

BULL SPERM AND SEMINAL PLASMA PROTEINS AND THEIR RELATIONSHIP WITH FERTILITY: A REVIEW

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↳ Supporting Information

ABSTRACT: The efficiency of artificial insemination (AI) is greatly influenced by the quality of semen. Sperm and seminal plasma are found in semen, which play a role in the reproductive process and its ability to fertilize an egg and maintain the development of an embryo. Various factors will determine the fertility capacity of a sperm, both from the intrinsic factors of the sperm and the plasma component of the semen. Seminal plasma proteins are crucial for maintaining the stability of the membrane, viability, motility of sperm, acrosome reactions, maintaining osmotic pressure and helping the fertilization process. Good quality semen will support the fertilization process. The purpose of this scoping review is to increase our understanding of protein from sperm and seminal plasma of bulls and their relationship with fertility. The sperm proteins that were significantly correlated with fertility were Outer Dense Fiber protein 2 (ODF2), Protamine (PRM), Testis specific histone 2B (TH2B), Phosphatidylethanolamine binding protein (PEBP4), and Ubiquinol-cytochrome-c reductase complex core protein 2 (UQCRSC2). Meanwhile, the seminal plasma proteins positively correlated with fertility were Osteopontin (OPN), Phospholipase 2 (PLA2), P25b, Acidic seminal fluid proteins (aSFP), Alpha-L-fucosidase (α -L-fucosidase), and Bovine seminal plasma (BSP).

Keywords: Bovine, Fertility, Semen, Seminal plasma protein, Sperm protein.

INTRODUCTION

Bull fertility determines the success of pregnancy in females and is an important factor in the sustainability of livestock. Bull fertility can be measured based on the ability of sperm to fertilize oocytes, reproductive efficiency (Kaya and Memili, 2016), pregnancy, number of births, non-return rate, pregnancy rate (Berry et al., 2014), libido, and testosterone hormone concentration (Iskandar et al., 2022). Bull fertility was phenotypically performed using the Breeding Soundness Examination (BSE) method at the AI center (Butler et al., 2020). A bull is assessed according to the BSE standards in three categories, including physical scrotal circumference, sperm progressive motility, and sperm morphology (Ugur et al., 2022). Molecularly, bull fertility can be carried out using proteomic analysis. It is a study to analyze how the molecular processes of sperm function are related to fertility (Aitken and Baker, 2008; Olivia et al., 2009).

Sperm contains proteins that support metabolic processes and help in redox regulation of cells (Barranco et al., 2019; Gaitskell-Phillips et al., 2020; Gaitskell-Phillips et al., 2020; Pena et al., 2021). Proteins are biomolecules that can be found in cells. Seminal plasma proteins interact with sperm transported in microvesicles; as a result, these proteins can affect and regulate sperm activity (Rodriguez-Martinez et al., 2021). Sperm proteins play a role in embryo growth and successful fertilization (McReynolds et al., 2014). Sperm are highly specialized and transcriptionally active cells; protein cascades may be involved in inducing sperm motility (Siva et al., 2010). The availability of sperm energy sources from seminal plasma in the form of fructose, sorbitol, plasmogen, and glycerylphosphoryl choline can also affect sperm motility (Sundari et al., 2013). Sperm and seminal plasma are sources for investigating bull fertility. Bull fertility has been defined as sperm's ability to fertilize oocytes and embryonic development (Kaya and Memili, 2016), as well as improvements in cattle genetic selection (Viana et al., 2018).

The seminal plasma of semen consists of various specific biochemical components that regulate the functions of sperm. Components in seminal plasma have functions in sperm cells and in the female reproductive tract (de Andrade et al., 2012; Caballero et al., 2012; Rodriguez-Martinez et al., 2021). Seminal plasma has been proven to have a helpful process for sperm and fertility, increasing longevity, and sperm mobility within the female ducts (Druart et al., 2019). The main components of seminal plasma are water and both organic and inorganic materials. The seminal components of plasma consist of ions, energy substrates (mainly fructose in the case of male sperm), organic compounds, peptides, and proteins. The seminal plasma components play a crucial role in the fertilization process, motility, capacitation, and interaction of egg and sperm cells (Juyena and Stalletta, 2012). The seminal plasma protein has been investigated

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previously, which is found to be a molecular marker in different species related to fertility. The majority of the investigation into the characterization of these proteins has been reported on boar, bull, buck, ram, stallion, and poultry (Jonakova et al., 2010). Seminal plasma has functions in membrane stabilization, sperm viability, the process of capacitation reactions, acrosome reactions, and fertilization (Barrios et al., 2000). The association between sperm and seminal plasma proteins of bull and fertility will be covered in this review. Conclusively, the proteins of sperm and seminal plasma of bulls can provide information and understand functions related to fertility.

