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Proteins and enzymes related to sperm motility, capacitation and acrosome reaction

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Abstract
Sperm motility is crucial for successful fertilization since it indicates a sperm cell's vitality. The reproductive health of female animals, the weather, and the quality of the semen (including sperm motility, capacitation, Acrosomal response, morphology, and plasma membrane integrity) all have a role in bovine reproduction. Capacitation typically affects the female reproductive tract, but it can also happen when certain capacitating inducer factors are present during cryopreservation. This is harmful because after capacitation, the acrosome lyses and all of the enzyme is liberated from it. Here, the mechanisms governing sperm motility, capacitation, and acrosome reaction are discussed.

Keywords: Semen, capacitation, acrosome reaction, proteins, sperm motility

Introduction
A crucial component of viable spermatozoa is sperm motility. Because sperm mobility is necessary for penetrating the outer membrane of the oocyte, including the zona pellucida, and allowing ejaculated spermatozoa to reach the female reproductive canal and the site of fertilization, according to Vijayaraghavan *et al*. (2003) [49]. Sperm motility typically indicates a population's viability. Numerous species have demonstrated a correlation between sperm motility and fertility (Catalano *et al*., 2011) [5]. In all domestic mammals, the spermatozoa that exit the testis are unable to fertilize the female gamete. Instead, they undergo distinct morphological, physiological, and biochemical changes during epididymal transit and acquire motility in the cauda epididymis (Amann, 1993; Cooper, 2012) [2, 50] and gain motility in cauda epididymis. The fertility of the samples varies greatly among various forms of motile spermatozoa in an ejaculate. Sperm movement and movement patterns are frequently regarded as the most crucial factors in determining fertility and predicting freez ability.

Sperm motility
Sperm motility typically indicates a population's viability. Numerous species have demonstrated a correlation between sperm motility and fertility (Catalano *et al*., 2011) [5]. In all domestic mammals, the spermatozoa that exit the testis are incapable of fertilizing the female gamete. They go through various morphological, physiological, and biochemical modifications to fertilize the female gamete during epididymal transit and acquire motility in the cauda epididymis (Amann *et al*., 1993) [2].
Assessing sperm motility entails estimating subjectively both the viability of spermatozoa and the level of motility. Using a phase contrast microscope, sperm motility can be evaluated in raw and extended semen and different patterns of sperm motility can be seen. However, in raw semen, sperm motility can be hindered or altered by high sperm concentration, so to get around this issue, an aliquot of semen should also be extended (Varner *et al*., 1991) [41]. Sperm motility in prolonged semen typically takes the form of a lengthy semi-arc pattern. Hyperactive sperm motility produces an x-pattern reaction. Sperm that are swimming in a small circular junction have experienced cold shock (Herman *et al*., 1994) [16]. Sperm motility patterns are dependent on the energy supply, and in low glucose conditions, mitochondrial oxidative phosphorylation is stimulated to make ATP.
The two main metabolic pathways that control sperm motility are ca+2 and cyclic adenosine monophosphate (cAMP) dependent protein kinase, also known as protein kinase A (PKA) (Darszon *et al*., 2006) [9]. Bicarbonate ions, calcium ions, adenyl Cyclase, various membrane channels, and phosphorylation processes are all involved in these pathways.

All are in charge of learning the skills necessary for sperm to fertilize the oocyte, specifically capacitation, hyperactivity, and acrosome response. Adenylyl Cyclase (ACs), which catalyse an intramolecular cyclization of ATP to cAMP under release of pyrophosphate, regulate the levels of cyclic adenosine monophosphate (cAMP) in cells (Steegborn *et al*., 2014) [51]. Transmembrane AC enzymes (tmACs) and soluble AC (sAC, commonly known as AC10) are two different forms of mammalian ACs. At many intracellular sites, soluble AC serves as a sensor for ATP, Ca+2, bicarbonate/CO_2, and pH. Soluble AC is directly activated by bicarbonate and Ca+2. Only signalling proteins that are soluble in ACs are known to be directly controlled by bicarbonate. As a subset of the G-protein coupled receptor pathways, tmACs, in contrast, are primarily controlled by heterotrimeric G-proteins and are not responsive to bicarbonate (Sunahara *et al*., 2002) [37]. Male fertility is significantly influenced by both ACs proteins. Transmembrane AC is engaged in both progressive motility and the fundamental mechanism for motility activation through cAMP-dependent protein phosphorylation (Dey *et al*., 2014) [10]. According to Hess *et al*. (2005) [17], soluble AC is the main adenyl cyclase that generates the majority of cAMP in spermatozoa and is essential for cAMP signalling as well as for the rise in spermatozoa beat frequency. Due to a lack of forward motility caused by the sAC gene's inactivation, male sterility results (Esposito *et al*., 2004) [12]. Thus, sperm motility regulation and fertility depend on cyclic AMP, and decreased cAMP levels are linked to decreased sperm motility (Esposito *et al*., 2004) [12].

