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# Review Article
# Bovine Fertility as Regulated by Sperm Binding Proteins: A Review

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## Abstract
Bovine seminal plasma proteins and its analogs are a family of structurally related proteins characterized by the presence of tandem fibronectin domains. Proteins of the bovine seminal plasma family have low molecular mass (12-30 kDa). Bovine seminal plasma is a protein rich fluid and is involved in sperm motility, capacitation, post testicular maturation event and sperm oocyte binding necessary for sperm to acquire the ability to fertilize an oocyte. Sperm binding proteins have been categorized based on its energy, structural and other functional proteins. As the name implies, these proteins play a vital role in sperm binding to the oviductal epithelium and formation of the oviductal sperm reservoir. Infertility is an important aspect of animal reproduction with much etiological ambiguity. Sperm binding proteins orchestrate the biochemical reactions which govern the fertility of cattle. Bovine seminal plasma contains fertility-associated proteins that are predictive of bull fertility.

Key words: Seminal plasma proteins, biomarkers, spermatozoa, bull fertility, acrosomal reaction, decapacitation

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Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Semen is composed of two major components, (i) The cellular fraction (spermatozoa and the non-spermatozoan cellular elements) and (ii) The seminal plasma (SP). The SP acts like a vehicle to transport the spermatozoa out of the male urethra at the time of ejaculation and direct them on their passage through the female reproductive tract. Semen is a complex fluid and has organic and inorganic components which provides nutrition and protection to the spermatozoa[1]. Seminal plasma fluid contains 35-55 g $L^{-1}$ protein, which plays major role in the fertility of sire. These proteins are important for sperm survival, sperm fertilizing capacity, puberty commencement and sexual maturity. The role of various proteins along with their metabolic functions mentioned in Table 1[2].

Normally the proteins present in semen are of blood origin and seminal plasma specific proteins. The binder of sperm proteins is major protein in seminal plasma, characterized by their ability to bind heparin like glycosaminoglycans. These proteins interact with glycosaminoglycans of female reproductive tract by destabilizing the spermatozoa membrane. Fertility is dependent on the blend complex reactions beginning with spermatogenesis right through sperm-oocyte penetration. Mineral mixture supplementation either in the organic or inorganic form in the diet of crossbred bulls influence quantitative and qualitative attributes of semen[3-5]. In addition, shelter management[6,7] and hormones like IGF-I and prostaglandin role have been found to regulate sperm quality and buffalo reproduction[8-12]. Any defect within this multi-step process will result in infertility or sub-fertility[13]. Infertility is an important aspect of animal reproduction and still presents much etiological ambiguity. Molecular investigations on sperm and seminal plasma reveal their 50% of infertility is related to the male partner[14]. Attempts have been made to increase female fertility in cattle but studies are still scanty to increase bull reproductive efficiency. Some studies have shown significant percentage of reproductive failure due to semen quality and not to the cow problems. Therefore, it is equally important to assess the fertility of sires in reproductive management of cows based on AI[15]. Spermatozoa from the caudal epididymis which have not been exposed to seminal plasma proteins are fertile[16], however, seminal plasma proteins also have some effect on fertility[17]. Queen *et al.*[18] reported that the spermatozoa binding with seminal vesicle proteins and its removal induces complete infertility resulting in decreased pregnancy rate and litter size in mouse. It has been hypothesized that fertility of sub fertile spermatozoa can be improved by combining with seminal plasma from fertile semen[19]. Subsequently prosaposin a peptide of seminal plasma protein was found to increase the fertility of bovine spermatozoa[20]. The fertility in animals is regulated by a number of factors, which are summed up in Fig. 1.