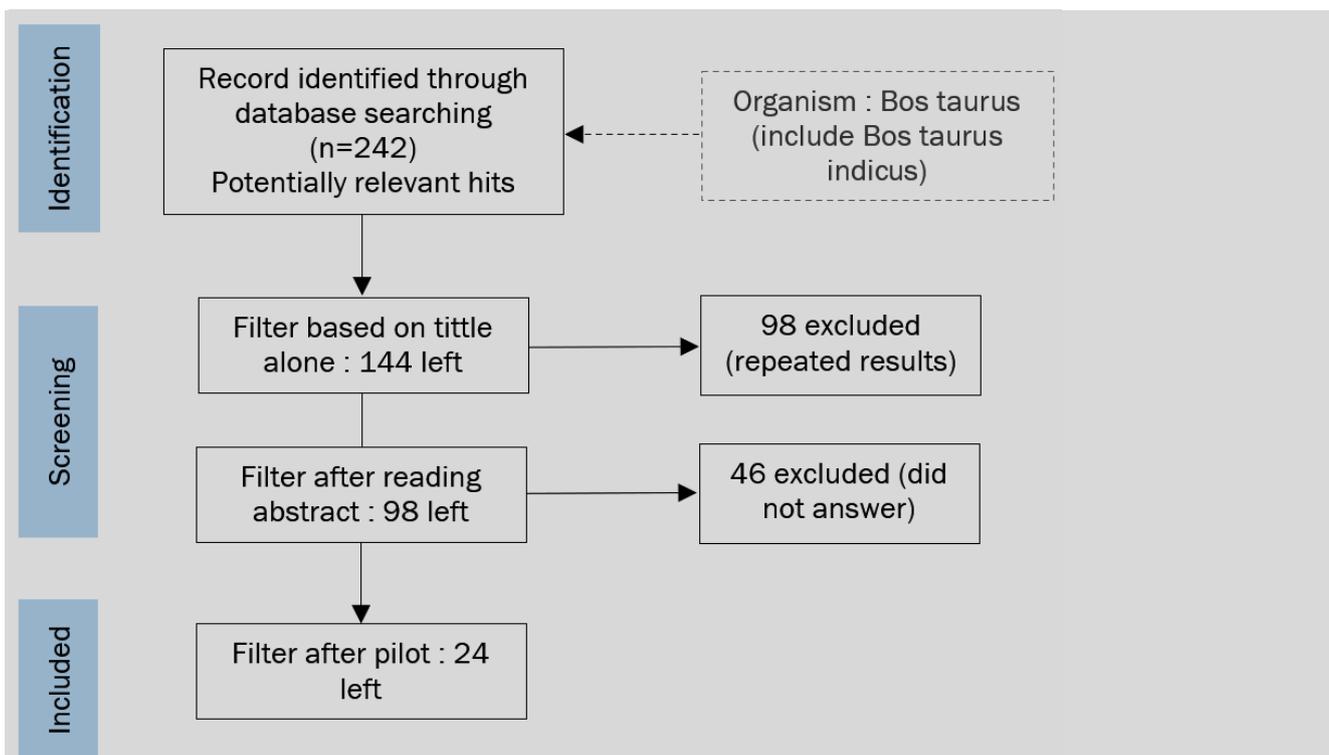


Figure 1 - Systematic review according to the protocol preferred reporting items for systematic reviews and meta-analyses (PRISMA).

Sperm chromatin dynamics

The plasma membrane, flagellum, cytoplasm, acrosome, and nucleus of the sperm include a variety of proteins that are important to the physiology of the sperm (Rawe et al., 2008). Chromatin is the physiological substrate for genetic processes in the nucleus of eukaryotic cells. Dynamic changes in chromatin emerge as key regulators of genomic function (Fischle et al., 2003). During the transition from spermatogonia to spermatozoa, there are histone changes (Figure 1). The complex process of spermatogenesis occurs in three stages. The first stage involves the process of mitotic cell division which allows the initial stage of the cell, the spermatogonia, to reproduce. The second stage of meiosis, in which diploid cells form haploid cells, is the process of division until the formation of spermatids. The final stage of spermatogenesis includes the production of sperm, mature, and motile sperm cells, through the process of spermiogenesis (de Kretser et al., 1998).

