al., 2000).

The intensity of apermadhesin Z13 in AGF showed an inverse relationship with fertility (Moura et al., 2006b). The low-molecular-weight antifertility peptide reported by Killian et al. (1993) in the seminal plasma of Holstein bulls was also identified as spermadhesin Z13. The isoform originally described in the seminal plasma by those authors appears to be more basic (pI 6.7) than the ones found in the AGF as antifertility factors pI 6.5 and 6.3 (Moura et al., 2006b).

The 2-D PAGE reference map of bull seminal plasma proteins provides information about the presence, in this particular fluid, of polypeptides of specifical biological significance (Mortarino et al., 1998). The PDC-109 represents more than 30% of the total protein contained in bull seminal plasma (Manjunath & Sairam, 1987). Comparison of individual 2-D PAGE maps with the reference map could provide a useful key to relate protein pattern changes to some physiopatological events influencing the reproductive sphere (Mortarino et al., 1998).

Spermadhesin Z13 is a peptide yhat displays 50% and 43% homology with the acidic seminal fluis protein and seminal plasma motility inhibitor (SPMI), respectively (Tedeschi et al., 2000). The former has positive effects on bovine sperm *in vitro* when as average concentratons, but it can inhibit both sperm motility and mitochondrial activity when at high levels (Schoneck et al., 1996).

### *3.1.3. Family of proteins (BSP proteins)*

Bovine seminal plasma contains a family of proteins designated BSP-A1/-A2, BSP-A3, and BSP-30-kDa (collectively called BSP proteins). These proteins are secretory products of seminal vesicles that are acquired by sperm at ejaculation, modifying the sperm membrane by inducing cholesterol efflux. Because cholesterol efflux is time and concentration dependent, continuous exposure to seminal plasma that contains BSP proteins may be detrimental to the sperm membrane, which may adversely affect the ability of sperm to be preserved (Manjunath et al., 2002). These proteins coat the surface of the spermatozoa after ejaculation and are believed to play an important role in membrane modifications occurring during capacitation. Isoforms of each BSP protein were found when purified iodinated proteins analysed by 2D-PAGE. BSP-A1was found at a M(r) of 16.5 kDa and in the range of pI of 4.7-5.0; BSP-A2 at 16 kDa and at a pI of 4.9-5.2; BSP-A3 at 14 kDa and a pI of 4.8-5.2, and BSP-30-kDa at 28 kDa and at a pI of 3.9-4.6 (Desnoyers et al., 1994). BSP protein are

acidic and have several isoforms. Furthermore, they heterogeneity of BSP-30-kDa is mainly due to its sialic acid content (Desnoyers et al., 1994).

The concentration of BSP-A1/-A2 was much higher compared with other seminal plasma proteins, and this protein alone represented a average of 38% of the total protein fraction, whereas BSP-A3 and BSP-30 kDa represented 3% to 4% of the total protein fraction (Nauc et al., 2000). The determination of BSP protein content on sperm surface may be an index of individual bull fertilizing ability or post-thaw status of sperm membranes (Nauc et al., 2000).

#### *3.1.4. Osteopontin (OPN)*

Osteopontin (OPN) is an acidic glycoprotein of about 41.5 kDa that has been isolated from rat, human and bovine bone. It is rich in aspartic acid, glutamic acid and serine and contains about 30 monosaccharides, including 10 sialic acids (Butler, 1989). The 55 kDa protein, shown to be more prevalent in higher-fertility males, was determined to be osteopontin (OPN) (Cancel et al., 1997). Results of immunofluorescence analyses of the male reproductive tract paralleled results for tissue extracts and fluids, indicating that bovine OPN is secreted by the ampulla and seminal vesicle. Tissue sections of the testis, epididymis (caput, corpus, cauda), vas deferens, prostate and bulbourethral gland were negative when reacted with antibodies against bovine seminal plasma OPN (Cancel et al., 1999).

Brown et al. (1992) studied the expression of OPN in normal adult human tissues. They showed that OPN was present on the luminal surfaces of epithelial cells of the gastrointestinal tract, gall bladder, pancreas, urinary and reproductive tracts, lung, bronchi, mammary and salivary glands, and sweat ducts. In general Brown et al. (1992) found that OPN accumulated on surfaces of epithelia bordering the luminal compartment.