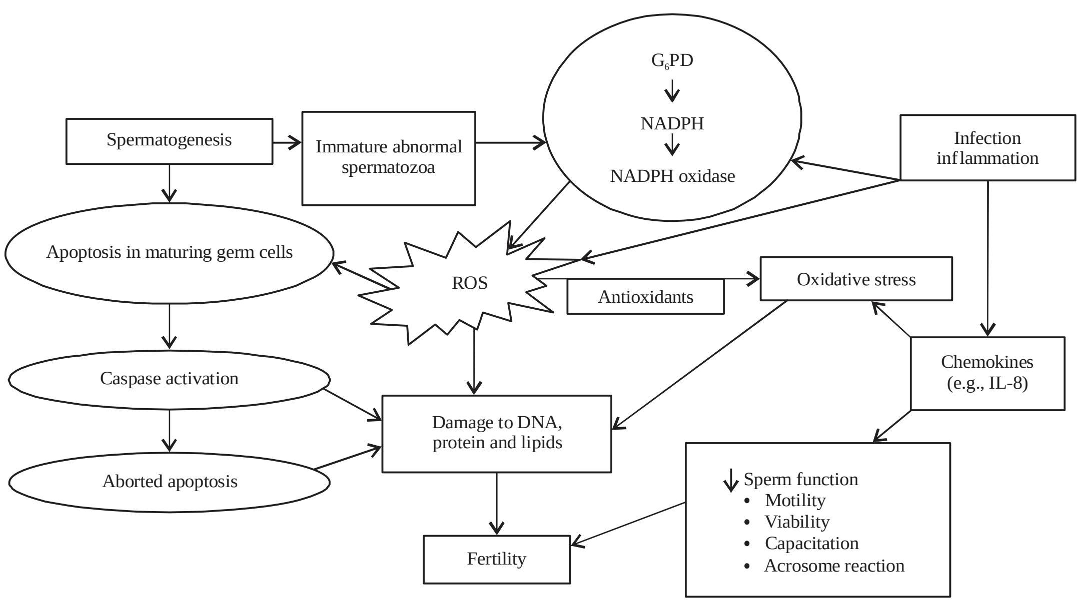


Fig. 1: Factors regulating the fertility in animals

Table 1: Fertility associated proteins and their respective metabolic functions

| Protein | Metabolic functions |
|---|---|
| Acidic seminal fluid protein (aSFP) | Related with sperm motility, metabolism and membrane integrity |
| Acrosin (ACR) | Positively related with sperm motility and semen concentration |
| Cathepsin D (CathD) | Supports seminiferous tubule maturation |
| Epididymal sperm-binding protein 1 (ESBP1) | It binds and tags sperm cells which would be dead upon ejaculation |
| Glutathione peroxidase (GPX) | Increases homeostasis and maintains cellular oxidative stress |
| Niemann-Pick type C2 (NPC2) | Regulates sperm cholesterol metabolism and semen concentration. |
| Osteopontin (OPN) | Assists the acrosome reaction and maintains sperm-oocyte interaction |
| Phosphoglycerate kinase 2 (PK2) | Supports homeostasis and sperm motility, oxidative stress |
| Prosaposin (PSAP) | Related with the structural improvement of the testis |
| Protein C inhibitor (PCI) | Defend sperm from premature acrosome reaction and degradation |
| Seminal plasma protein 1 (BSP1) | It regulates sperm energy use for motility and binds sperm, forms sperm reservoir in the female reproductive tract |
| Seminal plasma protein 3 (BSP3) | Binds sperm, forms sperm reservoir in the female reproductive tract |
| Spermadhesin Z13 (SZ13) | Helps in sperm decapacitation, associated to low fertility |

## MECHANISM OF ACTION

Sperm needs to be hyper-activated to acquire the competency to reach the oocyte, bind into and penetrate the zona pellucida and oolemma. The capacitation is achieved through the release of proteins from the sperm membrane and biochemical reactions related to sperm motility, like cholesterol efflux[21]. Sperm-zona binding is achieved through several proteins and glycoproteins which facilitates acrosome reaction and sperm-zona penetration[22].

Bovine seminal plasma (BSP) proteins coat the spermatozoal surface with phospholipids by specific interaction, decapacitate spermatozoa and prevent a premature acrosome reaction. High density lipoprotein docks to BSP proteins that bind to the sperm membrane and may sequester cholesterol and certain phospholipids resulting in the modification of the sperm membrane lipid composition[23]. The altered permeability of the spermatozoal membrane, allow calcium ions to enter to activate phospholipase enzyme. The later converts phospholipid to lysophospholipids which destabilize membranes and trigger fusion (acrosome reaction). Hence, it is possible that the BSP proteins not only decapacitate the spermatozoa but eventually promote capacitation[24].