Spermatogenesis is the process of producing functionally mature sperm from precursor sperm (Hao et al., 2019). Lysine and cysteine residues in protamine are distinct from those in core histones. Chromatin condensation and the substitution of protamines for histones occur in conjunction with the last stages of spermatogenesis (Sahoo et al., 2021). The nucleohistone complex, which contains histone H2A, H2B, histone 3 (H3), histone 4 (H4), and protamines (PRM), is firmly coiled around sperm DNA (Miller et al., 2010). Transition proteins 1 (TP1) and 2 (TP2) replace core histones during spermatogenesis, and transition proteins (TPs) are replaced by testis-specific protamines (Zhao et al., 2001; Balhorn, 2007). One of the most important phases in sperm chromatin remodeling is histone-to-protamine exchange, because it controls the degree of chromatin condensation, which is required for fertilization (Ugur et al., 2019). Round spermatids' chromatin structure and cellular shape undergo substantial changes throughout spermiogenesis. It results in sperm to have severely compacted chromatin and to experience transcriptional quiescence (Özbek et al., 2021).

Sperm chromatin during spermatogenesis is mostly packed with protamine (85-98%), forming toroid DNA (Kutchy et al., 2017). Histone-bound DNA loci encodes transcription and takes a pivotal part during postfertilization and early embryonic development (Hammoud et al., 2009). Sperm and chromatin are packaged efficiently, because the replacement of histones with protamine is aimed at egg fertilization, egg activation, and embryonic development (Balhorn et al., 1988). The association of protamines with DNA brings about a unique chromatin remodeling in sperm and appears to facilitate the hydrodynamic shape of the sperm head (Brunner et al., 2014). Sperm chromatin integrity is critical for successful fertilization, healthy egg activation, embryo development, and species survival (Gawecka et al.,

2013). Advanced techniques are required to evaluate the health of sperm chromatin, since it has such a significant influence on the bull's ability to reproduce (Kutchy et al., 2017). Linker histones are progressively replaced by testis-specific variations during spermatogenesis, and histones are replaced by transition proteins, protamines, and finally protamine-like substances (sperm chromatin health) (Kimmins and Sassone-Corse, 2005).

