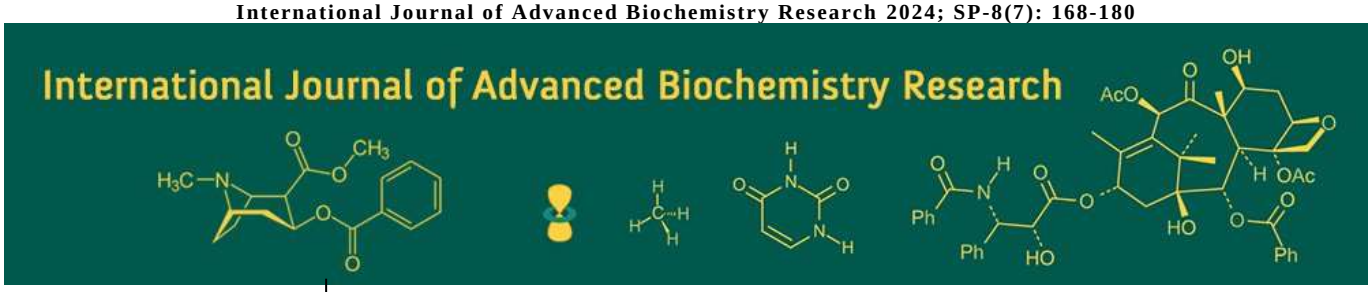

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Fertility markers in bull

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Abstract
Fertility is most important for enhancement of livestock populations. Sperm and seminal plasma contain a variety of proteins, including protamine, which can be utilized as a fertility marker. Every protein involved in sperm function is essential for its efficient assembly, motility, capacitation, cell protection, acrosome responses, effective fertilization, activation of the egg, and embryonic development. Marker-Assisted Selection (MAS) utilizes the genetic information at markers. Although metabolites are crucial to biological processes, little is known about the precise identities and importance of the seminal plasma metabolome in relation to bull fertility. Bull seminal plasma's metabolome was examined using GC-MS, and compounds including fructose and 2-oxoglutaric acid were identified as possible indicators of bull fertility. While several of these metabolites have been identified and their activities established, a larger number of sperm and seminal plasma metabolites remain unidentified. The epididymis, testis, and male accessory sex glands secrete seminal plasma. While there has been relatively little research on them, a variety of seminal plasma proteins are linked to male fertility. Recently, proteomics has demonstrated the variations in the protein profiles found in the seminal plasma of bulls with high and poor fertility. Osteopontin, phospholipase A2, P25b, acidic seminal fluid proteins, a-L-fucosidase, and cathepsin D have positive correlations with bull fertility, but lipocalin-type prostaglandin D synthase, spermadhesin Z13, clusterin, and ubiquitin have negative correlations. Further research is needed to understand the physiological functions of metalloproteinase-2 (TIMP-2), ecto-ADP-ribosyltransferase 5, nuclobindin, Niemann-Pick C2, and epididymal sperm-binding protein 1 and how they relate to bull fertility. The current review article explains the roles played by several blood, sperm, and seminal plasma proteins as well as how bull fertility is related to them.

Keywords: Fertility markers, bull, proteins, profile, proteomics

Introduction
To ensure maximum reproductive efficiency, bull fertility is necessary. There is virtually little information available regarding a bull's potential for fertility from traditional semen quality tests. Although the computer assisted semen analyzer (CASA) is another tool for assessing semen quality, it does not accurately correlate with bull fertility.

The principal reason of involuntary culling in cattle herds and the increase in infertility in dairy herds can be attributed to the use of markers for genetic improvement in animal breeding. It threatens the dairy industry's sustainability and has an impact on the cost dynamics of dairy farms (Ashwell *et al*., 2004) [3]. Because of the negative association between the qualities, it is generally believed that the increased emphasis on milk production was linked to lower reproductive rates in cows (Yang *et al*., 1999) [136]. Subsequently, it was discovered that fertility in connection to either the bull or the cow should be taken into account overall because there was a positive genetic correlation between the two (Taylor *et al*., 2018) [120] and infertility of a bull may be responsible for poor conception rates in cows (Raina *et al*., 2020) [94]. Bull or Sire is a significant factor determining the rate of conception in dairy cattle (Yang *et al*., 1999) [136] and effects both fertilization and the viability of the pre-implantation embryo (Cropp *et al*., 2017) [50]. Understanding the intricacies of bull fertility may be greatly enhanced by the discovery of fertility markers. Separating high-fertile bulls from low-fertile bulls would be helpful in order to select the former for AI (Raina *et al*., 2020) [94]. Antimicrobial activity, oxidative stress avoidance, capacitation, acrosome response, and early embryonic development are all impacted by seminal plasma proteins (Odet *et al*., 2013) [86-87]. Therefore, knowledge of the physiological roles played by seminal plasma proteins will help to increase breeding bull fertility.

The variations in the protein profiles of the seminal plasma from bulls with high and low fertility were demonstrated by proteomics. Some significant proteins, such as osteopontin, phospholipase A2, P25b, acidic seminal fluid proteins, a-L-fucosidase, and cathepsin D, are positively correlated with fertility and may serve as fertility markers, while lipocalin-type prostaglandin D synthase, spermadhesin Z13, clusterin, and ubiquitin are negatively correlated with fertility in bulls.

Marker-assisted Selection

Certain genes are linked to quantitative traits, and these genes are significant for selection because they may have a relatively greater impact on trait values (Raina *et al*., 2020) [94]. The Candidate gene approach is the most used method for tracking genes from the mapped genomic region. This strategy focuses on genes with established functions and identifies the relationships between phenotypic and molecular variations at each potential locus (Falconer, 1996) [21]. The gene's physiological and biochemical functions, its polymorphic DNA sequence, and its statistical correlation with a particular trait should all be understood in this context (Raina *et al*., 2020) [94]. As a result, the detected marker can be applied straight to Marker Assisted Selection (MAS).

