

# **SEMINAL PLASMA PROTEINS**

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# Introduction

Two major components

Sperm cells (Spermatozoa)

Seminal plasma

- Seminal plasma : Testis, Epididymis, Seminal vesicles, Ampullae, Prostate and Bulbourethral glands
- SP contains proteins, amino acids, enzymes, fructose and other carbohydrates, lipids, major minerals and trace elements

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# Seminal Plasma Proteins

- **Seminal plasma proteins of blood origin**
- **Seminal plasma specific proteins**

# Seminal Plasma Proteins of Blood Origin

- **Prealbumin, Albumin,**
- **Globulin, Transferring,**
- **$\alpha$ -Antitrypsin, B-lipoprotein,**
- **$\beta$ -glycoprotein,**
- **Orsomucoid, Kininogen,**
- **Peptide hormones,**
- **IgG, IgA and IgM**
- **Regulation of osmotic pressure and pH of seminal plasma, transport of ions, lipid and hormones**

# Seminal Plasma Specific Proteins

- BSP –proteins, BSA-A1, BSA-A2, BSA-A3 and BSA -30 kDA proteins
- Forward motility proteins
- IgG –Fc binding proteins
- Immobilin
- Cellular retinol binding proteins
- Androgen binding proteins
- Seminal plasmin
- Clustrin
- Heparin binding proteins

- **Osteopontin**
- **Calsemin**
- **$\alpha$ -lactalbumin**
- **Ferrisplasn**
- **Gossact**
- **Insulin like growth factors system**
- **Interleukins**
- **Calmodulin-Binding Protein**
- **Spermadhesins**

- **Cysteine-rich secretory proteins**
- **Ubiquitin**
- **Macrophage migration inhibitory factor**
- **Seminal protein 40**
- **Fertilization-Associated Antigen**
- **Homologue protein of HBP**
- **Non-heparin binding protein**
- **Heat shock protein**
- **DE (ARP)**
- **Extracellular matrix proteins**
- **Fucose-binding protein**

# Techniques for using of separation proteins

- **Ion exchange chromatography**
- **Sephadex gel filtration**
- **Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)**
- **2- Dimensional high resolution polyacrylamide gel electrophoresis (2DHR-PAGE)**
- **Fast performance liquid chromatography (FPLC)**
- **Immunodiffusion**
- **Immuno-electrophoretic analysis (IFA)**



- **Quantitative reverse transcription polymerase chain reaction (QT-PCR)**
- **Recombinant DNA technology**
- **cDNA probe**
- **Single radial immunodiffusion (SRID)**
- **Radio receptor assay**
- **Nuclear protection assay**
- **Tissue culture immunohistochemistry**
- **Radioimmunoassay assay**
- **Time resolved fluoroimmunoassay**
- **Blot analysis**

# Bovine Seminal Plasma Proteins

- BSP-A1, BSP-A2, BSP-A3, and BSP-30 kDa
- Secretory products of seminal vesicles
- BSPA1, A-2, and -A3 have a molecular mass between 15 and 17 kDa
- BSP-30 kDa has a molecular mass of 28–30 kDa.
- BSP-A3 is not glycosylated, BSP-A1, -A2 and -30 kDa are glycosylated
- BSP-A1 and -A2 are considered to be glycoforms of the same protein named BSP-A1/-A2 and also known as PDC-109

- **Homologs**  
Boar : pB1  
Stallion :HSP-1, HSP-2, and HSP-12 kDa  
Goat :GSP-14 kDa, GSP-15 kDa, GSP-20 kDa, and GSP-22 kDa
- **BSP proteins have to interact with choline phospholipids on the sperm membrane, with high- and low-density lipoproteins (HDL and LDL) and with heparin**
- **Biological role in sperm membrane stabilization (decapacitation) and destabilization (capacitation) during the fertilization process**
- **BSP proteins are mixed with sperm upon ejaculation and induce a cholesterol efflux from the sperm membrane, results in reorganization of its components.**
- **BSP proteins also bind to the choline phospholipids of the sperm membrane, which may sterically hinder phospholipids movements and thereby stabilize the membrane**