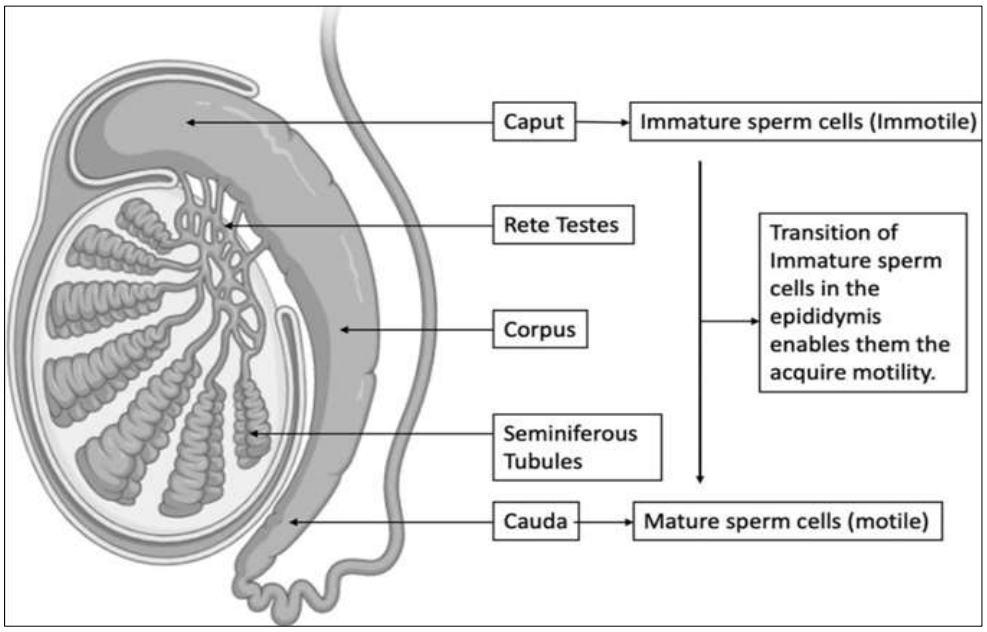

Fig 1: Diagram of the epididymis (Chakraborty *et al*., 2022) [6]