There are two methods for identifying the potential gene: physiological and comparative methods. Loci are locations of polymorphisms that have been identified and are known to have a similar impact on a species' phenotype. Moller *et al*. (1996) [72] provided a great illustration of how genes that were first discovered in mice may also regulate color and patterning in livestock through loci that govern these traits. Consequently, the technique is dependent on linkage disequilibrium occurring at the gene level within a population (Raina *et al*., 2020) [94].

Identified Genetic Markers Using Candidate Gene Approach

Researchers used various sorts of markers to target the Y chromosome in bulls in order to uncover potential markers for bull fertility. Deb *et al*. (2013) [12] evaluated microsatellite markers specific to the Y chromosome in order to study bull fertility, Mukhopadhyay *et al*. (Mukhopadhyay *et al*., 2011) [81] found helpful SNPs (single nucleotide polymorphisms) in the TSPY gene. Yue *et al*. (Yue *et al*., 2014) [138] used copy number variants in the Y linked HSFY and ZNF280BY genes to find that these differences were positively correlated with SCR and negatively correlated with testis size. In an effort to find and confirm putative genes influencing bull fertility, a number of researchers looked at the differential expression of genes (Kumar *et al*., 2015) [51, 52, 54]. The PRM1 gene was identified by Ganguly *et al*. (Ganguly *et al*., 2013) [26] as a potential gene influencing semen quality indicators and, eventually, fertility in bulls of HF crossbreds. Based on a comparison of the proteomic profiles of crossbred bulls that were high and low fertile, The POT1 and PTGER3 genes discovered in seminal plasma changed with bull fertility, according to Aslam and colleagues (Aslam *et al*., 2014) [4], and they might be genes that affect bull fertility.

Identified Genetic Markers based on Genome-wide association Studies

The Candidate gene technique played a major role in Marker Assisted Selection (MAS) in identifying bull fertility markers. However, strict criteria for SNPs to be considered "significant" and irrational presumptions, such as the majority of SNPs having no influence in MAS, proven to be a barrier. Due to this, the concept of genomics in animal breeding was developed. It was based on the identification of SNP markers across the entire genome, genotyping using SNP chips, and the estimate of SNP effects using genomic selection as opposed to significance testing (Meuwissen *et al*., 2016) [70]. As a matter of fact, the commercialization of SNP genotyping technology has been crucial in the fields of genomic prediction and animal selection, making it one of the most effectively commercialized genomics technologies (Raina *et al*., 2020) [94]. All of this so heralded in the era of "Genomewide Association Studies" in animal breeding, making dense genomic coverage using high-density SNP panels possible for more accurate measurement of QTL (quantitative traits loci) effects (van Binsbergen *et al*., 2014) [126].

It was discovered that significant SNP markers found by GWAS or other methods significantly increased the precision of genomic prediction. To improve prediction accuracy, Abdollahi-Arpanahi *et al*. (Abdollahi-Arpanahi *et al*. 2017) [1] recommended include pertinent markers that show nonlinear effects. Rezende *et al*. (Rezende *et al*., 2019) [97] confirmed that choosing markers increased the prediction model accuracy by deriving SNP subsets using various methods, such as SNPs considered significant, Gene Ontology (GO) SNPs, or genic SNPs. According to Nani *et al*. (Nani *et al*., 2019) [82], five markers on BTA 8, 9, 13, 17, and 27 had strong dominance effects; as a result, when included as fixed effects, these markers improved the accuracy of predictive models. According to Pacheco *et al*. (Pacheco *et al*., 2020) [88], including markers found on the X chromosome enhanced the models' prediction power in comparison to the conventional method that solely took autosomal markers into account.

Single Nucleotide Polymorphism (SNP)

In a high-density SNP genome association research, loci potentially related to dairy cattle fertility were found; noteworthy findings in two of the four loci were confirmed in a second cohort. According to functional studies, the ITGB5 gene, which is one of the genes with a repeated locus, may be involved in spermegg contact (Feugang *et al*., 2009) [22].

***Genic SNP*:** Using the R program biomaRt, the SNP were assigned to genes based on the UMD3.1 bovine genome assembly. An SNP was associated with a particular gene when it was discovered inside the genomic sequence (beginning of the first exon and ending of the last exon) or within 15 kilobases upstream or downstream of the gene. According to Abdollahi-Arpanahi *et al*. (2017) [1], a distance of 15 kilobases was utilized to capture regulatory regions and other functional places that might be outside the gene but extremely close to it, such as the promoter region.

It is noteworthy that the predictive power of each functional SNP subset was compared to that of a random marker set (i.e., an SNP subset containing the same number of markers but chosen at random from the genome). The goal was to look into the advantages of employing SNP with assumed functional roles that go beyond just taking genetic linkages (population structure) into account (Rezende *et al*., 2019) [97].

Significant SNP: A single marker linear model was used to evaluate the relationship between each SNP marker and SCR, with the SNP allele count acting as a linear covariate and the SCR evaluation acting as a categorical variable. Significant SNPs were those SNP markers having a nominal P-value of ≤0.05. Because predicting yet-to-be-observed symptoms was the main objective rather than identifying the causative mutations, reducing type I error was not given top priority (Abdollahi-Arpanahi *et al*., 2017; Rezende *et al*., 2019) [1, 97].

Every SNP subset's performance was compared to that of a different subset that was randomly picked throughout the genome and had the same amount of markers. The advantages of employing markers with biological functions beyond population accounting should be apparent from this comparison (Abdollahi *et al*., 2017) [1].

Osteopontin

Osteopontin was first found to be involved in cell adhesion in mineralized bone tissues. Its functions include cell migration, chemotaxis, calcification, tumor formation, and macrophage activation. The oviduct, seminal vesicles, ampullae, and potentially the epididymis all express this ubiquitous protein. Cell adhesion and migration may be facilitated by OPN in the seminiferous tubules, where it is produced by germ cells and Sertoli (Moura, 2018).

Although the expression of OPN in the epididymal epithelial cells of cows is still unknown, epididymal spermatozoa may absorb OPN released by these cells. The idea that the latter are transferred from the tubules to the epididymis already linked to OPN is suggested by the association between OPN and Sertoli and germ cells in the seminiferous tubules; however, such a notion has not yet been confirmed experimentally. Additional OPN may be obtained from the accessory sex glands for ejaculated sperm. According to Moura (2018) [73-74], integrins (αv and α5) that can attach to oviductal OPN have been found in the membrane of bovine sperm.

