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REVIEW ARTICLE

Current status of research on seminal plasma proteins

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ABSTRACT

Seminal plasma is a highly complex biological fluid containing proteins, amino acids, enzymes, hormones, carbohydrates, lipids, major minerals and trace elements. During the past two decades several novel seminal plasma proteins viz. forward motility protein (FMP), IgG-Fe binding protein, immobilin, cellular retinol-binding protein (CrBP) androgen-binding protein (ABP), transferrin, seminalplasmin, inhibin, clusterin, calcemin, ferrisplan, gossact, heparin-binding (HBPs), osteopontin, acrosome reaction potentiating protein and BSP proteins BSP-A_1, BSP-A_2, BSP-A_3 and BSP-30 kDa proteins have been reported. These proteins are synthesized and secreted by various reproductive organs such as the testis, epididymis, ampullae of the vas deferens, seminal vesicles, prostate, and bulbouretheral glands. The origin, tissue localization, isolation, purification, characterization and the potential role of some of these proteins in the biology of reproduction is discussed briefly.

Key Words: Seminal plasma protein, forward motility protein, ABP, BSP, Inhibin

The ejaculated semen consists two major components viz. Sperm cells or spermatozoa and the fluid part obtained after centrifugation called seminal plasma. The spermatozoa originate from the seminiferous tubule and are suspended in the seminal plasma. The seminal plasma is composed of secretions contributed by the testis, epididymis, seminal vesicles, ampullae, prostate and bulbouretheral glands. About 60-80% of the ejaculated semen of the bull originates from these sources. Seminal plasma is highly complex biological fluid containing proteins, amino acids, enzymes, fructose and other carbohydrates, lipids, major minerals and trace elements (Mann and Lutwak Mann, 1981). This paper deals briefly with seminal plasma proteins, their origin, characteristics and role in the biology of reproduction.

Origin of Seminal Plasma Proteins

Seminal plasma proteins partly originate from the blood plasma by exudation through the lumen of the male genital tract and partly are synthesized and secreted by various reproductive organs and are known as seminal plasma specific proteins.

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Seminal Plasma Proteins of Blood Origin

Several seminal plasma proteins of blood origin viz. Prealbumin, albumin, globulins, transferrin, α-antitrypsin, β-glycoprotein, β-lipoprotein, orsomucoid, kininogen, peptide hormones, FSH, LH, prolactin and immunoglobulins, IgG, IgA and IgM (Fowler and Meriono, 1983, Dondero *et al.*, 1984) have been identified and characterized. These proteins are involved in the regulation of osmotic pressure and pH of seminal plasma, transport of ions, lipids and hormones.

Seminal Plasma Specific Proteins

A major part of seminar plasma proteins originate from the testis, epididymis, vas deferens, prostate, seminal vesicles and bulbourethral glands (Matousek, 1985, Kulkarni *et al.*, 1998). The biosynthesis and secretion of these proteins is regulated by testosterone levels in the blood. During the past two decades extensive studies on seminal plasma proteins of the man and animals with special reference to their role in the maturation of spermatozoa, development of forward progressive motility and fertilizing capacity have been reported. The development of highly sensitive modern analytical techniques such as ion-exchange chromatography, sephadex gel filtration, sodium

dodecyl sulfate polyacrylamide gel electrophoresis (SDS_PAGE), 2-demensional high resolution polyacrylamide gel electrophoresis (2DHR-PAGE), fast performance liquid chromatography (FPLC), immunodiffusion, immunoelectophoretic analysis (IFA), quantitative reverse transcription polymerase chain reaction (QT-PCR) recombinant DNA technology, cDNA Probe, single radial immunodiffusion (SRID), radioreceptor assay, nuclear protection assay, tissue culture immunohistochemistry, immunocytochemistry, radioimmunoassay (RIA), time resolved fluroimmunoassay (TR-FIA) and blot analysis for isolation, purification, characterization and quantification of proteins, peptides, growth factors, their receptors and mRNA has contributed significatnly towards the advancement of the present knowledge of seminal plasma proteins. This has lead to the identification of several novel seminal plasma proteins viz., forward motility (FMP), IgG-Fe binding protein, immobilin, cellular retinol-binding protein, (CrBP), androgen bindings protein (ABP) seminaplasmin (SPLN), clusterin, heparin-binding proteins (HBPs), osteopontin, calsemin, alphalactalbumin, ferrisplasn, gossact, BSP-Proteins, BSP-A_1, BSP-A_2, BSP-A_3 and BSP-30kDA proteins, insulin-like growth factor system (IGF), and interleukins (ILS). The origin of some of these proteins, their characteristics and role in the biology of reproduction is discussed in brief.