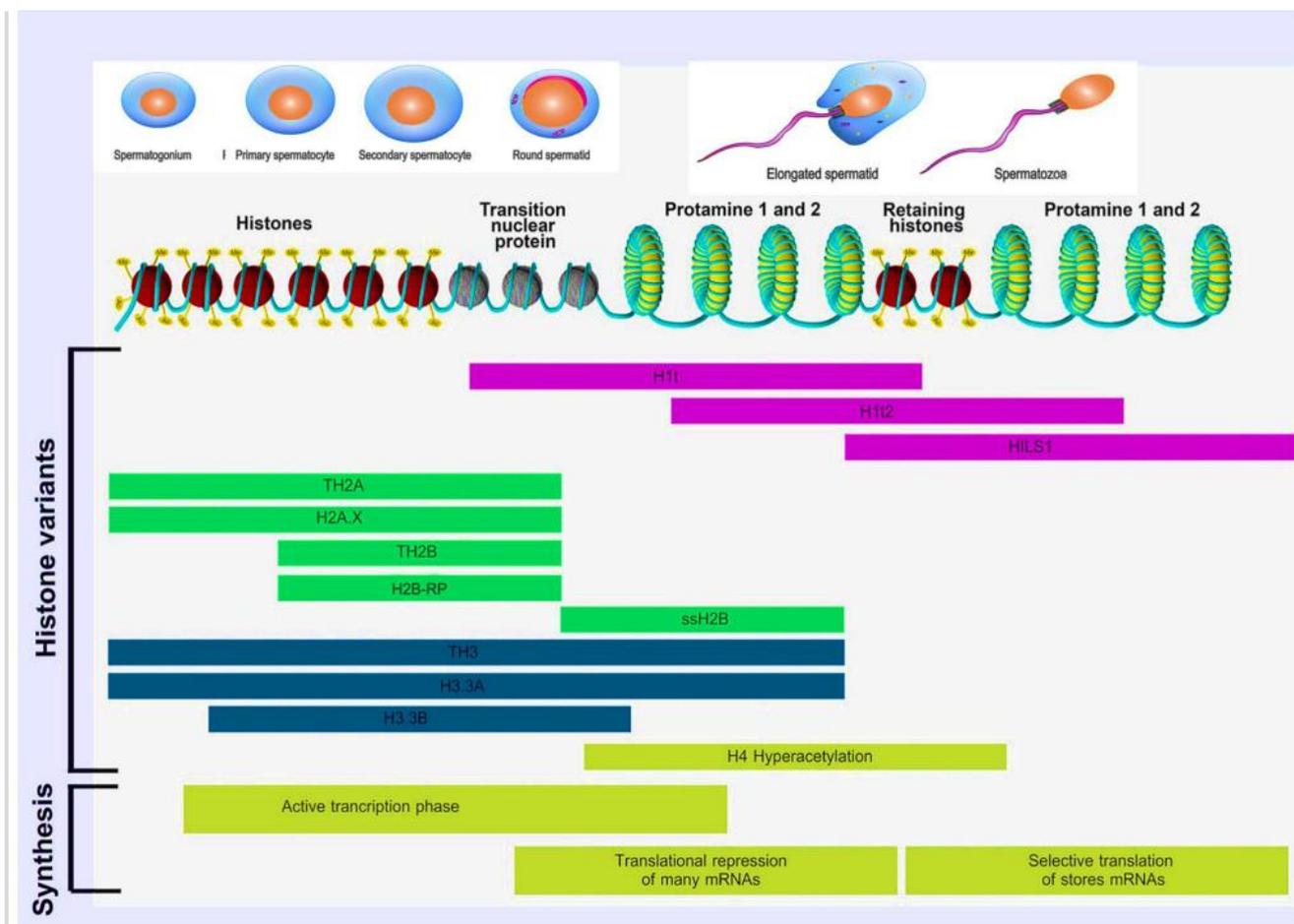


Figure 2 - Histone changes from spermatogonia to spermatozoa (adapted from Kimmins and Sassone-Corsi, 2005; Rathke et al., 2005; Özbek et al., 2021).

Sperm proteins

Proteins from sperm and their relationship with fertility are presented in Table 1. The ability of sperm to fertilize an egg is determined by the role of sperm proteins. Proteins such as ODF2, PAWP, PRM, TH2B, PEBP4, and UQCRC2 are sperm proteins associated with fertility. Sperm contains intracellular proteins, membrane proteins from the cell, and proteins bound to them from fluid derived from the epididymal and auxiliary sex glands (Rego et al., 2016; Kenny and Byrne, 2018; van Tilburg et al., 2021).

Outer Dense Fiber protein 2 (ODF2) is the protein detected in sperm and is associated with fertility. Kaya et al. (2021) reported that ODF is found in the sperm tail axoneme. In sperm, ODF2 is a cytoskeletal structural protein in flagella. The ODF2 signal is strongest on the main tail, followed by a decrease in strength at the tip and no signal in the middle, indicating that the mitochondrial sheath prevents anti-ODF antibodies from reaching the ODF fibers. The ODF2 gene produces a protein that is a major component of the sperm tail (Hoyer-Fender et al., 1998; Salmon et al., 2006; Hüber et al., 2008). ODF2 consists of nine fibers with doublet tubules of axonemy on the outer edge (Donkor et al., 2004). ODF2 has a function in maintaining the sperm tail which is needed to protect the sperm tail from shearing forces during the epididymis and ejaculation process (Kumar and Singh., 2021).

Protamines (PRMs), the key proteins in spermiogenesis for chromatin condensation, are potential candidate genes for sperm motility markers (Kumar et al., 2018) and as a protein biomarker of semen quality and production (Pardede et al., 2020). In the sperm head, PRM is one of the most prevalent core proteins. In humans (Balhorn et al., 2018), mice, rats, and hamsters (Bower et al., 1987), two kinds of PRM, namely PRM1 and PRM2, have a role in sperm function. According to Beletti et al. (2005), only one type of PRM, PRM1, plays a dominant role in the normal operation of bull sperm. However, PRM1, PRM2, and PRM3 are all expressed in bovines, according to Ferraz et al. (2013). Protamine is an arginine-rich proteins required for efficient compression of sperm DNA into a 10-fold more compact condition, compared to other spermatogenic cell types such as spermatogonia, until protamine toroid activities cause differentiation into spherical spermatids (Ward, 2010). During spermiogenesis, PRM replaces histone somatic cells in a complicated process (Bao and Bedford, 2016).