There is little doubt that OPN's contact with oocytes may initiate intra-cellular communication through second messenger pathways based on the effect it has on processes that follow sperm-egg binding. Although sperm exit the seminal fluid environment when semen is deposited in the female reproductive tract, osteopontin expression in both seminal plasma and oviductal fluid suggests a redundancy of roles The presence of OPN in the oviduct, the site of fertilization, would ensure appropriate integrin and CD44 receptor saturation on the surface of the sperm, increasing the likelihood of oocyte attachment. OPN released in the oviduct lumen may have an effect on oocytes prior to their encounter with sperm, considering the options available for mRNA splicing, post-translational modifications, and OPN's adaptability (Moura, 2018) [72-73].

According to Kumar *et al*. (2012) [53], OPN is generally linked to immune cell activation, cell adhesion, tissue remodeling, chemotaxis, and cell survival. It's fascinating that this multifunctional protein is connected to fertility and can also be present in the male reproductive system (Kumar *et al*., 2012) [53]. This protein is expressed in several species' ampullae, seminal vesicles, and epididymis, according to Kumar *et al*. (2012) [53]. It's probable that the different OPN isoforms located in different regions of the reproductive tract of bulls have different functions. These germ cells attach to nearby Sertoli cells as well as the seminiferous tubule's basement membrane (Luedtke *et al*., 2002) [61]. Only the seminiferous tubules harboring elongated spermatids in bulls exhibited OPN expression, indicating a stage-related expression pattern. Through unidentified means, OPN increases sperm vitality and facilitates sperm capacitation, potentially by obstructing apoptotic pathways (Erikson *et al*., 2007) [19]. According to Moura *et al*. (2007) [77], OPN affects interactions with the membrane of ejaculated bull sperm, sperm-oocyte binding, and early embryonic development.

In their 2008 study, Souza *et al*. postulated the mechanisms by which OPN attaches to sperm during ejaculation. It's possible that OPN has an indirect functional impact on male fertility. In inflammatory conditions, luminal OPN influences host defenses by binding to integrin receptors on the surface of epithelial cells, shielding them from bacterial infections (Brown *et al*., 1992) [10]. By preventing bacterial infections on the accessory sex glands' epithelial surfaces, OPN indirectly contributes to male fertility. High fertile bulls' accessory sex gland fluid contained higher concentrations of OPN than poor fertile bulls' (Moura *et al*., 2006) [75, 76, 78].

Phospholipase A2

Additionally, the plasma membrane, acrosome, and post-acrosomal material of ejaculated bull sperm have been reported to contain phospholipase A2 (PLA2) (Weinman *et al*., 1986). According to Soubeyrand *et al*. (1997) [114], it has a molecular weight of 60 kDa and a pI of 5.6. A 16 kDa PLA2 isoform has also been found (Ronkko *et al*., 1991) [102]. In contrast to epididymal sperm, PLA-2 is bound to the surface of ejaculated bull sperm, according to Ronkko's 1992 [101] study. It is crucial for the acrosomal reaction, spermegg fusion, and other late maturational processes of spermatozoa (Sato *et al*., 2010; Yuan *et al*., 2003) [107, 137]. Arachidonic acid is produced by the PLA2 affixed to sperm membranes, and this is subsequently transformed into prostaglandin E2, triggering processes associated with the acrosome reaction (Breitbart and Spungin, 1997) [9]. The local concentration of BSPs can either activate or inhibit the membrane-bound PLA2 (Manjunath *et al*., 1994) [65]. BSPs can sequester choline phospholipids on the surface of sperm, stopping PLA2 from reacting with these phospholipids and preventing sperm from going through an early acrosome reaction, as reported by Manjunath *et al*. (1994) [65]. Additionally, studies have shown that PLA2 has antibacterial action in the seminal plasma and activates immune cells (Granata *et al*., 2005; Bourgeon *et al*., 2004; Weinrauch *et al*., 1996) [31, 8, 132]. In the fluid of their accessory sex glands, bulls with high fertility express more PLA2 than bulls with low fertility (Moura *et al*., 2006) [75, 76, 78].

P25b

P25b, a member of the xylulose reductase family, is connected to the plasma membrane that encloses the acrosomal cap of spermatozoa. During the epididymal transit, it is released by the epididymal epithelium and attaches itself to the surface of testicular sperm (Parent *et al*., 1999) [89]. This protein, which is dependent on pH, temperature, and zinc, is transferred by the epididymosomes from the epididymal lumen to the sperm surface (Frenette *et al*., 2002) [25]. It might have a role in the spermatozoa's attachment to the egg's surface and development of the

ability for sperm fertilization (Saez *et al*., 2003; Kumar *et al*., 2012) [104, 53]. Individual differences exist in the P25b content of spermatozoa, with lower P25b content identified in spermatozoa from bulls with poorer fertility (Parent *et al*., 1999) [89]. This surface protein, P25b, is subjected to cryoinjuries throughout the cryopreservation process and is also thought to have partially cryoeluted off the surface of frozen-thawed sperm (Lessard *et al*., 2000) [57]. The capacity of frozen-thawed semen to fertilize is reduced by the loss of P25b that occurs during cryopreservation (Lessard *et al*., 2000) [57]. The amount of P25b on the surface of sperm is influenced by the length of time the spermatozoa are stored in liquid nitrogen as well as the extender that is used to cryopreserve them (Lessard *et al*., 2000) [57]. Lessard *et al*. (Lessard *et al*., 2000) [57] discovered that sperm are better protected against protein cryoelution during cryopreservation by a milk-based extender than by egg yolk. P25b may therefore be a sign of sperm maturation and impaired fertility related to freezing-thawing procedures.