Bovine Sperm Forward Motility Protein (FMP)

Accott and Hoskins (1978) first reported a 37.00 kDa glycoprotein present in the bull seminal plasma which initiates forward motility in immuature and immotile caput spermatozoa and designated this protein as forward motility protein (FMP). FMP has been partially purified by gel chromatography and affinity chromatography on concavalin-A agarose. It is synthesized by caput epididymis. Treatment with trypsin completely abolishes its activity. Further, studies have indicated the presence of FMP in the seminal plasma of several mammalian species. Raynaud and Kann (1986) reported FMP in hamster epididymal spermatozoa and demostrated that it develops forward morility in epididymal spermatozoa and prevents head-to-head agglutination of motile spermatozoa. Besides stimulating forward motility of immotile spermatozoa during epididymal transit, FMP causes stiffening of the flagella and thereby prevent the frequent reversal in the direction that typify the motility patterns of caput sperm without FMP (Accott and Hoskins, 1983). The specific biochemical mechanism at cellular/molecular level by which FMP initiates and develops the forward motility in immotile spermatozoa is not clear.

IgG-Fc binding Protein

A heat stable, 94.00kDa, IgG-Fc binding protein has been reported in human seminal plasma, which specifically binds with the Fc-region of the IgG molecule and does not bind with the $(Fab)^2$ fragments of IgG molecule nor with the Fc fragments of IgA and IgM (Witkin *et al.*, 1983, Kamada *et al.*, 1991). IgG-Fc binding protein has been isolated by affinity chromatography. This protein is synthesized by the prostate and is sensitive to pronase, but is resistant to glycosidase and deoxyribonuclease. The specific function of this protein is not clear. Fc region of the IgG molecule is responsible for biological functions such as complement fixation (Butler 1983). It is postulated that the seminal plasma Fc bindings 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 protein is adsorbed to the sperm surface after ejaculation and is responsible for the binding of IgG molecule to spermatozoa.

Immobilin

Immobilin is a high mol. wt (400 kDa) glycoprotein which leeps the rat cauda epididymal spermatozoa in fully immobilized state (Usselman and Cone, 1983, Turner and Reich 1987). This protein has been isolated by sephadex G200 gel filtration and ultracentrifugation of the rat cauda epididymal flui (Turner and Reich 1987). Immobilin is synthesized by the principal cells of the caput epididymis and is secreted in the lumen of the tubule which travels with the sperm in the cauda epididymis (Norka, 1988). Immobilin inhibits sperm motility in the rat and hamster cauda epididymis mechanically by creating a highly viscoelastic environment in the cauda epididymis, which is the store house of spermatozoa.

Seminalplasmin (SPLN)

A highly potent antimicrobial, transcript inhibitory, 5.4 kDa, 48, amino acids, basic protein with PI 9.6 to 9.8, present in the bull seminal plasma was reported by Reddy and Bhargwa (1979) from India, which was named seminalplasmin by them. In subsequent studies this protein was isolated and

purified form the bull seminal plasma and seminal vesicle extract and it's tissue localization, structure, physicochemical and biological characteristics were investigated (Reddy *et al.*, 1983, Shivaji *et al.*, 1984, Seheit *et al.*, 1985, Murti *et al.*, 1994). Seminalplasmin is synthesized and secreted by seminal vesicles, prostate and ampullae of the vas deferens of the bull not by the testis and epididymis (Shivaji *et al.*, 1984, Vempe *et al.*, 1990).