After ejaculation, binding of BSP proteins and albumin contribute to capacitation. In the female reproductive tract, defense of sperm against oxidative stress, damage caused by hydrophobic molecules, protein precipitation and complement-induced attack could be facilitated by accessory sex gland fluid (AGF) proteins such as acidic seminal fluid protein (aSFP), clusterin, 5 ´ -nucleotidase and albumin. When the sperm comes in contact with the cumulus layer, interaction with and remodeling of the extracellular matrix (ECM) must be of importance. It is viable thus that proteins such as TMIP-2, clusterin and possibly cathepsin L contribute in these events. Phospholipase A2 (PLA2) secreted into the AGF acts during acrosome reaction, membrane fusion and has reported antimicrobial activity. Sperm-oocyte interaction also includes osteopontin. Sperm motility for fertilization can be affected by aSFP, spermadhesin Z13 and 5'-nucleotidase. However, role of ADP-ribosyltransferase, Nucleobindin, etc has not been elucidated fully[25] (Fig. 2).

## SPERM BINDING PROTEINS

Research studies have found presence of at least 6-7 proteins in buffalo and 5-6 in cattle seminal plasma having 19-20 bands and molecular weight range of 1-92 kDa[26]. In cattle, 21kDa proteins are in abundance whereas 11 and 66 kDa proteins are in higher concentration in buffalo[27]. Three groups of bovine seminal plasma heparin binding proteins 14-17, 24 and 31 kDa has been reported[28]. The 14-17 kDa had a pI of 4.1-6.0 associated with sperm membrane and fertility. A closely related heparin binding protein (HBP), which binds to gelatin, was isolated from bovine seminal plasma (BSP) and designated as BSPA1, BSPA2, BSPA3, BSP-30 kDa[29]. These proteins have an apparent molecular masses of 15-16.5 kDa with isoelectric point (pI) 4.7-5.2, except for BSP 30 kDa which has a molecular mass of about 28-30 kDa with pI 3.9-4.6. Further 8 major HBP with molecular weight range of 13-71 kDa in buffalo seminal plasma has been found[30]. The HBP with a molecular weight of 31 kDa was named as fertility associated antigen (FAA) and may also relate to fertility[31].

## ROLE OF SBPs

The SBPs prolong the maintenance of sperm motility in the oviductal epithelium, presumably by enabling sperm to react with epithelium. The BSPs play an important role in sperm storage and when sperm lose binding affinity for the