Testis specific histone 2B (TH2B) is a biomolecular marker that can be used to assess the quality of sperm and predict bull fertility and sire suitability for artificial insemination (AI). [Kutchy et al. \(2017\)](#) reported that TH2B is localized in the sperm head and is related to sperm chromatin dynamics and bull fertility. [Shinagawa et al. \(2015\)](#) found that TH2B creates an open structure in sperm chromatin and engages in the replacement of proteins between the nuclei. During the process of spermatogenesis, chromatin undergoes structural reorganization. TH2B is involved in remodeling chromatin structure during spermatogenesis ([Lu et al., 2009](#)). Histone variant from TH2B is identified as testis H2B ([Trostle-Weige et al., 1982](#)). TH2B affects sperm chromatin's inter-nuclear protein replacement and produces open chromatin shape ([Shinagawa et al., 2015](#)).

Phosphatidylethanolamine binding protein 4 (PEBP4) has been identified as a secretory protein with an N-terminal signal peptide involved in serine protease control ([Wang et al., 2004](#)). PEBP is a highly conserved protein in mammals that is mostly expressed in the testis, as well as the parathyroid gland, spleen, gallbladder, small intestine, salivary gland, rectum, stomach, and kidney ([Uhlen et al., 2015](#)). PEBP4 enhanced sperm motility in boar sperm, hence it's possible that PEBP4 regulates sperm production and maturation in bovines ([An et al., 2012](#)). PEBP4 protein was higher in the high-fertility bulls group compared to the low-fertility and infertile groups ([Somashekar et al., 2017](#); [Selvaraju et al., 2018](#)). PEBP4 in the seminiferous tubules' elongated spermatids and Leydig cells but not in the Sertoli or spermatogonia cells. PEBP4 also affects sperm metabolism and motility via protein phosphorylation signaling ([Silva et al., 2015](#)). In addition, PEBP4 inhibits serine proteases and protects cells against TNF-induced apoptosis ([Wang et al., 2014](#)), and it is linked to spermatogenesis and sperm motility ([Somashekar et al., 2017](#)). PEBP4 has also been annotated particularly in relation to fertility traits like age at puberty and spermatogenesis ([Somashekar et al., 2017](#); [Stafuzza et al., 2020](#)).

Ubiquinol-cytochrome-c reductase complex core protein 2 (UQCRC2) has been reported to be correlated with bull fertility ([Park et al., 2019](#)). [Park et al. \(2019\)](#) revealed that UQCRC2 can be used to predict below-normal bull fertility. After capacitation, UQCRC2 was shown to be strongly expressed in sperm with large litter sizes ([Kwon et al., 2015](#)). UQCRC2 is a protein involved in the electron transport chain's (ETC) complex III and IV assembly ([Lopes et al., 2021](#)), associated with oxidative stress ([Shibanuma et al., 2011](#)) and oxidative phosphorylation ([Filipović et al., 2020](#)). Increased ROS generation occurs due to UQCRC2 deficiency ([Park et al., 2012](#)). UQCRC2 was reported to be significantly lower in high-fertility bulls than in low-fertility bulls ([Park et al., 2012](#)).

Table 2– Sperm proteins

| Preferred name | Annotation | Function | References |
|----------------|---|--|--|
| ODF2 | Outer Dense Fiber protein 2 | Activation and fertilization | Kaya et al., 2021 |
| PRM | Protamine | Changes in histones in sperm spermatid chromatin to the developmental stage of spermatogenesis, to condense sperm DNA into a complex, dense, and stable condition | Fortes et al., 2014 ; Dogan et al., 2015 |
| TH2B | Testis specific histone 2B | Testicular-specific histone variants required for the transformation of dissociated nucleosomes to protamine in male germ cells | Kutchy et al., 2017 |
| PEBP4 | Phosphatidylethanolamine binding protein | Regulation of sperm motility and fertility | Somashekar et al., 2017 |
| UQCRC2 | Ubiquinol-cytochrome-c reductase complex core protein 2 | Component of the multisubunit transmembrane complex known as the ubiquinol-cytochrome c oxidoreductase, which powers oxidative phosphorylation as part of the mitochondrial electron transport chain | Park et al., 2012 |