Antimicrobial activity of seminalplasmin

Seminalplasmin is a potent antimicrobial protein present in the bull seminal plasma and seminal vesicle fluid acting on a wide range of gram positive and gram negative becteria. It inhibits the growth of different species of bacteria such as E.coli, Staphylococcus aureus, Staphylococcus feacalis, Bacillus subtilis, Pseudomonas aeroginosa, Klebsiella, Pnemoias, Enterobactor aerogenes, Solmonella typhimurium and various other bacteria. Seminalplasmin is active against various yeasts and several retero viruses (Reddy *et al.*, 1983, Bhargava 1985). The specific function of SPLN in the biology of reproduction is not clear. In view of its potent antimicrobial activity against a wide variety of bacteria, viruses and yeasts it is postulated that SPLN could act as an antimicrobial agent providing protection to the male genital tract and also female genital tract which could receive seminalplasmin during mating (Bhargava 1985).

Gossact

A heat stable, leucine rich seminal plasma protein of 16 kDa has been isolated and purified from human seminal plasma and is designated "Gossact" (Nakamura *et al.*, 1991). Gossypol is a yellow phenolic pigment in cottonseeds which is a potent antifertility agent in the human, rat, hamster, boar and other animals. Gossypol inhibits lactate dehydrogenase (LDH) in human spermatozoa viz. Mitochondrial damage, and acrosomal fragmentation leading to infertility. The inhibitory effect of gossypol on LDH is neutralized by gossact (Nakamura *et al.*, 1991). Studies on the effects of gossypol on seminal quality of the bulls have been reported recently. Chenoweth *et al.* (1994) studied the effects of gossypol on semen quality, sperm motility, morphology and sperm production in young bulls. The experimental animals were fed cottonseed meal equivalent to 8.2 g of free gossypol per bull/day, and the control animals were fed soybean meal. Sperm production and sperm motility was significantly lower in experimental bulls that the control. Sperm abnormalities were significantly higher in experimental bulls than the control. Pereria *et al.* (1999) investigated the effects of long term feedings of cottonseed meal equivalent to free gossypol, 14 mg/kg body weight/day in bulls. In experimental animals sperm motility %, normal and live sperm % and daily sperm production were significantly lower than the control bulls. Abnormal sperm % was significantly higher in experimental animals. In many parts of India cottonseed meal feeding to cattle and buffalo breeding bulls is a common practice. However, information on the effect of gossypol feeding on the semen quality and reproduction are largely not available and require investigation. Similarly, the status of gossact like protein in the bovine seminal plasma in unknown and need to be studied.

Clusterin

Clusterin is a recently discovered acidic (PI 3.6-6.7) sulfated glycoprotein of mol. wt. 75-85 kDa, that was first identified in the ram rete testis fluid, which caused aggregation of Sertoli cells (Fritz *et al.*, 1983). During the past two decades extensive studies on tissue localization and distribution, isolation, purification and characterization of clusterin (Laslop *et al.*, 1993, Ibrahim *et al.*, 1999, 2000, 2001) from various species have been reported. The role of clusterin in the male reproductive tract and development expression of clusterin in the epididymis, seminal vesicles and prostate (Tenniswood, *et al.*, 1998) and the biosynthesis, tissue distribution and potential function of clusterin (Baily *et al.*, 2000) have been reported.

In a recent study Ibrahim *et al.* (1999) identified and characterized clusterin isomers in the rete testis fluid, cauda epididymal fluid and on cauda epididymal spermatozoa of the bull using SDS-PAGE and Western blot analysis. In another study Ibrahim *et al.* (2001) investigated the localization of clusterin on the bull spermatozoa before and after glass wool sephadex gel filtration, using polyclonal antibodies against the bull cauda epididymal clusterin. Immunofluorescence analysis detected clusterin on only a small numbers of morphologically abnormal spermatozoa, imdicating that the sperm clusterin in the bull semen is associated with morphologically abnormal sperm. These results further indicate that clusterin is implicated in the process of trapping of abnormal spermatozoa in grasswool-sephadex columns. Clusterin positive spermatozoa in the bull

semen could be a potential marker for poor quality of the bull semen (Ibrahim *et al.*, 2001).