Ubiquitin

The 8.5 kDa protein ubiquitin was first isolated from the bovine thymus in 1975, however it was later discovered in all eukaryotic cells (Goldstein *et al*., 1975) [30]. Epididymosomes transport proteins released by the epididymis to the bull sperm plasma membrane through the use of prostasome-like secretory particles seen in the bull epididymal fluid (Frenette *et al*., 2002) [25]. The production of such ubiquitin-containing epididymosomes is caused by the discharge of apical blebs from the epididymal epithelial lining (Hermo and Jacks, 2002) [34]. One of the sperm surface proteins that is ubiquitinated in the faulty spermatozoa is arylsulfatase A (Sutovsky *et al*., 2001) [117]. Bull sperm with elevated ubiquitin levels are a sign of low-quality semen and low fertility in bulls. Because it covalently attaches to the surface of aberrant mammalian spermatozoa, ubiquitin is a useful diagnostic for sperm abnormalities (Sutovsky *et al*., 2001) [117].

Fertility-associated proteins

Proteomics has recently yielded potential biomarkers for sperm fertility, but definitive research establishing a causal link between sperm fertility and the proteome has not yet been conducted. To determine the clinical importance of the protein markers discovered by proteomic analysis, spermatozoa from bulls with high and low fertility were subjected to 2-dimensional electrophoresis and their protein expression profiles compared. Then, using Western blot analysis, we looked into the connection between protein expression and each bull's fertility. Three proteins were found to be overrepresented in low fertility bulls compared to five other proteins: voltage dependent anion channel 2 (VDAC2), ATP synthase H+ transporting mitochondrial F1 complex beta subunit, phospholipid hydroperoxide glutathione peroxide, apoptosis-stimulating of p53 protein 2, and ropporin-1. ENO1, VDAC2, and UQCRC2 showed a substantial correlation with individual fertility among these proteins. According to Park *et al*. (2012) [90], these results imply that comparing protein expression levels with other fertility tests simultaneously could be a useful *in vitro* method for sperm fertility assessment.

Fertility-associated antigen

It has been discovered that the sperm membrane of bulls contains a particular heparin-binding protein called fertility-associated antigen (FAA). According to the results, bull groups with positive FAA had higher fertility than those with negative FAA. Additionally, more fertile FAA-positive bull groups than FAA-negative bull groups were produced by bulls chosen for their strong serving capacity—that is, their ability to mount and breed estrual heifers. Furthermore, earlier in the reproductive season, cows exposed to FAA-positive bulls had impregnation, leading to a higher proportion of older and heavier calves at weaning. To detect subfertile bulls, FAA profiles can be determined in addition to serving capacity and breeding soundness assessments. Bull fertility and cow/calf performance increased when FAA-positive bulls were chosen for the breeding stock (Bellin *et al*., 1998) [5].

Sperm-specific proteins

Phospholipase C zeta (PLCZ1): A spike in intracellular free Ca2+ concentration, which results from the phosphoinositide signaling pathway being activated, is what causes oocyte activation (Jones, 2003) [44]. IP3, or inositol 1,4,5-triphosphate, is produced subsequent to this process. Malcuit *et al*. (2005) reported that PLCZ1, a sperm factor, is the cause of the induction of IP3 synthesis and Ca2+ release. In bovines, the 5th chromosome contains the gene encoding PLCZ1. Manjunath (1984) [63] found a correlation between semen quality parameters and genetic polymorphism in the PLCZ1 promoter region.

Zonadhesin: A protein of the mosaic type called zonadhesin binds to zona pellucida in a species-specific manner and localizes to the apical head of spermatozoa. Due to its ability to bind, bovine zonadhesin—which is found on its 25th chromosome—is one of the most studied sperm ligands in mammals (Tardif *et al*., 2010) [119].

Calmegin: First expression of the calcegin (Clgn) gene occurs in the meiotic prophase of primary spermatocytes. According to Ikawa *et al*. (2001) [40], mice lacking the Calmegin gene exhibit male sterility due to abnormalities in oviduct movement and non-binding to the zona pellucida.

Fertilin: Fertilin, which was formerly known as PH-30, is a heterodimer made up of a and a and it actively mediates the binding and fusing of the sperm oocyte membrane. A homologous area between the subunit and the disintegrin family of integrin ligands exists (Evans *et al*., 1997) [20]. The sperm domain contains the protein fertilin, which is crucial in attaching to the egg membrane. The ADAMs gene family, which stands for "A Disintegrin and a Metalloprotease domain," is a novel group of transmembrane proteins that includes the components of fertilizin. The number of ADAM family members that have been identified is about thirty. Two subunits of bovine fertilin have been localized to the 17th and 27th chromosomes, respectively, Adam1 and ADAM2, respectively (Arcelay *et al*., 2004) [2]. The disintegrin domain of fertilin is thought to interact with the integrin receptor found on the surface of eggs. It has been suggested that fertilizin contributes to the union of the membranes of the egg and sperm.

Serine/threonine phosphatase: Sperm capacitation requires phosphorylation of c-AMP-regulated proteins and calcium. Consequently, it has been discovered that capacitation is the reliable biochemical indicator of sperm

capacitation and is invariably linked to an increase in the tyrosine phosphorylation of several proteins (Gross *et al*., 1987) [32]. Sperm motility is enhanced by an increase in c-AMP levels through the activation of protein kinase A (PKA). Calcium can modify c-AMP levels to indirectly influence protein phosphorylation, or it can directly effect it through calcium-activated protein kinases or phosphates (Vijayaraghavan *et al*., 1997) [129].

Lactate dehydrogenase C: The first glycolytic isozyme specific to the testis observed in male germ cells may be lactate dehydrogenase (Moura *et al*., 2006) [75, 76, 78]. Encoding the A, B, and C subunits of LDH, respectively, are three different genes called LDHA, LDHB, and LDHC, which also encode the three different forms of the protein. Male fertility was found to be affected in LDHC gene knockout mice (Odet *et al*., 2013) [86-87]. It might be the result of decreased sperm motility, ATP synthesis, and glucose consumption.