Bulls with the highest fertility scores had 2.3 times more of a 55 kDa osteopontin than bulls with above-average fertility and at least 4 times more than bulls with below average (Moura et al., 2006b). A secreted form of phospholipase $A_2$ ($PLA_2$) 58 kDa present in the accessory gland fluid was more prevalent in bulls of high fertility (Moura et al., 2006b).

### 3.2. Proteins of the cauda epididymal fluid and fertility

Factors isolated from epididymal fluid or epithelial cells in culture have been linked to sperm motility and protection of membranes against damage caused by cryopreservation (Reyes-Moreno et al., 2002), anticapacitation effects (Roberts et al., 2003), or sperm number (Gatti et al., 2004), but evidence linking epididymal proteins to male fertility indexes is limited (Moura et al., 2006a).

Because most proteins from the rete testis are not present in the milieu of the epididymis lumen (Olson & Hinton, 1985; Dacheux et al., 1989), there is a general assumption that proteins of the epididymal fluid are mainly the product of the epididymis itself. Numerous proteins have been detected in the epididymal milieu of mammalian species (Cornwall et

al., 2002; Dacheux & Dacheux, 2002) but the exact roles of most of them in sperm maturation are yet to be determined (Gatti et al., 2004).

Immature spermatozoa newly formed in the seminiferous tubules have a period of transit through the epididymis where they become motile and undergo a series of events that include changes in the composition of membrane lipids and proteins (Sullivan et al., 2005). The epididymal epithelium secretes proteins that potentially affect not only sperm maturation (Dacheux and Dacheux, 2002) but also other aspects of sperm physiology while these cells are stored in the cauda compartment (Hinton et al., 1995).

Fluid produced by the epididymis is diluted about 8- to 10- fold when mixed with accessory sex glands secretions at ejaculation (Gerena et al., 1998). This makes it difficult to accurately identify epididymal proteins in the seminal plasma milieu, particularly those secreted in low abundance or if they are also secreted by other organs, such as the accessory sex glands (Moura et al., 2006a).

An average of 118 spots was detected in the 2-D maps of the cauda epididymal fluid (CEF) in Holstein bulls. The intensity of alfa-L-fucosidase and cathepsin D was 2.3- 2.4-fold greater in high-fertility bulls than in low-fertility bulls (Moura et al., 2006a). The intensity of 3 isoforms (24-27 kDa, pI 6.3-5.8) of prostaglandin D-synthase (PGDS) were from 3.2 to 2.2 fold greater in low-fertility sires. The findings suggest that molecular markers of male fertility are associated with both epididymal sperm physiology and postejaculation eventus regulated by accessory sex gland components (Moura et al., 2006a). PGDS could influence male fertiling by mediating the action of hydrophobic molecules on sperm during epididymal transit or cauda epididymal storage (Moura et al., 2006a).

P25b, a protein with predictive properties for bull fertility, is transferred from prostasome-like particles present in the cauda epididymal fluid (PLPCd) to the sperm surface. The pattern of distribution of the PLPCd transferred varied from one sperm cell to the other, with a bias toward the acrosomal cap (Frenette et al., 2002).

## 4. Seasonal variation of zebu bull seminal plasma proteins

### 4.1. Heat-shock proteins and seminal quality in (Bos taurus indicus)

There were a number of suggestions in the earli er literature exposed to heat can produce sperm which do not produce normal offspring in unexposed females (Setchell, 1998). Bulls were subjected to scrotal insulation for 48 hours and semen collected and cryopreserved 2 or 3 weeks later, Following *in vitro* fertilization with swin-up sperm from these samples, there were decreased rates of spermpenetration, pronuclear formation (Walters et al., 2006) embryo cleavage, development and blastocyst formation (Walters et al., 2005) with semen collected from two of the bullsthree weeks after the insulation, but not which semen from two others bulls, or with semen collected after two weeks.

In bulls the heat is lost from the testis and scrotum to the environment through the scrotal skin, which is well endowed with sweat glands (Setchell, 2006). The temperature on the

surface of the scrotum is lower at its base than near the neck, but the temperature inside the testis is almost uniform, even slightly warmer at the base (Kastelic et al., 1996).