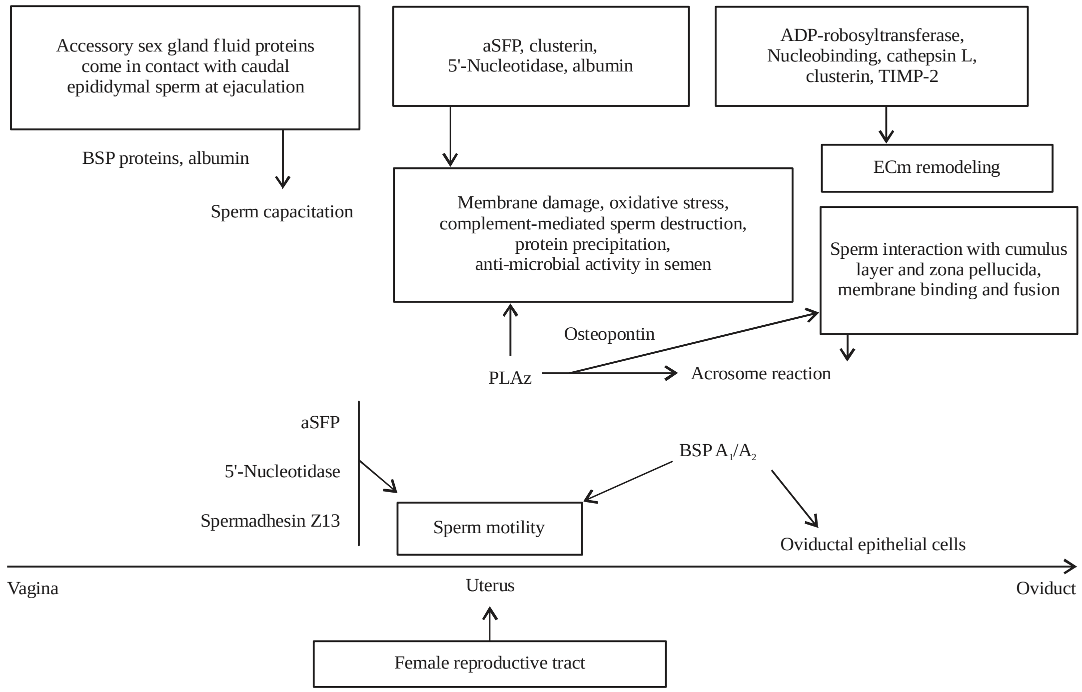


Fig. 2: Sperm functioning and fertilization potentially influenced by the proteins present in the accessory sex gland fluid

Table 2: Role of different enzymes which downregulate or upregulates Asthenozoospermia

| Enzyme name | Symptoms | Protein regulation | References |
|---|---|---|---|
| Isocitrate dehydrogenase subunit α (IDH-α) | Asthenozoospermia | Down | Zhao *et al.*[35] |
| Phosphoglycerate mutase 2 | Asthenozoospermia | Up | Zhao *et al.*[35] |
| Triose phosphate isomerase | Asthenozoospermia | Up | Siva *et al.*[37] |
| Triose phosphate isomerase | Globozoospermia | Down | Siva *et al.*[37] |
| Cytochrome C oxidase subunit 6B | Asthenozoospermia | Down | Martinez-Heredia *et al.*[36] |
| Glycerol kinase testis specific 2 (GKP2) | Asthenozoospermia | Up | Siva *et al.*[37] |
| Glutamate oxaloacetate transaminase-1 | Asthenozoospermia | Up | Zhao *et al.*[35] |
| Fumarate hydratase precursor | Asthenozoospermia | UP | Martinez-Heredia *et al.*[36] |

oviductal epithelium. The loss of BSPs during capacitation plays a major role in release of sperm[32]. The BSPs function as a molecular chaperone *in vitro*, suggesting a role of it in the proper folding of proteins involved in the bovine sperm capacitation pathway[33].

**Sperm motility and differentiation related proteins:** Sperm motility related proteins have been categorized as (i) Energy related enzymes in mitochondrial and glycolytic pathways (ii) Structural proteins of outer dense fiber and A-kinase anchoring proteins (AKAPs) in the flagella and (iii) Activating signal transducers e.g., protein kinase-A (PKA) and serine-threonine-tyrosine kinase/phosphatases[34].

**Proteins related to energy:** Experiments on sperm protein expression profiles with that of normozoospermic donors led to identification of proteins that are associated with sperm energy metabolism[35]. It is well known that ATP is consumed to production takes place through glycolysis and oxidative phosphorylation in mammalian sperm mitochondria. Proteins like isocitrate dehydrogenase subunit α (IDH-α) show lower expression in asthenozoospermic patients (Table 2) and glycolytic enzymes (phosphoglycerate mutase 2 and triosephosphate isomerase) and glutamate oxaloacetate transaminase-1 enzymes were highly expressed in most of the patients[35].

Martinez-Heredia *et al.*[36] observed 17 protein spots in asthenozoospermic group to be either increased or decreased and identified COX6B, DLDpre, FHpre and ECH1pre - energy related enzymes[36]. Cytochrome C oxidase was characterized as the terminal enzyme of the respiratory chain which catalyses the transfer of electrons to oxygen for ATP synthesis. The COX6B is rate limiting and could lead to lower ATP production and decrease in motility in the asthenozoospermic sperm samples.