# Calmodulin-Binding Protein

- **Basic protein of molecular weight 15 kD (bSVSP15 )**
- **Secreted : bovine seminal vesicle**
- **Capacitation :Removal or modification of certain surface components as well as an increase in the permeability of the plasma membrane to metal ions**
- **Influx of ions triggers the acrosome reaction, which is characterized by the fusion of the plasma membrane and outer acrosomal membrane**
- **Calmodulin has to stimulate various calcium-dependent enzymatic activities through specific associations with calmodulin-binding proteins**
- **Several calmodulin-binding proteins present in bovine epididymal and ejaculated spermatozoa**
- **Increased binding of calmodulin in the absence of  $Ca^{2+}$  has also been observed with proteins in plasma membrane preparations and outer acrosomal membrane complexes of bovine epididymal spermatozoa, and with proteins (21-14 kDa) of ejaculated boar spermatozoa and human spermatozoa.**
- **Interestingly, the proteolytic fragments of BSP-A1/-A2 and-30 kDa proteins exhibit higher calmodulin-binding activity than the native BSP proteins, particularly in the absence of  $Ca^{2+}$ .**

# Spermadhesins

- **Boar** :(AWN-1, AWN-2, AQN-1, AQN-3, PSP-I, and PSP-II ) -30%
- **Stallion** :HSP-7
- **Bovine** :aSFP and Z13
- **Secreted** :seminal vesicles (all)
- **Rete testis and tubuli recti**: AWN-1
- **Not glycosylated** :HSP-7, AQN-1, Z13, and aSFP proteins
- **Glycosylated**: PSP-I and PSP-II
- **Both non - glycosylated and glycosylated** :AQN-3 and AWN

- **Interaction with heparin and zona pellucida glycoconjugates, and bind to the sperm surface upon ejaculation ( except PSP-I )**
- **The spermadhesins adhere to sperm by binding to phosphatidyl ethanolamine present in the sperm membrane**
- **Porcine spermadhesin AWN-1 and its equine homolog (HSP-7) are to play a role in gamete recognition and sperm–egg binding at fertilization**
- **Spermadhesins can also bind heparin and are released from the sperm membrane during capacitation**
- **PSP-I and PSP-II do not seem to play a role in sperm capacitation or binding to the oocyte**
- **In fact, the PSP-I/ PSP-II heterodimer displays a proinflammatory effect and may modulate immune responses in the porcine uterine environment**

# Seminalplasmin

- **A highly potent antimicrobial having transcript inhibitory**
- **MW:5.4 kDa containing 48 amino acids and basic protein with PI 9.6 – 9.8**
- **Synthesised and secreted :seminal vesicles, prostate and ampullae of the vas deferens of the bulls and not by the testis and epididymis**
- **Specific function in the biology of reproduction is not clear**
- **The major basic protein of bull semen**
- **An important regulator of calcium transport in bovine sperm and a positive modulator of the zona pellucida induced acrosome reaction**
- **Is shown to be a recently created member of the neuropeptide Y gene family**

# Clusterin

- **Glycoprotein and sulphated glycoprotein-2 (SGP-2)**
- **Blood, seminal plasma, and several solid tissues**
- **Secreted by the Sertoli cells and epididymis**
- **Bind to the plasma membrane of spermatozoa during germ cell maturation**
- **Implicated in a number of processes including lipid transport, complements inhibition, secretory processes, and programmed cell death**
- **Epididymal cells in the proximal corpus of the stallion secrete clusterin abundantly, allowing the concentrations to remain high throughout the epididymis**
- **Clusterin-positive spermatozoa have lower fertility, as determined by nonreturn-to-estrus rates and had many morphological abnormalities**
- **Presence of clusterin on ejaculated sperm may indicate improper spermatogenesis or irregular epididymal maturation**



# Seminal protein 40 (SP-40)

- **SP-40, is a glycoprotein of Mr 80 kDa comprising two distinct 40-kD polypeptide chains**
- **Inhibits C5b-6 initiated complement hemolysis and it is likely that both proteins combine with nascent C5b-7 preventing membrane insertion and cell lysis**
- **Cloning and sequencing of SP-40, 40 cDNA from a human liver library**
- **Revealed a strong sequence homology with a major rat Sertoli cell product, sulfated glycoprotein-2 in the rat and the ram**
- **SP-40, 40 has been identified in Western immunoblots of human seminal plasma**
- **Normal human serum : 35-105 ug/ml**