There is considerable variation between individual animals in their response to heat exposure. Of the six bulls subjected to scrotal insulation by Vogler et al. (1993), two showed a large increase in abnormal spermatozoa (to more than 60%) whereas others had as few as 23% abnormal cells. Likewise, 4 bulls used for semen collection for in *in vitro* fertilization showed widely variable effects of 48h scrotal insulation on pronuclear formation, embryo development and apoptosis, with two bulls classed as severe responders, one a moderate responder and one showing no response to scrotal insulation (Walters et al., 2006).

Progress has been made in developing reliable indicators of ejaculate quality that allow exclusion of low quality ejaculates for use in artificial insemination (AI). Physical semen characteristics and sperm morphology measurements are not always indicative of fertility and reproductive performance in animals, and accurate and predictive genetic and protein markers are still needed (Foxcroft et al., 2008).

There is evidence revealing that seminal plasma prevents premature capacitation of sperm (Eng & Oliphant, 1978) and protects sperm from peroxidative damage (Jones et al., 1979; Schönech et al., 1996). It`s well known that low temperatures alter the function of spermatozoa (Watson, 1995). Cold shock results in the destabilization of sperm membranes and impairment of sperm function, and it is also well known that animal spermatozoa are sensitive to cold-shock stress as the bull, rabbit and man (Schönech et al., 1996).

Specific proteins in seminal plasma have been identified as potential markers of male fertility or infertility in the human (Martinez-Heredia et al., 2008; Yamakawa et al., 2007). Comprehensive proteomic analyses have been conducted in the bull *Bos taurus taurus* (Moura et al., 2006b). The literature on the effects of seasons on the semen characteristics and upon seminal plasma proteins in Nellore and Tabapua (*Bos taurus indicus*) and Limousin, Brown-Swiss and Brangus cattle under natural conditions in Brazil has already been recently studied (Chacur et al., 2003; 2004; 2006a; 2006b; 2007; 2008; 2009; 2010a; 2010b; 2011a; 2011b; 2012).

## 4.2. Potential markers of relative fertility in *Bos taurus indicus*

*Bos indicus* bulls are less sensitive to the effects of high temperatures than *Bos taurus* or crossbred bulls, but as they are actually more sensitive to the effects of scrotal insulation (Brito et al., 2003). This would appear to be due to the greater ability of *indicus* animals to keep their testes cool (Brito et al., 2002). *Bos indicus* bulls have greater testicular artery length to testicular volume ratios, and smaller testicular artery wall thickness and arterial to venous distances, which may be responsible for greater cooling of the arterial blood in the spermatic cord (Brito et al., 2004).

In Brazil sixty-eight Nellore (*Bos indicus)* bulls were used, with twenty of the padron variety and forty-eight of the mocho variety with mean of 4 years old. There was no difference ($P>0.05$) for the spermatic morphology between padron and mocho variety, respectively

with 5.06±8.20% and 5.32±6.40% of major defects; 9.91±6.7% and 8.36± 6.06% for minor defects; and 14.76±13.20% and 13.82±12.61% for the total defects. The electrophoresis of the seminal plasma showed protein bands with weights between 5- and 105-kDa. In 100% of the bulls with good semen the 13kDa protein were present, the same happens with the 18- and 20-kDa bands. The varieties padron and mocho revealed similar reproductive adaptation in front of the handling conditions and weather and looking very efficient (Chacur et al., 2006b).

Disruptions in sperm production include decreased sperm motility and increased of abnormal sperm. Seminal plasma appears to exert important effects on sperm function. The objective was to evaluate the dry and rainy season influence on the seminal characteristics and semen plasma proteins. Eleven bulls (*Bos taurus indicus*) with ages ranging from 34 to 38 months were submitted each one to 12 semen collect with eletroejaculation 6 on dry season and 6 on rainy season with 14 days interval, totalizing 144 samples. Qualitative and quantitative semen characteristics were evaluated. Samples of semen were centrifuged (1.500 g / 15 minutes) and conditioned and stored (–20ºC) until further processing. The proteins were extracted and quantified to electrophoresis performed. Variance analysis and Tukey test 5% was used. The semen vigor ($P<0.01$), minor defects and total defects ($P<0.05$) showed statistical difference between seasons, while the volume, motility and minor defects did not ($P>0.05$). The number of bands occurred between 6- and 125-kDa, see Table 1. The molecular band of 26 kDa was present in 100% of bulls in rainy season. The molecular bands of 6-, 9- and 125-kDa showed a high frequency in dry and rainy season. In conclusion, these results showed a band distribution variation throughout the season and the year seasons changed the semen quality with increase sperm vigor and reduction of abnormal sperm on dry season (Chacur et al., 2011b).