Angiotensin-converting enzyme (ACE): Two isoenzymes of ACE have been found in bovines: somatic and germinative, or testicular. Gene duplication results in the double domain of somatic ACE, which is mostly expressed in endothelial cells. Langford *et al*. (1993) [55] reported that germminative ACE is a single-domain condition that arises in the male reproductive system. Both isoforms are structurally similar because they are created via alternate transcription initiation and post-transcriptional splicing, and they are transcribed by a single gene. ACE mostly changes angiotensin 1 into angiotensin 2. It is a nonspecific peptidase, though, and can cleave a variety of substrates that impact numerous physiological processes, including blood pressure regulation, hemopoiesis, reproductive, renal, and immunological functions (Bernstein *et al*., 2013) [6].

Testis-specific protein on Y chromosome (TSPY): On the Y chromosome, the TSPY genes are grouped together (copy counts in bulls can reach up to 200). The bovine TSPY possesses seven exons, and its expression appears to be restricted to male germ cells from the beginning of the fetal stage (Vogel *et al*., 1997) [130]. The TSPY gene's protein product may interact with type B cyclins and initiate BCDK complexes between cyclins. Spermatogonia cell renewal, spermatocyte proliferation, and differentiation are all aided by the active complex (Vogel *et al*., 1997) [130]. While a single nucleotide polymorphism in the TSPY gene's fourth intron was found in buffalo bulls, there was no discernible correlation between it and the spermatogenic traits. The TSPY gene's absolute copy number and fertility rate are connected (Mukherjee *et al*., 2013) [80].

Ubiquitin specific peptidase 9 (USP9Y): The USP9Y gene is located on the long arm of the Y chromosome and belongs to the USP (ubiquitin-specific peptidases) family. Though its precise role in spermatocyte formation is uncertain, it is assumed to be related. However, with the identification of several unique SNPs, the USP9Y mutant was linked to severe spermatogenic failure and infertility (Bonfiglio *et al*., 2012) [7]. Rather than performing a vital role, USP9Y is more likely a fine tuner that increases spermatogenesis efficiency.

Semen Metabolome

According to Kumar *et al*. (2015) [51, 52, 54], metabolomics is a relatively new approach that has demonstrated potential in finding biomarkers associated with male fertility and infertility. As the systems biology's downstream, metabolomics has attracted a lot of attention for research and understanding of basic biological processes pertaining to tissues, organs, and reproduction (Kovac *et al*., 2013) [49]. (Dunn *et al*., 2005) [15]. Gas chromatography-mass spectrometry (GC-MS) has also been shown in a recent study to be a novel technique for the quick and noninvasive diagnosis of male infertility (Velho *et al*., 2018) [127]. Cryopreserved spermatozoa were shown to have lower amounts of glycine betaine (Longobardi *et al*., 2020).

Seminal Plasma Metabolites

The bull seminal plasma included the following metabolites in varying amounts: androstenedione, 4-ketoglucose, D-xylofuranose, 2-oxoglutaric acid, and erythronic acid were the least common, while fructose, citric acid, lactic acid, urea, and phosphoric acid were highly prevalent. Bull seminal plasma is primarily composed of fructose, citric acid, lactic acid, urea, and phosphoric acid as metabolites. Additionally, it was shown that the metabolite profiles of bulls with high and low fertility were clearly distinguished from one another. Fructose and 2-oxoglutaric acid were identified as possible candidates for bull fertility biomarkers.

Among the proteins specific to seminal plasma include heparin-binding proteins, calmodulin-binding proteins, forward-motility proteins, osteopontin, spermadhesin, androgen binding proteins (Kumar *et al*., 2015) [51, 52, 54].

High-fertility bulls had low levels of citrate and isoleucine in seminal plasma, one of the differential biomarkers, but high levels of taurine, tryptamine, and leucine. According to Ford and Harrison (1984) [24], citrate is the primary anion in seminal plasma and it chelates calcium ions to prevent sperm capacitation and spontaneous acrosome reactions. According to Kumar *et al*. (2015) [51, 52, 54], low citrate concentrations may thereby stimulate bull sperm for capacitation and the acrosome response necessary for fertilization.

Bovine Seminal Plasma Proteins/ Binding of sperm proteins (BSPs)

Around 70% of the total protein content of bovine seminal plasma is made up of bovine seminal plasma proteins (BSPs), which are released by the seminal vesicles. They belong to the heparin-binding protein family (Nauc and Manjunath, 2000) [83]. The three acidic proteins that are most commonly identified in bovine seminal plasma are BSP-A1/-A2, BSP-A3, and BSP-30 (collectively, BSPs) (Kumar *et al*., 2012) [53]. The molecular weights of BSP-A1/-A2 and BSP-A3 are 15–16 kDa, while BSP-30 has a molecular weight of 28–30 kDa (Kumar *et al*., 2012) [53]. The BSP-A1/-A2 combination, which is composed of BSP-A1 and BSP-A2, differs only in glycosylation, is sometimes referred to as PDC-109 (Protein with N-terminus aspartic acid D and carboxy terminus Cystine, comprising 109 amino acids) (Manjunath and Therien, 2002) [64].

In cooperation with the HUGO Gene Nomenclature Committee, Manjunath *et al*. (Manjunath *et al*, 2009) [66] proposed a new nomenclature; BSP-A1/A2, BSP-A3, and BSP-30 are now referred to as BSP1, BSP3, and BSP5,

respectively, and BSPs are collectively referred to as sperm binder (Kumar *et al*., 2012) [53].

The potential effects of BSPs in seminal plasma on mitotic cells can vary based on the exposure duration and dosage. It was hypothesised that this protective role is probably insufficient for bulls whose seminal plasma or accessory sex gland fluid had large concentrations of BSPs (Moura *et al*., 2006) [75, 76, 78]. BSP-A3 was detected in greater amounts in the membrane of the poor fertile bull by Roncoletta *et al*. (Roncoletta *et al*., 2006). The BSP proteins found in seminal plasma therefore have a dual effect on sperm, acting as a net positive and negative.