# Heparin-binding Proteins

- **Molecular mass of 31 kDa (14 to 31 kDa )**
- **Seminal vesicles and prostate glands**
- **Allow spermatozoa to respond to a challenge with lysophosphatidylcholine and undergo the acrosome reaction**
- **Heparin-binding affinity of porcine spermatozoa is related to acrosome morphology, retention of cytoplasmic droplets, and resistance to osmotic lysis**
- **Spermatozoa of bulls with more of a group of heparin- binding proteins in their membranes had 17% greater fertility than spermatozoa from bulls**
- **Bind to cauda epididymal sperm in vitro and increase the frequency of zonae pellucidae induced acrosome reactions by epididymal sperm incubated with heparin**

- **Five families of HBP with differing affinity for heparin**
- **Addition of the heparin binding seminal plasma proteins (HBP) in the semen has improved the progressive motility, BCMPT and HOST response at prefreeze level**
- **HBP at a concentration of 40µg/ml showed better results than HBP at a concentration of 80µg/ml**
- **Subjecting the HBP treated spermatozoa to cryopreservation resulted in significant reduction of motility, viability, acrosomal integrity and response to BCMPT and HOST in the HBP treated groups when compared to those in control group.**
- **The deleterious effect of HBP was found to be concentration dependent with the higher concentration causing higher post-thaw damage.**

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# Homologue protein of HBP

- Molecular mass of 24 kDa also may be related to fertility
- Has an amino acid sequence related to tissue inhibitor of metalloproteases- 2.

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# Non-heparin binding protein

- Provide some degree of protection to buffalo spermatozoa against cryopreservation stress
- Protection from the deleterious effect of HBP during cryopreservation

# Fertilization-Associated Antigen (FAA)

- **31-kDa protein**
- **Sequence similarity to DNase-I**
- **The amount of FAA on spermatozoa is related to fertility.**
- **Inseminated with FAA-positive spermatozoa pregnancy rates were about 15% higher than in females inseminated with FAA-negative spermatozoa.**

# Heat shock protein

- Identified in proteins extracted from bull and boar sperm, and in seminal plasma of bulls
- Part of a super family of molecular chaperones, which protect cells from chemical and heat shock
- Two main intracellular isoforms :HSC70 and HSP70  
HSC70 : 70 kDa and is constitutively expressed  
HSP70 :Low levels in normal cells and high levels in stressed cells
- HSP70 helps maintain protein conformation, stabilize unfolded precursor proteins prior to assembly into macromolecular complexes and participate in transfer of proteins across intracellular membranes
- During cellular stress, HSP70 synthesis is enhanced and the protein protects cells by refolding denatured protein, removing damaged proteins at degradation and by blocking apoptosis

- **Various isoforms of HSP70 :stage specific and developmentally regulated manner during spermatogenesis and germ cell differentiation**
- **In mitigating effects of elevated temperatures on spermatogenesis and post-ejaculatory function of sperm**
- **An important role in sperm function after ejaculation**
- **HSP 70 has been implicated in fertilization and embryo development in cattle**
- **Gene expression was significantly down-regulated in ejaculated sperm from infertile animals with oligoteratozoospermia**
- **Anomalies in the expression of this gene are associated with spermatogenic and/or spermiogenic dysfunction involved in the pathogenesis of some cases of male infertility, and sperm mRNA analyses may thus be a useful tool in evaluating the infertile man.**
- **HSP70 may also be induced following exposure to a wide variety of physio-chemical insults: temperature variation, steroids, amino acid analogues, heavy metals, and oxidative damage**
- **Mammalian cells produce HSP70 in response to infection by viral or bacterial pathogens, and this response has been indicated to exert a cytoprotective effect during disease pathogenesis**