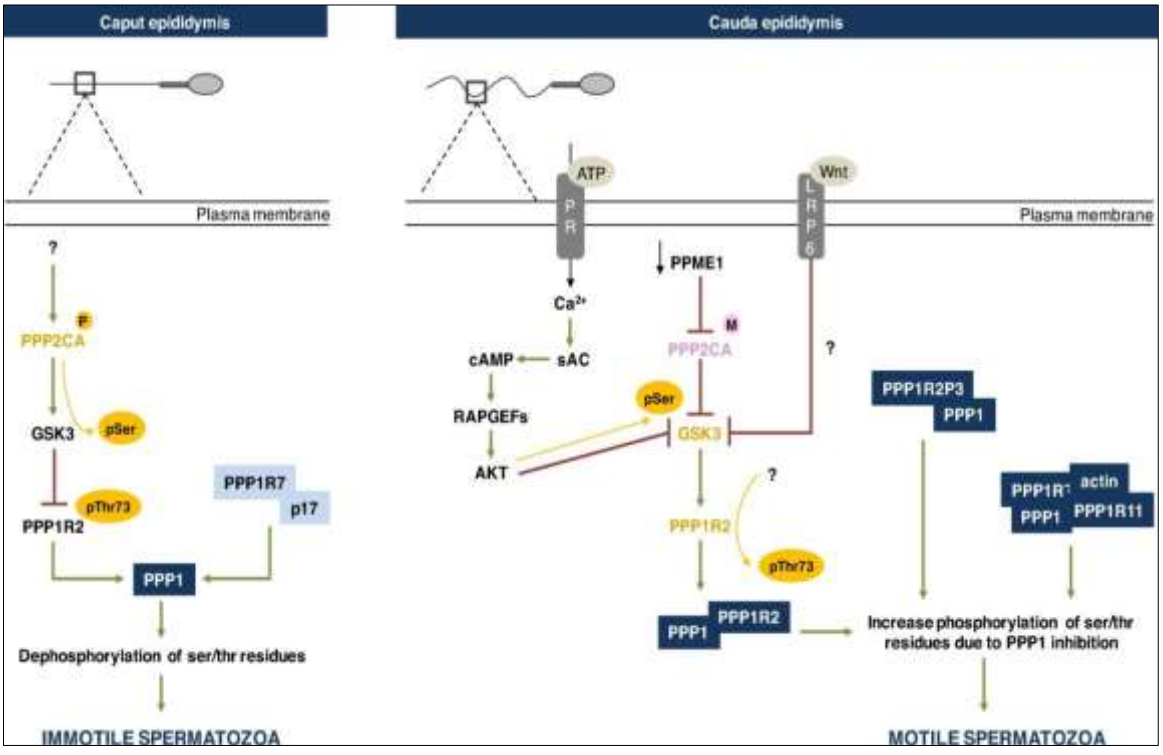

Fig 2: Schematic representation of the signaling events required for sperm motility acquisition in the epididymis (Freitas *et al*., 2017) [14]

Sperm capacitation

Sperm that has just been ejaculated cannot quickly fertilize the eggs. To be able to fertilize ova, they must spend a specific amount of time in the female genital tract after semen is deposited in the vagina. 71 years ago, Chang and Austin separately described this phenomenon in rats and rabbits, respectively. Capacitation (Puga *et al*., 2018) [28] is the term used to describe the activation of spermatozoa inside the female reproductive canal. Once the capacitation process has begun, it cannot be stopped; the spermatozoa's end outcomes are either death or reaching the oocyte. Mammalian spermatozoa must undergo a sequence of metabolic changes in the female reproductive tract known as capacitation before they may fertilize the oocyte right away after ejaculation. After being capacitated, spermatozoa attach to the egg's zona pellucida (ZP) and proceed through the acrosome reaction (AR), which permits the sperm to enter and fertilize the oocyte. According to a recent study (Jin *et al*., 2011), mouse sperm that undergo AR before coming into touch with the ZP can fertilize the egg. However, a number of intracellular alterations, such as an increase in cholesterol efflux, a rise in membrane fluidity, an increase in intracellular Ca+2 concentration, and others are known to occur (Breitbart, 2005) [4]. There is no distinctly trustworthy sign to characterize capacitation. De-capacitation factor is a molecule that is present in seminal plasma and has the ability to promote capacitation at the proper moment during incubation as well as prevent premature capacitation at the wrong period. Three acidic proteins known as BSP-A1/-A2, BSP-A3, and BSP-30 (also known as BSPs) are the most prevalent proteins found in bovine seminal plasma. BSP-30 has a molecular weight of 28–30 kDa compared to BSP–A1/–A2 and BSP–A3's 15–16 kDa. The BSP-A1/-A2 is a blend of BSP-A1 and BSP-A2, which only differ in glycosylation (Kumar *et al*., 2015) [21]. It is also known as PDC-109 (Protein containing N-aspartic acid D and carboxy terminus Cysteine, comprising 109 amino acids). High density lipoprotein (HDL) and glycosaminoglycans can bind with BSP-1, 2, and 3 to facilitate the activation of sperm. Additional processes that take place during the capacitation of human, mouse, ram, and bull sperm include actin polymerization and changes in swimming pattern. Actin is a protein that joins to other actin proteins during a process known as actin polymerization, resulting in filamentous actin (F- actin). Actin protein can be found in the tail and post-acrosomal regions (Clarke *et al*., 1982) [7]. Actin protein may serve a significant function in regulating sperm motility, and its presence in the head raises the possibility that it also plays a role in sperm capacitation and the acrosome reaction.