| bulls | Proteins (kDa) | Dry season (n=144) | Rainy season (n=144) |
|---|---|---|---|
| a, b, c, d, e, f, h, i, j, k, | 6 | 9/11 (81.81%) | 10/11(90.90%) |
| a, b, c, d, e, f, g, h, j, k | 9 | 10/11(90.90%) | 8/11(72.72%) |
| d, e, f, h, i, j, k | 12 | 7/11(63.63%) | 5/11(45.45%) |
| a, b, c, d, e | 13 | 5/11(45.45%) | 1/11(9.09%) |
| a, b, c, e, f, g, j | 17 | 3/11(27.27%) | 7/11(63.63%) |
| a, d, e, f, j | 20 | 3/11(27.27%) | 3/11(27.27%) |
| a, b, c, d, e, f, g, h, i, j, k | 26 | 6/11(54.54%) | 11/11(100%) |
| a, b, c, e, f, h, i, j, k | 35 | 4/11(36.36%) | 7/11 (63.63%) |
| a, b, e, f, g, h, j, k | 44 | 2/11(18.18%) | 8/11(72.72%) |
| a, c, d, e, f, h, i, j | 55 | 7/11(63.63%) | 3/11(27.27%) |
| b, d, h, i, k | 66 | 2/11(18.18%) | 4/11(36.36%) |
| a, c, d, e, f, i, j, k | 75 | 6/11(54.54%) | 6/11(54.54%) |
| a, b, c, i | 80 | 1/11(9.09%) | 4/11(36.36%) |

| bulls | Proteins (kDa) | Dry season (n=144) | Rainy season (n=144) |
|---|---|---|---|
| a, c, f, i, j | 105 | 4/11(36.36%) | 3/11(27.27%) |
| a, b, c, e, f, g, h, i, j, k | 125 | 9/11 (81.81%) | 10/11(90.90%) |

Chacur et al. (2011).

**Table 1.** Frequency of proteins bands in dry season (may-july) and rainy season (october-december).

Seminal plasma is a complex of secretions of the male accessory reproductive organs and appears to exert important effects on sperm function (Shivaji et al., 1990). The protein quality of the seminal plasma may affect positively the bulls' fertility (Killian *et al.*, 1993). Peptides of 55- and 66-kDa were present in bulls with excellent spermatic conditions for example motility and vigor. On the other hand, 16- and 36-kDa peptides were observed with unfavorable spermatic conditions (Chacur *et al.*, 2009). The objective was to determine the influence of season on seminal plasma proteins in Brown Swiss bulls. Semen from 33 Brown Swiss bulls 24 months of age were collected by electroejaculation during winter (from June to August) and summer (from December to February) in the southern hemisphere in 2008. Semen samples were collected with 14-day intervals totalizing 196 ejaculates. Samples of semen were centrifuged (1500*g*/15 min) and the seminal plasma was conditioned in cryotubes and stored at –20°C until further processing. Proteins were extracted from 200 μL of each sample in 2 mL of extraction buffer composed of 0.625 M Tris-HCl, at pH 6.8, in 2% SDS, 5% β-mercaptoethanol, and 20% of glycerol. Percentages of different plasma proteins by season were statistically compared by the chi-square test with significance level ($P < 0.05$). Proteins were quantified according to Bradford (1976) and electrophoresis was performed according to Laemmili (1970). Gels were fixed with isopropanol:acetic acid:water (4:1:5 v/v) for 30 minutes and stained in the same solution with 2% of Coomassie Blue R250. In 26 bulls, the absence of high molecular weight (HMW; 55 kDa, 66 kDa, and 80 kDa) proteins was found in the summer. There was a significant increase ($P < 0.05$) in total spermatic defects, acrosome defects, and distal cytoplasmatic droplets in these bulls. The 40-kDa protein that reflected low fertility was observed in 10 bulls in the summer with semen quality decreases. The 11 bulls showed presence of HMW (55 kDa) in the winter. In 11 bulls, HMW (55 kDa, 66 kDa, or 80 kDa) proteins were present with a satisfactory semen condition according to Killiam *et al.* (1993). In conclusion, the seasons of the year may influence the presence of proteins in seminal plasma. There was a direct relationship of the season with seminal plasma proteins. The presence of the proteins of 20 kDa, 55 kDa, 66 kDa, and 80 kDa suggested an increase of the semen quality during the winter (Chacur et al., 2010a; 2011a).