- **The actuality of such induction would suggest an additional mechanism limiting immunity to spermatozoa: prevention of pro-inflammatory cytokine synthesis and T-lymphocyte activation secondary to initiation of Hsp70 gene transcription**
- **The Hsp70 mRNA was also identified in cells of the endo-cervix from sexually active women after intercourse**
- **correlation between the amount of a protein, originally thought to be a form of creatine kinase, and human spermatozoal fertility and maturity**
- **HspA2 content per spermatozoon is positively correlated with morphological defects in spermatozoa and is negatively correlated ( $r = 0.70$ ) with spermatozoal concentration in human semen samples**
- **It was proposed that larger amounts of HspA2 are associated with immature spermatozoa**
- **Elevation of HspA2 may indicate that spermatozoa have not extruded their cytoplasm during spermiogenesis and have not completed plasma membrane remodeling during epididymal maturation because men with diminished fertility had spermatozoa with larger amounts of cytoplasmic proteins.**

# Androgen-binding protein

- **Androgen-binding protein (ABP) is a glycoprotein (beta-globulin) and 70-90 kDa**
- **Produced by the Sertoli cells in the seminiferous tubules of the testis ABP is also synthesised by the epididymis**
- **Binds specifically to testosterone (T), dihydrotestosterone (DHT), and 17-beta-estradiol**
- **ABP has the same amino acid sequence as sex hormone-binding globulin (SHBG) and the difference is the site of production and the addition of different sugar moieties**
- **ABP's production is regulated under influence of FSH on Sertoli cell, enhanced by insulin, retinol, and testosterone**

- **synthesised by sertoli cells and is secreted in seminiferous tubules and transported with spermatozoa to the lumen of the epididymis and creates a specialised androgen microenvironment in the epididymal tubules for the maturation of spermatozoa**
- **Heterogeneity of ABP : glycosylation, phosphorylation , methylation , sulphation and acetylation pattern of ABP molecules , physiological**
- **Epididymal maturation of spermatozoa, acquisition of sperm motility and fertilizing ability, in the regulation of function of epididymis including the synthesis and secretion of epididymal sperm maturation proteins, regulation of  $5\alpha$ -DHT, which mediate androgen action in the epididymis**
- **Information on ABP status in cattle and buffalo bulls and their role in the bovine male reproduction is not available and need investigation.**

# Forward Motility Protein (FMP)

- **FMP was 37.5 kDa in its molecular weight by SDS–PAGE gel and also a heat stable glycoprotein**
- **Mammalian sperm acquire their capability for motility as they traverse the epididymis**
- **Sperm motility is a key factor in allowing us to determine semen quality and fertilizing capacity**
- **Motility :surrounding metabolites and ions (Ca<sup>2+</sup>, Mg<sup>2+</sup>, etc.), pH, and temperature**
- **Typical index of sperm motility, sperm forward motility capacity**

- **Mechanisms: cAMP activates sperm cytosolic cAMP dependent protein kinases, in turn, phosphorylate multiple intra-sperm phosphoproteins that may regulate flagellar motility**
- **Exogenous forward motility-related proteins, in concert with elevated levels of cAMP, initiates flagellar motility during the epididymal transit of sperm**
- **FMPs seem not to be species-specific in mammals, these proteins could perform their biological functions in spermatozoa in various species**
- **These proteins could also play a vital role in diagnosis and treatment of male infertility, development of contraceptive vaccines, and population control**

# Osteopontin (OPN)

- **55 kDa**
- **Extracellular matrix phosphoprotein : the male and female reproductive tracts**
- **High fertility marker in bull seminal plasma, produced by the ampulla and the vesicular gland**
- **Immunofluorescent analysis of ejaculated and cauda epididymal sperm showed OPN localization in a well-defined band in the postacrosomal region of the sperm head and also on the midpiece**
- **Sperm treated with an antibody to OPN fertilized fewer oocytes than sperm treated with control medium while increasing incidence of polyspermy, suggesting a role of sperm-associated OPN in fertilization and a block to polyspermy**
- **This OPN exists at multiple molecular weight forms in the bull reproductive tract and its presence on ejaculated sperm may signal its importance in fertilization by interacting with integrins or other proteins on the oocyte plasma membrane**
- **OPN functioned as an adhesion molecule, binding these germ cells to the basement membrane of the seminiferous tubule and to adjacent Sertoli cells**