Table 3– Seminal plasma proteins

| Preferred Name | Annotation | Function | References |
|----------------|-------------------------------|---------------------------|--|
| OPN | Osteopontin | Sperm viability | Erikson et al., 2007 |
| PLA2 | Phospholipase 2 | Acrosome reaction | Kumar et al., 2012 |
| P25b | P25b | Fertilizing ability | Kumar et al., 2012 |
| aSFP | Acidic seminal fluid proteins | Motility and freezability | Kumar et al., 2012 |
| a-L-fucosidase | Alpha-L-fucosidase | Fertilizing ability | Kumar et al., 2012 |
| BSP | Bovine seminal plasma | Maturation process | Manjunath et al., 2009 |

Seminal plasma proteins

Proteins from seminal plasma and their relationship with fertility are presented in Table 2. Proteins such as OPN, PLA2, P25b, aSFP, α -L-fucosidase, and BSPs are seminal plasma proteins associated with fertility.

The candidate specification associated with fertility is osteopontin (OPN). OPN is an extracellular phosphoprotein matrix protein that releases identified chemicals in various tissues and fluids, including those of the male and female reproductive tracts. OPN was previously identified as a marker of high fertility with a molecular weight of 55 kDa in Holstein bull seminal plasma, produced by the ampulla and vesicular glands (Erikson et al., 2007). In a previous study of OPN, a 55 kDa isoform was detected in seminal plasma in Holstein bulls, which had a positive correlation with fertility (Cancel et al., 1997). OPN can be found in bull's accessory sex glandular fluids (AGF), seminal vesicle fluids and ampullary fluids (Cancel et al., 1997), the epithelium of male reproductive tract in humans, and rat's testis (Brown et al., 1992) and epididymis (Manjunath, 1984; Siiteri et al., 1995; Luedtke et al., 2002). However, previous studies were unsuccessful in detecting OPN in immunofluorescent bovine sperm. According to Cancel et al. (1997), OPN has several relationships and functions in ejaculated bovine sperm due to its presence in the male reproductive tract and seminal plasma, as well as its correlation with male fertility. OPN has been described as a sperm surface molecule in mice (Siiteri et al., 1995), such as those associated with sperm during development in the testes (Luedtke et al., 2002) and while sperm are transported and stored in the epididymis (Manjunath, 1984; Luedtke et al., 2002) and are present in AGF (Cancel et al., 1997). Therefore, the AGF protein is known to bind to sperm during ejaculation (Weinman et al., 1986). Osteopontin was also reported to be positively correlated with freezability of bull (Rego et al., 2016), several peptides (Willforss et al., 2021), sperm-egg binding and embryo development (Moura, 2005; Erikson et al., 2007; Gonçalves et al., 2008; Monaco et al., 2009).

Phospholipase A2 (PLA2) is found in the plasma membrane, acrosome, and post-acrosome substantiation of ejaculated bull sperm (Weinman et al., 1986). The molecular weight of 60 kDa and pI 5.6 (Soubeyrand et al., 1997) and the 16 kDa PLA2 isoform were also detected (Ronkko et al., 1991). It was demonstrated that PLA2 adhered to the surfaces of ejaculated bull sperm but not epididymal sperm (Ronkko, 1992). PLA2 forms a superfamily of proteins, hydrolyzes the sn2-ester bonds of glycerophospholipids, and is involved in many biological functions (Six and Dennis, 2000). PLA2 activity in seminal plasma and sperm heads is known to be associated with motility and fertility (Anfuso et al., 2015). PLA2 is also involved in capacitation, acrosome reaction, and early stages of fertilization, sperm binding and sperm-oocyte fusion in mammals (Roldan and Fraggio, 1993; Pietrobon et al., 2005; Roldan and Shi, 2007; Stival, 2016). Furthermore, PLA2 is classified into PLA2 (cPLA2), PLA2 (sPLA2), PLA2 (iPLA2), and platelet-activating factor (PAF) (Alberghina, 2010). PLA2 is a protein found mostly in male reproductive organs (Koizumi et al., 2003; Bao et al., 2004; Masuda et al., 2005; Roldan and Shi, 2007).