In Brazil semen from eleven Tabapua bulls, 30 months old, were collected by electroejaculation during winter (from June to August) and summer (from December to February) of 2007. From each bull a total of 132 semen samples were collected in an interval of 14 days. Samples of seminal plasma were centrifuged (1500g/15min) and conditioned in criotubes and stored at –20ºC until further processing. Proteins were extracted from 200 μL of each sample in 2 mL of extraction buffer composed by 0.625 M Tris-HCl, pH 6.8, 2% SDS, 5% β-mercaptoethanol and 20% of glycerol. Proteins were quantified according to Bradford (1976) and electrophoresis was performed according to Laemmli (1970). Gels were fixed

with isopropanol: acetic acid: water (4:1:5 v/v) for 30 minutes, and stained in the same solution with 2% of Comassie Blue R250. Percentage of different seasons including plasma proteins were statistically compared by the Chi-square test with significance level at $P<0.05$. In two bulls, the absence of high molecular weight (HMW 55kDa, 66kDa and 80kDa) proteins was verified in the summer. There was a significant increase ($P<0.05$) in total spermatic defects in these two bulls. The protein of 40kDa which suppose to be of low fertility was observed in eight bulls in the summer with semen quality decrease. The eight bulls showed presence of HMW (55kDa) in the winter. In nine bulls HMW (55kDa, 66kDa or 80kDa) proteins were present with a satisfactory semen condition in accordance with Chacur et al. (2006a). The two bulls showed presence of HMW proteins (66kDa and 80kDa) in the summer. The results suggest that different seasons of the year may influence the presence of a variety of proteins in seminal plasma. There was a direct relationship of the season upon seminal plasma proteins. The presence of the proteins of 20kDa, 55kDa, 66kDa and 80kDa suggests an increase of the semen quality during the winter (Chacur et al., 2008).

Peptides of 55- and 66-kDa were present in bulls with excellent spermatic conditions for example motility and vigor (Chacur et al., 2009a). On the other hand, 13- and 33-kDa peptides were observed in association with unfavourable spermatic conditions (Chacur et al., 2009b). The objective of this study was to determine the profile SDS-PAGE of seminal plasma and evaluate the semen characteristics in Brangus and Brown-Swiss bulls. Semen from 14 Brangus, 36 months old, was collected by electroejaculation during summer of 2009-2010. A total of 84 semen samples were collected in an interval of 14 days. Semen volume, motility, vigor, major defects and minor defects were evaluated according to Brazilian College of Animal Reproduction (Manual..., 1998). Animals were divided in two groups: poor semen (motility <50% and major defects >10%) and good semen, and subsequently compared regarding the composition of seminal plasma proteins. Samples of seminal plasma were centrifuged (1500g/15min) and conditioned in criotubes and stored at –20ºC until further processing. Proteins were extracted from 200 μL of each sample in 2 mL of extraction buffer composed by 0.625 M Tris-HCl, pH 6.8, 2% SDS, 5% β-mercaptoethanol and 20% of glycerol. Proteins were quantified according to Bradford (1976) and electrophoresis was performed according to Laemmli (1970). Gels were fixed with isopropanol: acetic acid: water (4:1:5 v/v) for 30 minutes, and stained in the same solution containing 2% of Comassie Blue R250. Each semen collection was used in duplicate. The concentration of proteins was measured using a spectrophotometer PF-901(Chemistry Analyser Labsystems). Gels were submitted to a photodocumentation system (Bio Doc-IT and Visidoc-IT Gel Documentation systems, UVP) and analysed by Doc-IT-LS 6.0 software. GLM from SAS, version 6, was used in order to evaluate possible variations of seminal variables and protein molecular mass. Statistical significance was accepted from $P<0.05\%$. The means of semen variables were: volume (5±1 mL), motility (75±5%), vigor (4), major defects (7±2%) and minor defects (12±4%). The results of analyses of gels revealed a variety of proteins in each animal and among bulls. There were 28 different major polypeptides, ranging from 15 to 24 bands in each individual bull. In six Brangus bulls the presence of low molecular weight (LMW 13kDa and 33kDa) proteins was associated with low motility (35-40%) in accordance with Chacur et al. (2009a). There was a significant increase ($P<0.05$) in