- **In the bull, OPN in the seminiferous tubule, but only in tubules that contain elongated spermatids, stage-related expression pattern**
- **The presence in the epididymis and on epididymal sperm may be important in regulating calcium content of the sperm and epididymal lumen**
- **OPN contains a calcium-binding site and causes calcium release in osteoclasts through an integrin-stimulated IP3 pathway**
- **Calcium crystal deposits have also been shown in human rete testis, efferent ducts, and epididymis.**
- **OPN in epididymal fluid may prevent calcium crystallization that can be detrimental to sperm motility and fertility.**
- **OPN is also a known ligand for the CD44 family of plasma membrane receptors**

- **CD44 and its splice variants are members of the hyaluronic acid receptor family, ubiquitously expressed and can bind extracellular matrix proteins, such as OPN in addition to its primary ligand hyaluronic acid.**
- **IVF : OPN involved in a block to polyspermy.**
- **Sperm treated with anti-OPN bound to oocytes in higher numbers than sperm incubated in control medium, and the incidence of polyspermic fertilization also increased with antibody-treated sperm**
- **The localization of OPN on sperm in the postacrosomal region makes it unlikely that it participates in zona interactions, but a likely candidate for interaction with the plasma membrane of oocytes.**



# Cellular Retinol binding Protein (CRBP)

- Retinol (vitamin A alcohol) is essential for male reproductive function
- Adluminal germ cells also contain two vitamin A-binding proteins: cellular retinoic acid-binding protein (CRABP) and, in lesser amounts, cellular retinol binding protein (CRBP)
- CRBP is present in relatively high levels in Sertoli cells, where its level of expression varies dramatically with the spermatogenic cycle
- Retinol is transported systemically and inter cellularly bound to retinol-binding protein (RBP)
- it has been speculated to be involved in retinol uptake and metabolism and/or in the transport of retinoids to the germ cells
- CRBP is believed to be involved in the transepithelial movement of vitamin A in the testis
- Sertoli cells also express this lecithin-retinol acyltransferase (LRAT) enzyme, it is possible that they synthesize retinyl esters for export to the germ cells. Sertoli cells have recently been shown to synthesize and secrete RBP
- Cellular retinol-binding proteins (CRBP) and cellular retinoic acid-binding proteins function in intracellular vitamin A transport, metabolism and homeostasis

# IgG – Fc binding proteins

- **A heat stable, 94.00 kDA**
- **Reported in human seminal plasma, which specifically binds with the Fc region of IgG molecule**
- **Does not bind with the Fab2 fragments of IgG molecule nor with the Fc fragments of IgA and IgM**
- **Synthesised by prostate and is sensitive to pronase, but is resistant to glycosidase and deoxyribonuclease**
- **Specific function of this protein is not clear**
- **Seminal plasma Fc binding protein may be one of the factors regulating the female humoral and cellular immune responses of the inseminated spermatozoa by protecting them from immune destruction**
- **IgG-Fc binding protein is adsorbed to the sperm surface after ejaculation and is responsible for the binding of IgG-Fc molecule to spermatozoa.**

# Immobilin

- **High mol.wt. (400 kDa) glycoprotein**
- **Isolated by sephadex G200 gel filtration and ultracentrifugation of the rat cauda epididymal fluid**
- **Synthesised by the principle cells of the caput epididymis and is secreted in the lumen of the tubule which travels with the sperm in cauda epididymis**
- **Immobilin inhibits sperm motility in the rat and hamster cauda epididymis mechanically by creating a highly viscous-elastic environment in the cauda epididymis, which is the store house of spermatozoa.**

# Gossact

- Heat stable, leucine rich seminal plasma protein of 16 kDa
- Isolated and purified from human seminal plasma
- Gossypol is a yellow phenolic pigment in cotton seeds which is a potent anti-fertility agent
- Gossypol inhibits lactate dehydrogenase (LDH) in human spermatozoa viz. mitochondrial damage, and acrosomal fragmentation leading to infertility
- The inhibitory effect of gossypol of LDH is neutralized by gossact
- The status of gossact like protein in the bovine seminal plasma is unknown and need to be studied