Numerous studies have used the measurement of seminal plasma proteins (Viana *et al*., 2018; Killian *et al*., 1993) [128, 47] and sperm proteins (Kasimanickam *et al*., 2019; Hou *et al*., 2019) [46, 36] to identify proteic markers of bull fertility. According to research done on bull and ram sperm, they show that sperm exposed to seminal plasma BSPs for extended periods of time during storage or freezing are harmful (Plante *et al*., 2015) [93]. Elevated levels of BSPs, particularly BSP1, in the seminal plasma may cause harm to spermatozoa when they are being preserved and frozen (Leahy and Graaf, 2012) [56].

Seminal vesicles release a 13 kDa protein called SPADH1 (Acidic Seminal Fluid Protein, Spermadhesin-1) to a much smaller amount by the epididymis (Wempe *et al*., 1992). According to Einspanier *et al*. (1991) [16], it falls into the growth factor category. SPADH1, a member of the spermadhesin family, is lost upon capacitation and adheres slackly to the surface of bull sperm (Dostalova *et al*., 1994) [14]. Bull SPADH1 may be involved in controlling sperm metabolism and shielding sperm membranes from oxidative stress, according to Schoneck *et al*. (1996) [108].

In fact, the quantity of SPADH1 in bull seminal plasma was favorably correlated with spermatozoa's freezing capacity and frozen semen's fertility (Jobim *et al*., 2004; Somashekar *et al*., 2015) [42-43, 112]. Bull fertility was also strongly correlated with SPADH2, or spermadhesin Z13, another parented protein (Somashekar *et al*., 2017) [113]. Willfross *et al*. (2021) [135] found that there is a positive or negative correlation between the BSPs and either freezability or fertility.

Lipase

Fertility has consistently been demonstrated to be adversely linked with the enzyme lipase (LIPA; full name: Lysosomal acid lipase (LAL)/cholesteryl ester hydrolase). Additionally, triglycerides and cholesterol esters that are ingested by lipoprotein particle endocytosis are known to be hydrolyzed intracellularly by it.

Furthermore, Willfross *et al*. (2021) [135] have reported that lipase is involved in mediating the effect of lipoprotein particle endocytosis on the inhibition of "hydromethylglutaryl-CoA" and stimulating the synthesis of endogenous cellular cholesteryl ester formation. It is a part of pathways that regulate cholesterol levels in both situations. Given that cholesterol homeostasis affects male reproductive function, these phenomena may have implications for fertility (Sedes *et al*., 2018) [109]. The process of capacitation includes the loss of cholesterol from the sperm membrane (Therien *et al*., 1998) [122]. Sperm freezability has been found to be positively correlated with membrane cholesterol content (Rajoriya *et al*., 2013; Singh *et al*., 2014) [95].

Prostaglandins D synthase

The 26 kDa seminal plasma protein linked to fertility was identified as lipocalin-type prostaglandin D synthase (L-PGDS) by enzymatic testing, N-terminal sequencing, and immunoblotting (Gerena *et al*., 1998) [28]. Essentially, L-PGDS belongs to the transport protein family and has a number of physiological activities, such as regulating body temperature, inducing sleep, and causing smooth muscle contraction and relaxation (Urade *et al*., 1995) [125]. However, its precise function in male reproduction is still unclear. However, L-PGDS have been detected on the acrosome's apical ridge on ejaculated bovine sperm, efferent duct epithelial cells, elongating spermatids, and rete testis. Sperm growth and maturation may be significantly impacted by it (Gerena *et al*., 1998; Gerena *et al*., 2000) [28, 27]. Given that it can act as a transmembrane lipophilic carrier protein to preserve the blood-testis and blood-epididymal barriers, its correlation with total male fertility may be explained (Gerena *et al*., 2000) [27]. Bulls with high fertility have been linked to higher expression of L-PGDS in seminal plasma (Gerena *et al*., 1998; Killian *et al*., 1993) [28, 47]; however, bulls with low fertility were shown to have higher expression of L-PGDS in their epididymal fluid (Moura *et al*., 2006) [75, 76, 78]. Additionally, Jobim *et al*. (Jobin *et al*., 2004) [42-43] discovered that seminal plasma samples from bulls with low semen freezability are more likely to contain L-PGDS than bulls with high semen freezability. Further investigation is necessary to examine the function of L-PGDS in bull fertility (Kumar *et al*., 2012) [53].

This protein is an extracellular transport protein that binds tightly to particular cell receptors and small hydrophobic ligands, according to Samanta *et al*. (2018) [106]. Fertility was previously believed to be associated with its isoform of prostaglandin D synthase, lipocalin-type (Killian *et al*., 1996) [48]. Poor semen characteristics, including reduced sperm count, motility, and normal morphology, have been linked to prostaglandin D synthase (Diamandis *et al*., 1999; Heshmat *et al*., 2008) [13, 35].

***N*-acetylglucosamine-1-phosphotransferase subunit gamma (GNPTG) and cartilage acidic protein 1 (CRTAC1)**

A higher amount of this protein was observed in the semen of bulls who performed badly, as indicated by the two-protein identified in the enlarged list that corresponded with the negative connection between GNPTG and fertility (Liu *et al*., 2018; Willfross *et al*., 2021) [58, 135]. Also, there was a positive correlation between CRTAC1 and fertility; however, further research on this topic may be necessary because other studies examining its association with freezing resilience and liquid preservation had revealed distinct patterns (Soleilhavoup *et al*., 2014; Rickard *et al*., 2015) [111, 98].