major spermatic defects in these six bulls (20.3±3.7%) associated with presence of proteins that had molecular weights of (23, 35 and 72KDa). In eight Brangus bulls, 55KDa, 66KDa or 80KDa proteins were present and associated with a satisfactory semen condition (motility 77±6% and major defects 5±2%) in accordance with Chacur et al. (2009b). In cattle, the 55-, 66- and 80-kDa proteins are associated positively with camp-dependent progressive motility (Shivaji et al., 1990). Consistently, in the present experiment, there was a positive relationship of presence of seminal plasma proteins 55kDa, 66kDa and 80kDa and semen quality (motility and major defects). The presence of these proteins suggests an increase in semen quality (Chacur et al., 2010b).

### 4.3. Interation between year seasons on the semen and hormones in *Bos indicus* and *Bos taurus* in Brazil

In Brazil the influence of four year seasons was study on semen characteristics and levels of testosterone and cortisol in Nelore and Simmental bulls. Five Nelore and five Simmental bulls with 48-72 months old, extensively managed were evaluated for sexual soundness using physical and morphological characteristics of semen and serum levels of testosterone and cortisol. There was decreased motility and vigor semen ($P<0.05$) during winter in Simmental bulls (Table 2). There was correlation ($P<0.01$) between testosterone x motility (0.69) and testosterone x vigor (0.57) in Simmental breed (Table 4) and cortisol x motility (0.68) and cortisol x vigor (0.65) in Nelore breed (Table 3). The effect of year seasons changed the semen quality with increase sperm motility and vigor on springer-summer in Simmental bulls. The cortisol level decreased on autumn in Nelore bulls (Chacur et al., 2012).

| characteristics | breed | spring | summer | autumn | winter |
|---|---|---|---|---|---|
| Volume (mL) | S | 8.80±0.65 Aab | 9.85±0.65 Aa | 8.76±0.58 Aab | 7.35±0.65 Ab |
| | N | 7.10±0.65 Aa | 7.55±0.65 Ba | 6.26±0.58 Ba | 6.05±0.65 Aa |
| Motility (%) | S | 70.00±5.83Aa | 70.00±5.83Aa | 60.80±8.21Aab | 48.00±5.83Ab |
| | N | 63.50±5.83Aa | 60.00±5.83Aa | 54.40±5.21Aa | 53.50±5.83Aa |
| Vigor (1-5) | S | 3.35±0.28 Aa | 3.55±0.28 Aa | 3.00±0.25 Aab | 2.20±0.28 Ab |
| | N | 3.05±0.28 Aa | 2.95±0.28 Aa | 2.32±0.25 Aa | 2.50±0.28 Aa |
| Major defects (%) | S | 11.33±1.31 Aab | 8.00±1.07 Ab | 10.75±0.98 Aab | 12.18±1.20 Aa |
| | N | 6.30±1.07 Ba | 6.31±1.10 Aa | 9.92±0.96 Aa | 9.42±1.10 Aa |
| Minor defects (%) | S | 7.88±0.83 Aa | 7.25±0.79 Aa | 8.25±0.72 Aa | 10.25±0.88 Aa |
| | N | 7.60±0.79 Aa | 5.63±0.81 Aa | 7.16±0.70 Aa | 6.84±0.81 Ba |
| Total defects (%) | S | 19.22±1.63 Aab | 15.25±1.54 Ab | 19.00±1.41 Aab | 22.43±1.73 Aa |
| | N | 13.90±1.53 Ba | 11.97±1.59 Aa | 17.08±1.38 Aa | 16.36±1.59 Ba |

| characteristics | breed | spring | summer | autumn | winter |
|---|---|---|---|---|---|
| Concentration (x$10^9$/mL) | S | 1.35±0.13 Aa | 1.38±0.13 Aa | 1.32±0.12 Aa | 1.00±0.13 Aa |
| | N | 0.88±0.13 Ba | 1.14±0.13 Aa | 0.95±0.12 Ba | 0.91±0.13 Aa |

Significance level 5% (P< 0,05); A, B – distinct letters in column (P<0,05); a, b – distinct letters in line (P<0,05).