Table 3: Flagella related proteins and their functions

| Protein name | Symptoms | Protein regulation | References |
|---|---|---|---|
| Outer dense fiber protein 2 | Globozoospermia and Asthenozoospermia | Down | Zhao *et al.*[35] Liao *et al.*[41] |
| Tektin 1 | Asthenozoospermia | Down | Siva *et al.*[37] |
| Septin 4 and testis anion transporter 1 | Asthenozoospermia | Down | Lhuillier *et al.*[42] |
| Secretory actin binding protein | Asthenozoospermia | Up | Capkova *et al.*[43] |
| Tubulin beta 2C chain | Asthenozoospermia | Down | Siva *et al.*[37] |
| Isoform 1 and 2 of tubulin $\alpha$-2 chain | Globozoospermia | Down | Liao *et al.*[41] |
| $\alpha$-tubulin isotype H2-$\alpha$ | Globozoospermia | Down | Liao *et al.*[41] |

Table 4: Role of various transducer proteins in sperm biochemical reactions

| Protein name | Symptoms | Protein regulation | Protein function | Reference |
|---|---|---|---|---|
| Dihydrolipoamide dehydrogenase precursor | Asthenozoospermia | Up | Hyper activation of spermatozoa during capacitation and acrosome reaction | Martinez-Heredia *et al.*[36] |
| Inositol-1 (or 4)-monophosphatase | Asthenozoospermia | Up | Key enzyme of the phosphatidylinositol signalling pathway | Martinez-Heredia *et al.*[36] |
| S100 calcium binding protein A9 | Asthenozoospermia | Down | Calcium binding protein | Martinez-Heredia *et al.*[36] |
| Cation channel sperm-associated protein 1, 2, 3 and 4 | Knock-out gene mice | - | Calcium influx to trigger tyrosine phosphorylation | Muratori *et al.*[34] |
| Soluble adenylyl cyclase | Knock-out gene mice | - | cAMP production | Muratori *et al.*[34] |
| Testis-specific serine/threonine kinases 1 and 4 | - | - | May involve in signalling pathway | Li *et al.*[44] |
| Proprotein convertase subtilisin/ kexin type 4 | Knock-out gene mice | - | Enzymatic activation of precursor proteins | Gyamera-Acheampong and Mikay[45] |

**Flagella related proteins:** Actin, the major cytoskeletal proteins participates in many important cellular functions including cell motility, vesicle and organelle movement, cytokinesis, cell signalling (Table 3) and the establishment and maintenance of cell juncture and cell shape[36]. Actin polymerization may serve as an important regulatory pathway that is associated with tyrosine phosphorylation in sperm[38]. A decrease in actin decrease the sperm motility since, without cytoskeletal support, the flagellum might not beat correctly. In this regard, involvement of prolactin induced protein has been found in some kind of actin binding function, in the post-acrosomal zone and in fertilization[39,40].

**Signal transducer proteins:** The information on signal transducer protein is very scanty (Table 4). The cell signalling and regulation have been detected an increased amount of IMPA1 in asthenozoospermic patients which is one of the enzymes involved in myo-inositol synthesis and embryonic development[44].

**Proteins related to acrosome biogenesis and acrosome reaction:** Sperm proteins are associated with the nucleus on the X chromosome (SPANX-A, B and C) and are expressed in post-meiotic spermatids. The SP play an important role in acrosome biogenesis and are found to be down regulated in globozoospermic sperm[46]. Seminal plasma secretory actin-binding protein is associated with capacitation and acrosome reaction and is the main cytoskeletal sperm protein in head, midpiece and tail[47]. The most abundant sperm nuclear proteins are Protamine 1 and Protamine 2 (P2, P3 and P4). Testis/sperm-specific histone is found to be over-expressed in infertile individuals and negatively correlated with protamine abundance[46]. In the infertile individuals, the P1/P2 ratio which is 1/1 in fertile individuals is increased as a consequence of under-expression of P2 and DNA fragmentation[48].