The role of calcium, bicarbonate and phosphorylation in sperm motility and capacitation

For sperm capacitation, hyperactivation, and acrosome response, calcium is a crucial regulator. Flagella beat symmetrically at low intracellular Ca+2 concentrations, but as sperm activation levels increase, the waveform becomes more asymmetric and the sperm become hyper-activated (Suarez *et al*., 2008) [36]. High Ca+2 levels, however, inhibit motility. This inhibition appears to be brought on by Ca+2 inhibiting substrate-kinase interactions by reducing protein phosphorylation (Either by substrate depletion or by conformational changes). According to Smith *et al*. (1992) [33], calcium has a role in controlling dynein-driven microtubule sliding. The calmodulin-dependent kinase may mediate this Ca+2 signal because calmodulin is an important axonemal Ca+2 sensor. The central pair complex and the radial spokes control these complexes, which are located at the sperm axoneme. Through direct interactions with protein kinases, phosphatases, and sAC, calmodulin controls motility. Additionally, bicarbonate (HCO3) ions play a significant role in controlling sperm activity. It is a female reproductive system anion that is transferred into sperm during capacitation. Ca+2 stimulation causes sAC to convert ATP to cAMP, which raises the amount of cAMP. HCO_3 has the same effects since it is necessary for Ca+2 absorption. HCO3 treatment *in vitro* causes Ca+2 entrance, which quickly raises flagellar beat frequency while lowering asymmetry. Proteins with PKA anchoring sites are found in the fibrous sheath (FS), which clearly suggests that one of the main functions of this structure is to anchor PKA in the main component of the flagellum. Cyclic AMP stimulates PKA activity and facilitates both capacitation and the acrosome response (Yanagimachi *et al*., 1994) [45]. Tyrosine phosphorylation and flagellar beat frequency are thought to increase when PKA is activated, preparing the capacitated sperm for fertilization.

Enzymes and proteins related to sperm motility

According to Hou *et al*. (2019) [52], proteomics technology provides a reliable way to look at the molecular mechanisms behind sperm motility and capacitation. Human low-motile sperm and normal-motile sperm were compared using proteomics, and the results indicated thirty-four proteins of interest (Zaho *et al*., 2007) [46]. Proteins related to sperm motility are categorized into (i) energy-related enzymes in mitochondrial and glycolytic pathways, (ii) structural proteins such as outer dense fiber (ODF) and a-kinase anchoring proteins (AKAPs) in the flagella, and (iii) activating signal transducers e.g. protein kinase-A like (PKA) and serine-threonine-tyrosine kinase/phosphatases (Muratori *et al*., 2009) [24]. Various enzymes and proteins related to sperm physiology in domestic animals are mentioned below in table no. 1 and 2, respectively**.**

Table 1: Enzymes related to sperm motility in mammals

Enzyme name	Species	Function	Location	Comment	References
Isocitrate dehydrogenase subunitα (IDH- α)	Human	For energy production in form of ATP.	Mitochondria	Reduced sperm motility associated with low IDH-α expression	Zaho *et al.*, (2007) [46]
Phosphoglycerate mutase 2 (PGK 2)	Human	Essential for sperm function and male fertility	Cytosol	PGK2catalyzes the first ATP-generating step	Zaho *et al.*, (2007) [46]
Triosephosphate isomerase (TPIS	Pig	-	Cytosol	Negatively correlated with sperm quality (Motility, integrity)	Vilagran *et al.*, (2016) [43]
Glutamate oxaloacetate transaminase-1	Human	Direct relationship between Transaminase activities with sperm concentration.	Cytosol	Transaminase values in oligospermic and a spermic ejaculate is always low.	Zaho *et al.*, (2007) [46]
Glycerol kinase, testis specific 2 (GK2)	Mouse	Essential for proper arrangement of crescent-like mitochondria to from the mitochondrial sheath during spermatogenesis	Mitochondria	abnormal bent tail and fragmented mitochondrial sheath were observed in Gk2 spermatozoa collected from the cauda epididymis	Shimada *et al.*, (2019) [30]
PLA2	Mice	Key enzyme in sperm capacitation and acrosome reaction.	Seminal vesicles	Deactivation of the PLA2 affect the disruption of sperm motility and reduce the fertility rates	Sato *et al.*, (2010) [29]