**Table 2.** Semen characteristics in spring, summer, autumn and winter for Simmental (S) and Nelore (N) bulls.

| | breed | spring | summer | autumn | winter | correlations |
|---|---|---|---|---|---|---|
| cortisol | S | 0.6 | 0.6 | 0.5 | 1.1 | |
| Volume (mL) | S | 8.800 | 9.444 | 10.800 | 7.500 | -0.87 |
| color | S | 1.700 | 2.111 | 1.400 | 1.200 | -0.60 |
| aspect | S | 1.800 | 1.889 | 1.400 | 1.900 | 0.55 |
| Mass moviment (1-5) | S | 1.800 | 4.000 | 2.800 | 1.800 | -0.52 |
| Motility (%) | S | 67.000 | 80.000 | 64.000 | 46.000 | -0.83 |
| Vigor (%) | S | 3.000 | 4.111 | 3.200 | 2.100 | -0.79 |
| Concentration (x$10^9$/mL) | S | 1.093 | 1.697 | 1.360 | 0.960 | -0.66 |
| Major defects (%) | S | 13.889 | 6.222 | 10.800 | 10.875 | 0.10 |
| Minor defects (%) | S | 7.889 | 6.222 | 7.400 | 8.250 | 0.60 |
| Total defects (%) | S | 21.778 | 12.444 | 18.200 | 19.125 | 0.22 |
| cortisol | N | 1.68 | 3.10 | 1,36 | 3.06 | correlations |
| Volume (mL) | N | 8.278 | 8.286 | 5.700 | 5.800 | 0.14 |
| color | N | 1.444 | 3.000 | 1.600 | 2.200 | 0.87 |
| aspect | N | 1.889 | 2.429 | 1.500 | 2.000 | 0.85 |
| Mass moviment (1-5) | N | 2.222 | 3.714 | 1.300 | 2.200 | 0.75 |
| Motility (%) | N | 63.333 | 80.000 | 33.000 | 56.000 | 0.75 |
| Vigor (1-5) | N | 3.111 | 4.000 | 1.500 | 2.600 | 0.65 |
| concentration (x$10^9$/mL) | N | 0.913 | 1.329 | 0.701 | 1.080 | 0.91 |
| Major defects (%) | N | 5.889 | 5.857 | 10.700 | 9.200 | -0.31 |
| Minor defects (%) | N | 7.000 | 4.571 | 8.000 | 7.500 | -0.60 |
| Total defects (%) | N | 12.889 | 10.429 | 18.700 | 16.900 | -0.43 |

color: 1 – white, 2 – White-Milk and 3 – White-yellow; aspect: 1 – aquous, 2 – viscous and 3 – cremous.

**Table 3.** Correlations between cortisol (μg/dL) and semen characteristics on year season in Simmental (S) and Nelore (N) bulls.

| | breed | spring | summer | autumn | winter | correlations |
|---|---|---|---|---|---|---|
| testosterone | S | 879.5 | 901.1 | 584.0 | 648.2 | |
| Volume (mL) | S | 8.800 | 9.444 | 10.800 | 7.500 | -0.16 |
| color | S | 1.700 | 2.111 | 1.400 | 1.200 | 0.86 |
| aspect | S | 1.800 | 1.889 | 1.400 | 1.900 | 0.62 |
| Mass moviment (1-5) | S | 1.800 | 4.000 | 2.800 | 1.800 | 0.31 |
| Motility (%) | S | 67.000 | 80.000 | 64.000 | 46.000 | 0.69 |
| Vigor (1-5) | S | 3.000 | 4.111 | 3.200 | 2.100 | 0.57 |
| Concentration ($x10^9$/mL) | S | 1.093 | 1.697 | 1.360 | 0.960 | 0.37 |
| Major defects (%) | S | 13.889 | 6.222 | 10.800 | 10.875 | -0.19 |
| Minor defects (%) | S | 7.889 | 6.222 | 7.400 | 8.250 | -0.47 |
| Total defects (%) | S | 21.778 | 12.444 | 18.200 | 19.125 | -0.26 |
| testosterone | N | 430.41 | 234.71 | 420.31 | 329.15 | correlations |
| Volume (mL) | N | 8.278 | 8.286 | 5.700 | 5.800 | -0,28 |
| color | N | 1.444 | 3.000 | 1.600 | 2.200 | -1.00 |
| aspect | N | 1.889 | 2.429 | 1.500 | 2.000 | -0.89 |
| Mass moviment (1-5) | N | 2.222 | 3.714 | 1.300 | 2.200 | -0.87 |
| Motility (%) | N | 63.333 | 80.000 | 33.000 | 56.000 | -0.71 |
| Vigor (1-5) | N | 3.111 | 4.000 | 1.500 | 2.600 | -0.70 |
| Concentration ($x10^9$/mL) | N | 0.913 | 1.329 | 0.701 | 1.080 | -0.93 |
| Major defects (%) | N | 5.889 | 5.857 | 10.700 | 9.200 | 0.36 |
| Minor defects (%) | N | 7.000 | 4.571 | 8.000 | 7.500 | 0.82 |
| Total defects (%) | N | 12.889 | 10.429 | 18.700 | 16.900 | 0.56 |