Acidic Seminal Fluid Protein

Growth factor-like activity was discovered upon the initial separation of an acidic seminal fluid protein (aSFP) from bovine semen, which had a molecular weight of 13 kDa (Einspanier *et al*., 1991) [16]. Later, ampulla and seminal vesicle secretions were shown to contain aSFP, while testicles, epididymis, or bulls' blood did not (Einspanier *et al*., 1993) [18]. Kumar *et al*. (2012) [53] have classified bovine aSFP as a member of the spermadhesin protein family. It is possible for the bull spermatozoa surface to completely lose

spermadhesin aSFP before the spermatozoa contact the investing egg (Dostalova *et al*., 1994) [14]. Instead of binding to the zona pellucida, aSFP may therefore function as a decapacitation factor on bull spermatozoa. At the highest aSFP concentrations, the motility of ejaculated bovine spermatozoa was markedly decreased *in vitro*; however, following dilution, the motility was recovered, indicating that a particular interaction between aSFP and spermatozoa may be the source of the reversible reduction of propagational motility. However, aSFP had no effect on the viability of spermatozoa (Schoneck *et al*., 1996) [108]. Prior to ejaculation, the bull stores its spermatozoa in the ampulla, where high levels of aSFP limit motility as an energy-preserving mechanism (Schoneck *et al*., 1996) [108], but in the female reproductive tract, where aSFP is diluted out, sperm motility can quickly return (Dostalova *et al*., 1994) [14]. High amounts of artificial stem cells (aSFP) in ampulla are thought to prevent spermatozoa from losing energy during ejaculation, but they may also stimulate them after they are diluted in the female vaginal canal (Schoneck *et al*., 1996) [108]. According to Einspanier *et al*. (1994) [17], the aSFP also prevents oxidative damage to spermatozoa by reducing lipid peroxidation at its physiological concentration. Bulls with high freezability had a greater concentration of aSFP in their semen compared to those with poor freezability (Jobim *et al*., 2004) [42-43].

Spermadeshins

When spermadhesin was found in high concentrations in the seminal plasma of bulls with low fecundity, Killian *et al*. (1993) [47] recognized it as an anti-fertility factor. It was later determined to be the spermadhesin Z13. In 2D SDS PAGE of bovine seminal plasma, spermadhesin Z13 is detected as two 13 kDa monomers, which are thought to be the product of a 26 kDa dimer (Tedeschi *et al*., 2000) [121]. Similarly, spermadhesin Z13 spot at 29 kDa was found in the accessory sex gland fluid gels by Moura *et al*. (Moura *et al*. 2006) [75, 76, 78]. Tedeschi *et al*. (2000) [121] report that spermadhesin Z13 shares 50% and 43% homology with aSFP and the seminal plasma motility inhibitor, respectively. Sperm surface is bound by spermadhesin Z13 (Topfer-Petersen *et al*., 1998) [128]. Because spermadhesin Z13 has a negative influence on sperm motility, Moura *et al*. (Moura *et al*., 2006) [75, 76, 78] proposed that bulls with high amounts of this protein would be less fertile. Hence, there is an inverse link between bull fertility and this protein.

Positive connections between spermadhesins (SPADH1 and SPADH2) and frozen semen fertility have been found (Jobim *et al*., 2004) [42-43]. Spermadhesins may have a protective role against oxidative stress (Somashekar *et al*., 2017) [113]. Moreover, Spermadhesin Z13 displayed a tendency toward a favorable correlation with fertility (Menezes *et al*., 2017; Willfross *et al*., 2021) [69, 135].

Cathepsin D

During the process of epididymal transit, sperm membrane components may undergo proteolytic alteration. This process may be aided by the presence of cathepsin D in the cauda epididymal fluid found in the male reproductive canal (Moura *et al*., 2006) [75, 76, 78]. In comparison to bulls with poor fertility, high fertility bulls showed a 2.4-fold increase in cathepsin D intensity in 2D maps of the cauda epididymal fluid (Moura *et al*., 2006) [75, 76, 78].

Apolipoprotein A 1

According to Therien *et al*. (1999) [123], apolipoprotein A-I, a component of HDL (Sparrow *et al*., 1992) [116], may have a role in sperm cholesterol efflux and capacitation. In bovine seminal plasma, the metalloproteinase-2 (TIMP-2) is identified as a 24 kDa heparin-binding protein (Calvete *et al*., 1996; Moura *et al*., 2007) [11, 77]. According to McCauley *et al*. (2001) [68], TIMP-2 may affect bull fertility through inhibiting metalloprotease activity in semen or through other unknown mechanisms unrelated to the suppression of matrix metalloproteinase (MMP). However, research is now being done to determine TIMP-2's exact function and mode of action in relation to bull fertility.

Clusterin

Clusterin is a 75–80 kDa heterodimeric protein bound by serine bonds. It is also referred to as sulfated glycoprotein-2, testosterone-repressed prostate message-2, apolipoprotein J, and complement lysis inhibitor. It is generated by Sertoli cells and main epididymal epithelial cells in the male reproductive tracts (Sylvester *et al*., 1984) [118], and it is translocated to aberrant germ cells and spermatozoa (Ibrahim *et al*., 2001) [38]. The study conducted by Ibrahim *et al*. (1999) [39] revealed that the bull reproductive system harbors many isoforms of clusterin. The researchers hypothesized that the biological or functional activity of the protein could be impacted by the variations in carbohydrate content among these isoforms. Clusterin has several physiological roles in the male reproductive tracts, including protecting sperm from oxidative damage, binding and agglutinating defective spermatozoa in bulls, and preventing complement-induced sperm lysis (Ibrahim *et al*., 1999; O'Bryan *et al*., 1990; Reyes-Moreno *et al*., 2002) [39, 84, 96]. Only aberrant sperm cells are bound by antibodies produced against the dimeric form of clusterin in human and bull semen (O'bryan *et al*., 1994) [85]. According to Ibrahim *et al*. (Kumar *et al*., 2012) [53], the proportion of sperm cells in semen that are positive for clusterin may serve as a helpful indicator for ejaculates of low quality. Clusterin on ejaculated sperm may be a sign of aberrant epididymal maturation or abnormal spermatogenesis (Ibrahim *et al*., 2000) [37]. Consequently, a protein called clusterin has an unfavorable connection with fertility.

Other Seminal Plasma Proteins

α-L-fucosidase has been found on bull spermatozoa, in seminal plasma, and in epididymal fluid. The primary source of a-L-fucosidase in bulls is the cauda epididymidis. In addition, a-L-fucosidase with a molecular weight of 54.4 kd and a pI of 6.6 was found in the cauda epididymal fluid of bulls by Moura *et al*. (Moura *et al*., 2006) [75, 76, 78]. These bulls were linked to high fertility. There was less a-L-fucosidase found in the seminal plasma of bulls who had a larger percentage of faulty sperm. According to Jauhiainen and Vanha-Perttula (1986) [41], this enzyme might be implicated in the changes that occur to the sperm membrane proteins' carbohydrate moieties during epididymal transit.