**Sperm peripheral proteins:** Sperm peripheral proteins are produced outside the sperm and then attach to the sperm that are fertility related. Beta defensin 126 is an epididymal protein which covers the whole sperm head of monkeys and helps in cervical mucus penetration[49]. Another protein guanylyl cyclase receptor-G (hGC-G) is expressed in human testis with receptor on the acrosome cap and equatorial segment of the mature sperm. It plays a role in zona binding in humans[50].

**Proteins related to post-translational modification:** The sperm is a translational and transcriptionally inactive cell. Due to this reason, post-translational modification plays a

Table 5: Functional role of different proteins in fertilization

| Protein name | Protein function | Reference |
|---|---|---|
| Spermadhesin | Primary zona-binding as a heparin binding protein | Gadbella[52] |
| N-acetylglucosaminidase | Initial sperm-zona binding in humans | Martinez *et al.*[55] and |
| Inner acrosomal membrane protein and zona-pellucida binding protein 2 | Interact with zona glycoproteins during sperm-oocyte penetration | Sutovsky[56] |
| Zonadhesin | Involved in sperm-oolemma interaction | Shur[57] |
| Tetraspanins CD9 and CD81 | Control sperm oolemma interaction and penetration | Sutovsky[56] |
| N-cadherin | Sperm-oolemma penetration | Marin Briggiler *et al.*[54] |
| Cystein-rich secretory protein (CRISPs) family | Sperm-oolemma penetration | Sutovsky[56] |
| Disulfide isomerase protein (ERp57, ERp72,PDI, P5) family | Protein refolding which might trigger sperm-oocyte fusion | Nixon *et al.*[50] |

crucial role in sperm activation and fertilization. Epididymal proteins like HSP60, HSP90 and endoplasmin render sperm-zona receptors ready for zona binding. Flagella calcium binding protein and fibrous sheet AKAP3 are highly tyrosine-phosphorylated during sperm capacitation leading to sperm hyper-activation[34]. It has been found that protein convertase type 4 has roles in sperm capacitation, hyper-activation and sperm-zona pellucida reaction[51].

**Proteins related to sperm-zona pellucida interaction and sperm-oolemma penetration:** Zona pellucida is comprised of sulphated glycoproteins such as ZP1, ZP2 and ZP3 which are produced by oocyte or granulosa cells. Zona pellucida sperm-binding protein 3 is the receptor in the zona pellucida which binds sperm at the beginning of fertilization[52]. Spermadhesin is a porcine protein which plays a role in sperm zona pellucida binding (Table 5). Zonadhesin is the inner-acrosomal protein in porcine sperm which is involved in sperm-oolemma interaction[53]. Disintegrin, cysteine rich domain and epidermal growth factor (EGF) are involved in sperm-oocyte penetration and sperm migration in the oviduct[54]. N-cadherin (Neural cadherin), a transmembrane glycoprotein is found in the equatorial segment of acrosome-reacted sperm and participates in sperm-oolemma interaction[55].

## CONCLUSIONS AND FUTURE RECOMMENDATIONS

Sperm binding proteins orchestrate many biochemical reactions and govern the fertility in dairy animals. The available literature indicates the importance of bovine semen fertility associated proteins that are predictive of bull fertility. Besides this, the role of factors like dietary regime, climatic variables and management of bulls may affect their concentration in the semen and their importance. Research interventions are required to identify specific sperm binding proteins that would have an impact on fertility and unravel their physiological significance to develop new diagnostic methods to correct infertility problems.

Infertility and sub-fertility are the major thrust areas of research work to improve the reproductive problems in both domestic animals and human beings. The secretions from the testis, epididymis and male accessory sex glands together constitute seminal plasma which contains number proteins. These seminal plasma proteins are associated with male fertility but most of these have not been studied in detail in bull semen till date, though some major differences in seminal plasma proteins of high and low fertility bulls have been found and their functions have been elucidated. If detailed studies of seminal plasma protein and their specific role in fertilization process is known than seminal plasma protein could be used as a promising biomarkers for bull fertility.

## SIGNIFICANCE STATEMENTS

Sperm proteomic study has elucidated existence of potential semen fertility markers which have been categorized as energy related or functional proteins. These sperm binding proteins are crucial for sperm motility, capacitation and binding with oocyte.

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