Table 2: Protein related to sperm motility in domestic animals

Protein	Species	Function	Location	Comments	References
Spermadhesin 2	Cattle bull	Exhibit carbohydrate-binding activity and interact with phospholipids.	Seminal plasma	Negative impact on sperm motility and fertility	Druart *et al.*, (2019) [11]
Cluster in	Cattle bull	However cluster in prevent oxidative damage and inhibits sperm lysis	Seminal plasma	More abundant in low-fertility bulls	Viana *et al.*, (2018) [42]
Bovine seminal plasma protein (BSP 1)	Cross-breed bull	Regulation of sperm motility sperm capacitation and acrosome reaction	Seminal plasma	High levels of BSP1 causes an imbalance in the plasma membrane, sos-perm is more sensitive during cryopreservation	Aslam *et al.*, (2018) [3]
BSP5	Cattle bull	useful in the process of membrane stability and sperm capacitation	Seminal plasma	High BSP5 concentrations are found in high fertility bulls	Viana *et al.*, (2018) [42]
ODF2	Buffalo bull	molecular marker of functional maturation of centrioles. intimately it connected with spermatogenesis,	Sperm tail	ODF2down regulated in the low-motility buffalo sperm, so the protein is associated with sperm motility	Huang *et al.*, (2015) [18]
ATP5A1	Buffalo bull	ATP5A1 expressed in the high-motility sperm provides more energy	Mitochondria	The concentration of the ATP5A1 in low motile buffalo sperm was lower than high motility buffalo sperm,	Huang *et al.*, (2015) [18]
Enolase (ENO-1)	Buffalo bull	Regulate energy for sperm motility and protect from oxidative stress.	Sperm tail	ENO1 enzyme concentration is higher in cauda epididymis of low sperm motile bull than cauda and caput of high sperm motile bull (Potential marker for low sperm motility).	Park *et al.*, (2021) [26]
Malate dehydrogenase-2 NAD (MDH2)	Cattle bull	MDH2 regulate energy in sperm and supports its potential involvement in regulating male fertility by facilitating capacitation and acrosome reaction.	Mid piece of spermatozoa	The abnormal expression of MDH2 will cause the disruption of internal energy distribution in spermatozoa and has an impact on sperm motility, capacitation an occurrence of hyperactivation.	Peddinti *et al.*, (2008) [27]
Heat shock protein 90(HSP90)	Cattle bull	HSP90 prevent cell damage from adverse environment and mediate in sperm motility.	Sperm tail	In fresh semen HSP90 level found higher than equilibrated and frozen hence acrosome integrity and motility was lower in equilibrated and frozen semen than fresh.	Zhang *et al.*, (2015) [47]
PEBP4	Boar	PEBP4 have N-terminal signal peptide and involved in regulation of serine proteases, regulate sperm motility and metabolic activity through protein phosphorylation.	Sperm tail	Stimulate sperm motility in semen. Higher abundances of PEBP4 in HM spermatozoa indicated that PEBP4 might be a potential proteomic marker for ram sperm motility.	Zhang *et al.*, 2015 [47]
SPATA18 and LETM1	Ram	Spata18 mitochondria-eating protein that orchestrates the degradation of unhealthy mitochondria during spermatogenesis, LETM1 is crucial for the maintenance of mitochondrial tubular networks and shapes in spermatozoa.	Sperm mitochondria	Higher abundance of SPATA18 and LETM1 indicated higher mitochondrial activity, and more energy supplementation in the high motileram spermatozoa.	Zhu *et al.*, (2019) [48]
NUP98	Ram	integral component of the basket filaments associated with the nuclear pore complexes	Sperm filament	In this study NUP98 found higher in high-motile than low-motile spermatozoa.	Zhu *et al.*, (2019) [48]