# DE (ARP)

- **37 kD glycoprotein**
- **Synthesized and secreted from the epididymal epithelium in an androgen-dependent manner**
- **Identified in the rat, mouse, and human by molecular cloning**
- **Localized to the dorsal region of the rat sperm head, and translocates to equatorial segment during capacitation, a sperm maturational process that renders the sperm capable of fertilizing an egg**
- **DE is one of the few reagents that affect sperm-egg fusion specifically**
- **Recent results show that penetration of ZP-free hamster eggs by human sperm is reduced in the presence of anti-ARP antibodies, but sperm-egg binding is unaffected.**

# Extracellular matrix proteins

- **Human sperm : fibronectin, laminin, and vitronectin**
- **The sources of these sperm-associated extracellular matrix proteins have not been definitively determined**
- **Fibronectin could associate with the surface of sperm during spermatogenesis in the testis, during epididymal transit, or during mixture with seminal plasma**
- **Vitronectin mRNA has been detected in Northern blots of human testis, although the seminal plasma is another potential source**
- **Fibronectin :associated with the equatorial segment, and also uniform distribution over the entire sperm**

- **Vitronectin appears to be localized in the acrosome of acrosome-intact sperm and released during the acrosome reaction**
- **Laminin : localized on the sperm tail or on the acrosomal membranes**
- **A possible role for fibronectin or vitronectin in sperm-egg binding**
- **Interestingly, an increase in sperm-egg binding is observed with lower concentrations of vitronectin, whereas a decrease is observed in higher concentrations**
- **The reduced sperm-egg binding may be due to agglutination of sperm in the higher concentrations, or perhaps is due to vitronectin serving as a "bridge" between a sperm vitronectin receptor and an egg vitronectin receptor**
- **Laminin present on sperm is capable of mediating any part of sperm-egg membrane interactions**

# Inhibin

- **Inhibin is a steroid free glycoprotein hormone of 15-30 kDa**
- **Present in seminal plasma and ovarian follicular fluid of various mammalian species**
- **Inhibin is mainly synthesised by sertoli cells in the male and granulosa cells in female**
- **Inhibin exerts its physiological effect at pituitary levels by decreasing the basal FSH secretion, which in turn modulates the reproductive function in the male and female**
- **Mechanism of action :at low concentration Inhibin rapidly suppress FSH synthesis and secretion**
- **At higher concentration the content of both FSH and LH is effectively by degradation of intracellular stores of these gonadotropins**



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# Fucose-binding protein

- **Oviductal sperm reservoirs have been found in cattle, mice, hamsters, pigs, and horses**
- **In cattle (*Bos taurus*), the reservoir is evidently formed when sperm bind to fucosylated ligands resembling Le(a) trisaccharide on the surface of oviductal epithelium**
- **This protein inhibited binding of sperm to oviductal explants**
- **epifluorescence microscopy, live uncapacitated sperm labeled over the acrosome with a fucose-BSA-fluorescein isothiocyanate (FITC) conjugate**

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# Cysteine-rich secretory proteins (CRISPS)

- Mainly found in the cauda epididymis
- It is synthesized by the principal cells and secreted into the lumen, accounting for about 15% of the protein content of the epididymal fluid
- Role in the late stages of sperm maturation

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# Ubiquitin

- As a protein capable of inducing B-lymphocyte differentiation
- Playing a central role in intracellular proteolysis
- Bovine ubiquitin standards were between 0.16 and 2.5 µg/ml.

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# Macrophage migration inhibitory factor

- **Secreted by Leydig cells**
- **MIF modulates Sertoli cell inhibin production**
- **MIF has been shown to possess a tautomerase activity and thiol-protein oxidoreduction properties**
- **T-cell cytokine**
- **MIF modulates insulin secretion by pancreatic cells and regulates the glucocorticoid mediated suppression of the immune response**
- **Differentiation in the testis the mammalian spermatozoon travels along the epididymis**

- **During this journey along this single convoluted tubule that the male gamete will acquire its fertilizing ability**
- **MIF is one of the proteins transferred to spermatozoa during the epididymal transit**
- **MIF associated with epididymosomes is transferred to the dense fibres of the sperm flagellum**
- **In sperm flagellar beating and acquire rigidity during the epididymal transit because of the formation of disulphide bonds that progressively increase during sperm maturation**
- **MIF human semen, originating from the prostatic secretion containing membranous vesicles similar to epididymosomes and described as prostasomes**

Thanking you