Moura *et al*. found nucleobindin (58 kDa, pI 5), a protein with DNA-binding motifs and Ca^{2+}, in the fluid of the accessory sex gland for the first time (Moura *et al*., 2007) [77]. It's interesting to note that nucleobindin and OPN have both been linked to odontoblasts and bone cells (Petersson *et al*., 2004) [91]. As a result, additional bone protein was found in the auxiliary sex gland fluid and bull seminal

plasma, albeit it is unclear how important it is for conception.

Various species' epididymis secretes a high-affinity cholesterol-binding protein called Niemann-Pick C2 (NPC2) (Kumar *et al*., 2012) [53]. Girouard *et al*. (2008) [29] speculate that this protein may have a role in the transfer of cholesterol during the maturation of sperm. A process that is independent of capacitation uses NPC2 in the seminal plasma of bulls to reorganize the membrane (Kumar *et al*., 2012) [53].

The epididymal sperm-binding protein 1 (ELSPBP1) is highly regionalized in its expression inside the epididymis (Saalmann *et al*., 2001) [103]. Sahin *et al*. (2009) [105] state that ELSPBP1's unique spatial expression patterns along the epididymal duct imply that it might be involved in sperm maturation.

Blood serum

Albumin, antitrypsin, b-lipoproteins, and orosomucoids are all found in blood; these substances work together to support osmotic regulation, pH maintenance, and the transport of ions, lipids, and hormones. Bulls with high fecundity have low serum levels of asparagine and isoleucine and high serum levels of glycogen and citrulline (Kumar *et al*., 2015) [51, 52, 54].

Conclusion

The indicators included in this review are those that could be utilized to mitigate the effects of low temperature storage, as well as those that could be employed for the *in vitro* evaluation of domestic mammalian fertility. The prediction of bull fertility necessitates a solid understanding of both statistical techniques and reproductive biology to avoid drawing erroneous or useless findings.

Bull fertility has always been an important factor in determining the profitability of a farm, but because it is not as heritable as cow fertility features, it has not gotten the attention it deserves. Recently, efforts have been made to pinpoint the powerful genetic markers that underlie bull fertility. The use of these genome-wide indicators for the selection of highly fertile bulls has garnered increased attention in light of the availability of genomic data. The availability of genetic data has brought the notion of Marker Assisted Selection back to the forefront, after it had faded owing to relevant drawbacks. Undoubtedly, MAS based on genetic data has attracted a lot of interest in this context even though the research is still sparse on its utility for choosing bulls with better fertility. Future developments in this field of study will undoubtedly provide light on the intricate nature of bull fertility.

The physiological functions of several seminal plasma proteins and their connection to bull fertility have been well studied. Sperm membrane stabilization, oviduct-forming sperm reservoir formation, sperm viability preservation in sperm reservoir, sperm capacitation, and PLA2 activity suppression are all facilitated by BSPs. BSPs in seminal plasma, however, have a dual effect on sperm, acting as both a benefit and a drawback. Sperm development and maturation are linked to L-PGDS. The blood-testis and blood-epididymal barriers are preserved by the trans membrane lipophilic carrier proteins. Contradictory effects are seen in L-PGDS on bull fertility. PLA2 has been linked to the semen of bulls with high fertility and plays a significant role in sperm-egg fusion and acrosomal response. OPN plays a role in early embryonic development, fertilization, sperm-oocyte binding, prevention of polyspermy, and defense against bacterial infections on the accessory sex glands' epithelial surfaces. Additionally, it attaches germ cells to nearby Sertoli cells as well as the basement membrane of the seminiferous tubule. OPN is abundant in the seminal plasma of fertile bulls and is a well-known marker of bull fertility. Spermatozoa are shielded from oxidative damage by aSFP, which also affects sperm motility. Bull semen with high freezability has higher concentrations of aSFP. The binding of spermatozoa to the ovum is facilitated by P25b, a marker of epididymal maturation of sperm. Spermatozoa from bulls with reduced fertility have lower amounts of P25b. Sperm motility is negatively impacted by the anti-fertility factor spermadhesin Z13. As a helpful indicator of low-quality ejaculates, clusterin binds and agglutinates aberrant spermatozoa, inhibits complement-induced sperm lysis, and shields sperm from oxidative damage. Ubiquitin binds covalently to the surface of aberrant mammalian spermatozoa, making it an appropriate marker of sperm abnormalities. In addition to these, other proteins with distinct roles that are also present in seminal plasma include a-L-fucosidase, cathepsin D, ART5, TIPM-2, and NPC2. However, more research is required to determine how these proteins relate to bull fertility. Bull fertility is also linked to sperm-specific proteins such as PLCZ1, zonadeshin, calmegin, fertilin, lactate dehydrogenase C, ACE, TSPY, and USP9Y. Apoptosis-stimulating of P53 protein2, alpha-2-HS-glycoprotein, END1, ATP synthase H+ transporting mitochondrial F1 complex beta subunit, and phospholipid hydroperoxide glutathione peroxidase were more common in high fertility bulls than in low fertility bulls. Bulls with positive fertility-associated antigens are more fertile than those with negative FAA. Albumin, antitrypsin, beta-lipoprotein, and orosomucoids are all found in blood; these substances work together to maintain pH, regulate osmotic pressure, and transport ions, lipids, and hormones. Bulls with high fertility exhibited low levels of isoleucine and asparagine in their serum, but high levels of glycogen and citrulline. The particular role of seminal plasma proteins and their relationship to fertility will be clarified by comprehensive proteomic research of low/abundant protein(s) components in fertile and subfertile bulls, made possible by the advancement of techniques and technologies in this field.

In conclusion, we have discussed the case of a multifunctional sperm, seminal plasma protein, and blood that have empirically been linked to high-performing sires' fertility indices utilized in artificial insemination centers. By identifying these elements, we can improve the accuracy of predicting male reproductive function as well as comprehend and diagnose cases of infertility and/or subfertility.

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