As chromatin condensation begins, transition proteins (TNPs) are essential for removing histone proteins, which are then replaced by protamines (Kistler *et al*., 1996) [20]. In a study on cattle conducted by Eugene *et al*. in 2000 [13], it was shown that the TNP1 protein is crucial for sperm motility since 60% of TNP1-null males were found to be infertile due to drastically decreased sperm motility. Male infertility is finally brought on by TNP2 protein anomalies that result in abnormalities in sperm heads due to acrosomal errors and spermatozoa's failure to penetrate zona pellucida (Adham *et al*., 2001; Eugene *et al*., 2000) [1, 13]. Sperm from TNP1 and TNP2 protein-deficient animals showed reduced chromatin condensation, normal motility, and shape (Shirley *et al*., 2004; Miyagawa *et al*., 2005) [31, 23]. Therefore, we can conclude that the TNPs protein is crucial for male fertility. According to Wang *et al*. (2004) [44], the PEBP4 has been discovered as a secretory protein with an N-terminal signal peptide that is involved in the control of serine proteases. Both in humans and domesticated animals, there has been research on the connection between PEBP4 and sperm motility. For example, it has been reported that PEBP4 could regulate human spermatozoa motility and metabolic activity through protein phosphorylation signaling cascades (Siva *et al.*, 2010) [32]. In boar semen, the PEBP4 may promote sperm motility. Additionally, it was discovered that PEBP4 was localized in the main tail portion of bull spermatozoa and that it positively correlated with sperm motility (Somashekar *et al*., 2017) [35]. PEBP4 may serve as a possible proteomic marker for ram sperm motility because to its higher presence in HM spermatozoa. Sperm mitochondria are essential parts that give the sperm energy to move around (Thangaraj *et al*., 2003) [39]. Both LETM 1 and SPATA 18 are mitochondrial proteins. According to Tsuneki *et al*. (2015) [40], SPATA 18 is a mitochondria-eating protein that coordinates the destruction of dysfunctional mitochondria during spermatogenesis. In order to maintain the networks and morphologies of the mitochondrial tubular networks in human spermatozoa, the mitochondrial inner-membrane protein LETM 1 is essential (Tamai *et al*., 2008) [38]. Higher levels of SPATA 18 and LETM 1 were found in the high motility ram spermatozoa, indicating increased mitochondrial activity and energy replenishment. The basket filaments connected to the nuclear pore complexes contain NUP 98 as a key part. The fidelity of gene regulation, which is necessary to accomplish the precisely regulated germ cell differentiation necessary for fertility, depends heavily on nucleocytoplasmic transport. Spermatogonia cells from Drosophila with NUP 98 mutants were shown to have increased cell mortality and decreased cell proliferation (Colozza *et al*., 2011) [8]. In the current investigation, sperm with high motility had a larger abundance of NUP 98 than sperm with poor motility. The information provided here may point to a decline in the spermatozoa's apoptotic rate in the group of highly mobile spermatozoa. Proteomic analysis by Park *et al*. (2012) [25] discovered potential indicators for sperm fertility, however no conclusive research has yet demonstrated a connection between the proteome and sperm fertility. Therefore, using 2-dimensional electrophoresis, the protein expression profiles of spermatozoa from high and poor fertility bulls were compared in order to ascertain the clinical significance of the protein markers discovered by proteomic analysis. Then investigated the relationship between protein expression and the fertility of individual bulls as assessed by Western blot analysis. Five proteins, enolase 1 (ENO1), ATP synthase H^+ transporting mitochondrial F1 complex beta subunit, apoptosis-stimulating of p53 protein 2, alpha-2-HS-glycoprotein, and phospholipid hydroperoxide glutathione peroxide, were more highly represented in high fertility bulls, whereas three proteins, voltage-dependent anion channel 2 (VDAC2), ropporin-1, and ubiquinol-cytochrome-c reductase complex core protein 2 (UQCRC2), were more highly represented in low fertility bulls. Among those proteins, ENO1, VDAC2, and UQCRC2 were significantly correlated with individual