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Functional insights into the role of seminal plasma proteins on sperm motility of buffalo

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ABSTRACT

The objective of the present study was to describe the proteins from the seminal plasma of buffalo and correlate these proteins with sperm motility. Ejaculates from sixteen Murrah buffalo were used. Semen collection was performed by electroejaculation, and the ejaculate was evaluated by macroscopic (volume) and microscopic analysis (subjective motility and vigor, as well as sperm concentration). After the analysis, the samples were centrifuged (800*g* for 10 min and 10,000 for 30 min at 4 °C), and the supernatant (seminal plasma) was used to determine total protein concentration by the Bradford method. Based on total protein concentration, an aliquot (50 µg) was taken to conduct protein in-solution digestion for nano-LC–ESI-Q-TOF mass spectrometry analysis. Samples were divided into two groups, minimal (little sperm motility) and greater (typical sperm motility), based on non-hierarchical clustering considering motility and emPAI protein value. The data were analyzed by multivariate statistical analysis using principal component analysis (PCA) and partial analysis of minimum squares discrimination (PLS-DA). Forty-eight proteins were detected in the seminal plasma, and fifteen were common to two groups. There were six proteins that were significantly different between the groups. The main functions of proteins in seminal plasma were catalytic and binding activity. Spermadhesin protein, ribonuclease, 14-3-3 protein zeta/delta and acrosin inhibitor were in greater amounts in seminal plasma from the group with greater sperm motility; prosaposin and peptide YY were in greater amounts in the group with little sperm motility. The proteins detected in the greater motility group were correlated with sperm protection, including protection against oxidative stress, lipid peroxidation, protease inhibition and prevention of premature capacitation and acrosome reaction. In the group with little sperm motility, one of the identified proteins is considered to be an antifertility factor, whereas the function of other identified protein is not definitive. Results from the present study add to the knowledge base about the molecular processes related with sperm motility, and these findings can be used for determining potential markers of semen quality.

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1. Introduction

Seminal plasma is a fluid composed of a complex mixture, which contains different macromolecules from the testes, epididymis and accessory sexual glands with the function of maintaining viability of sperm cells. Proteins are the main macromolecules contained in seminal plasma, which have been correlated with male fertility in different species (Alvarez and Storey, 1995; Calvete et al., 1997; Daskalova et al., 2014; Kumar and Swamy, 2016), including cattle (Killian et al., 1993; Manjunath and Thérien, 2002). The functions of these proteins were related to sperm motility (Govindaraju et al., 2012), maintenance of a sperm reservoir in the female reproductive duct system (Singleton and Killian, 1983), capacitation (Killian et al., 1993; Manjunath and Thérien, 2002; Souza et al., 2011), acrosome reaction (Killian et al., 1993; Riffo and Párraga, 1997; Kummar et al., 2012), gamete fusion (Souza et al., 2008; Monaco et al., 2009), cell protection (Alvarez and Storey, 1995; Moura et al., 2007; Roncoletta et al., 2006) and fertilization (Thérien et al., 1997; Erikson et al., 2007). Nevertheless, in buffalo, few studies have been conducted to assess seminal protein content (Huang et al., 2015).

There is increasing interest in heparin binding proteins (HBPs) in buffalo, because most studies have focused on a seminal plasma proteomic approach in evaluation of HBPs (Arangasamy et al., 2005; Harshan et al., 2006, 2009; Kumar et al., 2008; Singh et al., 2007, 2013, 2014). Moreover, there may be an influence of the seminal plasma composition on the capacity to freeze semen from buffalo, because this species has a poor fertility rate after artificial insemination with frozen/thawed semen (Anzar et al., 2003; Akhter et al., 2007; Andrabi, 2009).

Harshan et al. (2006) studied effects of HBPs addition to epididymal spermatozoa of buffalo and found a poor freezing capacity, when there were greater concentrations of these proteins as a result of semen supplementations with HBPs. A similar interaction mechanism was proposed to buffalo as occurs between HBPs from sperm cell with lipids of egg yolk during cryopreservation of bovine spermatozoa (Singh et al., 2007). Lipids of egg yolk, after dilution of semen to freeze, interact with the HBPs, particularly the BSP proteins, forming complexes that prevent the binding of HBPs to the sperm membrane and inhibit the action of proteins (Manjunath and Thérien, 2002). In bovine, although the BSPs are beneficial to sperm functions, are associated with cholesterol and phospholipid efflux (Thérien et al., 1998, 1999), which is detrimental to the sperm membrane during cryopreservation (Manjunath et al., 2002).

Other hypotheses have been put forth in attempts to explain the lesser fertility rate of buffalo after cryopreservation of semen as compared to what occurs in cattle. Buffalo have a lesser semen protein concentration, which is thought to result in a lesser sperm motility, viability and fertility (Kulkarni et al., 1998; Dixit et al., 2016). The addition of bull seminal plasma to epididymal spermatozoa from buffalo, however, had detrimental effects on these semen quality markers (Herold et al., 2004). These results were confirmed by Singh et al. (2014), where a specific protein was identified, PDC-109 (BSP1), which is an HBP that was isolated from bull seminal plasma that when added to semen from buffalo there was a lesser freezing capacity of the treated samples.

Even though there have been these previous studies on protein composition of seminal plasma and the freezeability of buffalo semen, the relationship of proteins with sperm motility has not been thoroughly elucidated. Sperm motility is modulated by proteins contained in seminal plasma (Govindaraju et al., 2012), although specific mechanisms of action remain unclear in buffalo (Huang et al., 2015). Thus, the objective of the present study was to describe the main proteins of seminal plasma of buffalo by using mass spectrometry and correlate these molecules with sperm motility.

2. Materials and methods

2.1. Reagents

All reagents used in the present study were of the greatest purity and obtained from Sigma-Aldrich (St. Louis, MO, USA), GE Healthcare Life Sciences (São Paulo, São Paulo, Brazil), Waters Corp. (Barueri, São Paulo, Brazil) and Thermo Fisher Scientific (São Paulo, São Paulo, Brazil), unless otherwise cited.

2.2. Ethical aspects

The study was performed in accordance with ethical recommendations of the National Council for the Control of Animal Experimentation (CONCEA), and with the approval of the Committee on Ethics in the Use of Animals protocol 95/2016.

2.3. Animals, collection and semen evaluation

The groups were composed for animals that allowed the collection by electroejaculation. Sixteen adult (2.5 to 5.0 years), Murrah, clinically healthy buffalo (*Bubalus bubalis*), a > 30 cm scrotal circumference of unknown fertility from a single farm were used. The animals were maintained in an extensive grazing condition (*Brachiaria decumbens*), receiving water and mineral salt *ad libitum*.

After collection, the semen was analyzed according to the macroscopic (volume) and microscopic (subjective analyses of sperm motility and vigor, and concentration) characteristics.

Volume was measured with a graduated tube. Motility and vigor were subjectively analyzed by placing a semen drop on a glass slide, overlaid by a coverslip, and observing by optical microscopy (Bioval, L1000b-AC, Hexasystems Group, Taboão da Serra, Brazil), at 100× magnification. Sperm motility was classified as a percentage (0% to 100%), where 100% indicates all cells with movement, and 0% indicates no cells with movement. Sperm vigor was evaluated by using a score from 0 to 5, where 0 represented no