P25b is a sperm protein found in bulls that is associated with the acrosome's plasma membrane. P25b is part of the xylulose reductase family, which is secreted by the epididymal epithelium and adheres to the sperm surfaces of the testes during epididymal transit. This corresponds to the plasma membrane that covers the acrosomal caps of the sperm (Khumran et al., 2020). Through apocrine secretion by epithelial cells bordering the epididymal lumen, epididymosomes are released in cauda epididymal fluids. Epididymosomes transfer these proteins from the epididymal lumen to the sperm surfaces depending on pH, temperature, and zinc (Frenette et al., 2002). Karunakaran and Devanathan (2017) reported that P25b was only present in high-fertility bulls and absent in low-fertility bulls. P25b plays a key role in the regulation of cellular activities that lead to acrosome reaction, recognition, binding, and sperm penetration of the oocyte's zona pellucida during fertilization (Iida et al., 1999; Girouard et al., 2009). Kumar et al. (2012) identified P25b as a potential sperm maturation and fertility marker associated with freezing and thawing techniques.

Acidic seminal fluid proteins (aSFP) are a protein family that significantly affects in vitro mitogenicity and steroidogenesis (Einspanier et al., 1993). aSFP, also known as Spermadhesin-1, is secreted by the accessory sex glands (Moura et al., 2007) and the cauda epididymis of bulls (Moura et al., 2010). aSFP has the ability to guard against oxidative stress triggers in semen; the effects of aSFP on sperm motility and mitochondrial activation are very important, when aSFP is at high concentrations (Einspanier et al., 1993; Schoneck et al., 1996).

Alpha-L-fucosidase was found in seminal plasma, epididymal fluid, and bull sperm (Jauhiainen and Vanha-Perttula, 1986; Srivastava et al., 1986). Cauda epididymidis is the main source of α -L-fucosidase in bulls (Srivastava et al., 1986). Moura et al. (2006) also detected α -L-fucosidase in the cauda epididymal fluid of bulls which had a molecular weight of 54.4 kDa and pI of 6.6 and was associated with high bull fertility. α -L-fucosidase involved in the modification of the carbohydrate portion of the sperm membrane protein during epididymal transit and is found in lower numbers in the seminal plasma of bulls with a higher percentage of abnormal sperm (Jauhiainen and Vanha-Perttula, 1986). Pure enzymes promote acrosome reactions of guinea pig sperm in vitro (Srivastava et al., 1986).

Bovine seminal plasma (BSP) is one of the proteins of the seminal plasma associated with fertility. BSP proteins are a subfamily of proteins that play a key role in sperm maturation and have been extensively researched in terms of biochemical, structural, and functional characteristics (Manjunath et al., 2009). They also take a pivotal part in the formation of the oviductal sperm reservoir (Gwathmey et al., 2006). BSP secreted by the seminal vesicle belongs to the heparin-binding protein family and represents approximately 70% of total protein content of bovine seminal plasma (Nauc and Manjunath, 2000). Bovine seminal plasma contains a protein family designated as Binder of Sperm (BSP) protein,

which has been characterized extensively (Calvete et al., 1995; Bourgeon et al., 2004). The BSP family of proteins is found in mammalian seminal plasma in various forms and is ubiquitous in nature (Villemure et al., 2003). The BSP protein superfamily includes the BSP-1 (PDC-109), BSP-3 (BSP-A3), and BSP-5 (30 kDa) proteins, which are found in all mammals and are involved in various fertility-related events such as sperm membrane modification during capacitation and acrosome reaction, and sperm motility maintenance during oviduct storage by binding to the oviductal epithelium (Nauc and Manjunanth, 2000; Gwathmey et al., 2006; Souza et al., 2008).

CONCLUSION

Research related to protein, specifically in bulls, would be very helpful to better understand and confirm fertile, infertile or subfertile bulls with various conditions in the field. However, proteins have been reported in sperm and seminal plasma that can assist in the selection of fertile bulls.

DECLARATIONS

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Authors' contribution

All authors contributed equally in conducting and writing the manuscript.

Conflict of interests

The authors declared that they had no conflicts of interests.

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