fertility. These findings therefore imply that an effective *in vitro* assay to assess sperm fertility may involve concurrent comparisons between protein expression and other fertility assays. Aslam *et al*. (2018) [3] found a small number of possible proteins in buffalo bull spermatozoa that may be helpful in determining fertility by comparative proteomics. The sperm proteome of high-fertile buffalo bulls was compared with that of low-fertile buffalo bulls using two-dimensional difference gel electrophoresis (2D-DIGE), and the differentially expressed proteins were identified through mass spectrometric method. The protein interaction network and the functional bioinformatics analysis of differentially expressed proteins were also carried out. In the spermatozoa of high-fertile bulls, 10 proteins were found overexpressed and 15 proteins were under-expressed at the level of twofold or more ($p \leq 0.05$). The proteins over expressed in high-fertile spermatozoa were PDZD8, GTF2F2, ZNF397, KIZ, LOH12CR1, ACRBP, PRSS37, CYP11B2, F13A1 and SPO11, whereas those over expressed in low-fertile spermatozoa were MT1A, ATP5F1, CS, TCRB, PRODH2, HARS, IDH3A, SRPK3, Uncharacterized protein C9 or F9 homolog isoform X4, TUBB2B, GPR4, PMP2, CTSL1, TPPP2 and EGFL6. The difference in expression between the two groups varied from 2.0 to 6.1 folds, with MT1A being highly abundant in low-fertile spermatozoa and CYP11B2 being highly abundant in high-fertile spermatozoa. The majority of the proteins that were overexpressed in low-fertile spermatozoa were involved in energy metabolism and capacitation factors, suggesting that premature capacitation and cryo-damages may have contributed to the reduced fertility of buffalo spermatozoa that have been cryopreserved. The entire characterization of sperm biology and the extensive identification of spermatozoa and seminal plasma proteins were the goals of an experiment conducted by Fu *et al*. in 2019 [15]. This work is crucial to understanding how seminal plasma affects sperm fertility. This work used a bottom-up method to analyse the proteome of buffalo spermatozoa and seminal plasma. In total, 864 and 2147 proteins were found in seminal plasma and mature spermatozoa, respectively. 371 of these proteins, or 42.9% of the total, were shared by seminal plasma and spermatozoa. The three most prevalent proteins in seminal plasma were ALB, CLU, and AZGP1, while the three most abundant proteins in spermatozoa were ODF2, AKAP4, and TUBB. Buffalo spermatozoa and seminal plasma proteome profiles. The Computer Assisted Semen Analysis (CASA) technology was used to analyze the buffalo semen samples. Sperm concentration was 5.240.71108, while motility and progressive motility were, respectively, 79.612.1% and 67.16.4%. Peptides from spermatozoa and seminal plasma were examined using LC-MS/MS after protein extraction and processing. A total of 8072 and 4058 distinct peptides, which were translated into 2147 and 864 proteins, respectively, were obtained. Datasets based on LC-MS/MS of bovine and swine were acquired for the purpose of analyzing the proteome profile of buffalo spermatozoa and seminal plasma and

creating a Venn diagram. The Venn diagram showed that 348 proteins were found in all three ungulate species together. 813 proteins in all were found to be shared among bovine and buffalo spermatozoa. The seminal plasma of buffalo and bovine showed a better similarity in protein content, with 325 proteins being jointly identified and 683 proteins co-present. In a study by Park *et al*. (2021) [26], it was discovered that the caput portion of the epididymis from bulls with low motile spermatozoa contained higher levels of the protein Enolase-1 (ENO 1) than the caput and cauda spermatozoa from bulls with high motility.

Conclusion
Sperm that have been released from seminiferous tubules and are immobile gain the ability to move and the capacity to fertilize in the epididymis. Acrosomal response, or the release of acrosomal enzymes, and successful fertilization require capping. The numerous proteins and enzymes have an impact on acrosomal response and capacitation.

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Conflict of interest
There is no conflict of interest

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