color: 1 – white, 2 – White-Milk and 3 – White-yellow; aspect: 1 – aquous, 2 – viscous and 3 – cremous.

**Table 4.** Correlations between testosterone (ng/dL) and semen characteristics on year season in Simmental (S) and Nelore (N) bulls.

## 5. Summary and conclusions

Seminal plasma is composed of secretions from the male accessory sex glands and epididymis, which contains many organic and inorganic components that have effects on sperm quality (Foxcroft et al., 2008). The proteins secreted into seminal plasma may play an important role during sperm capacitation and fertilization (Rodriguez-Martinez et al., 1998), and may also serve to protect sperm from damage or to maintain their longevity.

Specific proteins in seminal plasma have been identified as potential markers of male fertility or infertility in the human (Martinez-Heredia et al., 2008; Yamakawa et al., 2007). Comprehensive proteomic analyses have been conducted in the bull (Moura et al., 2006a; Moura et al., 2006b; Chacur et al., 2010a; Chacur et al., 2010b; Chacur et al., 2011a; Chacur et al., 2011b). The literature on the effects of seasons on the semen characteristics and upon seminal plasma proteins in Nellore (*Bos taurus indicus*) cattle under natural conditions in Brazil has already been recently studied in Brazil (Chacur et al., 2003; 2004; 2006a; 2007; 2009a; 2010a; 2010b; 2011a; 2011b; 2012).

Its well known that low temperatures alter the function of spermatozoa. Cold shock results in the destabilization of sperm membranes and impairment of sperm function, and it is also well known that animal spermatozoa are sensitive to cold-shock stress as the bull (Watson, 1995).

Seminal plasma has also been shown to have deleterious effect on bovine sperm during semen storage at ambient temperatures and a damaging effect during semen cooling and freezing. Recent studies have shown that proteins from bovine seminal plasma (BSP) may modulate sperm properties. Many autors believe that the bull seminal plasma contains fertility associated protein markers. Comparison of individual 2-D PAGE maps with the reference map could provide a useful key to relate protein pattern changes to some physiopathological events influencing the reproductive sphere.

Factors isolated from epididymal fluid or epithelial cells in culture have been linked to sperm motility and protection of membranes against damage caused by cryopreservation (Reyes-Moreno et al., 2002), anticapacitation effects (Roberts et al., 2003), or sperm number (Gatti et al., 2004), but evidence linking epididymal proteins to male fertility indexes is limited (Moura et al., 2006a).

## 6. Future prospects

The determination of protein content on sperm surface and seminal plasma may be an index of individual bull fertilizing ability or post-thaw status of sperm membranes (Nauc & Manjunath, 2000). A reference map of seminal plasma proteins could be useful in relating protein pattern changes to physiopathological events influencing the reproductive sphere (Mortarino et al., 1998). PGDS could influence male fertility by mediating the action of hydrophobic molecules on sperm during epididymal transit or cauda epididymal storage (Moura et al., 2006a; 2006b). Although known functional attributes of these proteins provide some understanding of how they may influence male reproductive performance (Moura et al., 2006b; Chacur et al., 2010a; 2011a).

## Author details

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