Ibrahim *et al.* (2000) investigated the correlation between clustein positive spermatozoa of bull semen and fertility. Clusterin positive spermatozoa were determined by flow cytometry (Fluorescence activated sperm sorting technique) in 48 bulls with known fertility and observed significant inverse relationship between the percentage of clusterin positive spermatozoa and adjusted non return rate (ANR) ($r-0.058$, $P\leq-0.01$) and estimated relative conception rate (ERCR) ($r=-0.60$, $P\leq-0.001$. Estimated relative conception rate is potentially accurate method for fertility determination and it resulted in higher correlation with clusterin positive spermatozoa. A direct relationship was observed between clusterin positive spermatozoa and primary, secondary, teriary and total sperm abnormalities ($r=0.52$, $P\leq0.001$, 0.77, $P\leq0.001$, 0.32, $P\leq0.05$ and 0.58, $P\leq0.001$) respectively. On the basis of these observations it was concluded that flow cytometry is a useful technique for objective and efficient detection of clusterin positive spermatozoa and is potentially a better predictor of fertility than sperm motility or abnormal sperm morphology.

Heparin – binding proteins (HBPs)

Heparin-binding proteins with high affinity for heparin in the seminal plasma and seminal vesicular fluid of the bull, boar, human and horse (Ronkko, *et al.*, 1994, Krause *et al.*, 2001, Petersen *et al.*, 1998, Calvete *et al.*, 1997) have been identified, isolated and characterized recently. These proteins are synthesized by the male accessory sex glands viz. peminal vesicles, prostate and bulbourethral glands and are implicated in sperm capacitation, acrosome reaction and fertilization (Petersen *et al.*, 1995). The biosynthesis and secretion of heparin-binding proteins is regulated by testosterone.

Bovine heparin-binding proteins

Bovine heparin-binding proteins from the bull seminal plasma (Miller *et al.*, 1990) and seminal vesicle fluid (Nass *et al.*, 190) have been isolated using heparin affinity HPLC, and reported the major HBPs were of molecular weight 13 and 15 kDa, alongwith some minor proteins of 16, 24 and 30 kDa. Each of these HBPs were shown to bind epididymal spermatozoa. Heparin binds to bull sperm membrane and induces sperm capacitation and acrosome reaction. The relationship of heparin binding proteins to fertility in bulls has been investigated (Bellin *et al.*, 1994). The bulls with greater affinity to heparin-binding proteins in sperm membrane had greater frequency of acrosome reacted sperm and higher ($P<0.05$) fertility than those with lower affinity to HBPs in sperm membrane. McCauley *et al.*, (1996) studied the localization of HBPs during sperm capacitation and immunofluorescence binding patterns of monoclonal antibody (M_1) against HBPs in higher fertility bulls. Immunofluorescence localization of M_1 binding sites revealed the presence of specific membrane domains containing HBPs in acrosomal and post acrosomal regions of the ejaculated but not epididymal spermatozoa.

In a recent study, the molecular basis of heparin induced bovine sperm capacitation has been investigated (Chamberland *et al.*, 2000). Bull spermatozoa were incubated without heparin (control) and with 10µg/ml of heparin to induce capacitation. Sperm protein phosphorylation was studied with a radioactive protein substrate of protein kinase. Sperm motion parameters viz. Percentage of motile sperm, amplitude of lateral head displacement, (ALH) and flagellar beat cross frequency (BCF) were studied by computer motion analysis to determine the effect of protein phosphorylation on motility parameters. Heparin significantly ($P<-0.0001$) increased ALH and BCF ($P<0.01$) motility parameters as compared with the control. Heparin showed a marked effect over time on percentage of motile spermatozoa due to hyperactivation. These results indicated that heparin induced physiological changes on sperm motility parameters and also increased intensity of phosphorylation of 50 kDa sperm protein.

Androgen-binding protein (ABP)

Androgen-binding protein of 70-90 kDa, with high affinity to testosterone and dihydrotestosterone (5α DHT) has been reported in the seminal plasma, rate testis fluid, seminiferous tubular fluid, testis, epididymis, and prostate of the ram, man, monkey and several other animals (Jegou *et al*, 1979, Barahona *et al.*, 1998). Androgen – binding protein is synthesized by Sertoli cells and is mainly secreted in seminiferous tubule and transported alongwith spermatozoa via the rete testis fluid and efferent ductuli to the lumen of the epididymis (Larrea *et al.*, 1981) and creates a specialized androgen microenvironment in the epididymal tubule for the maturation of spermatozoa. ABP is also synthesized by the epididymis. Numerous studies have reported the heterogeneity of purified ABP in respect of molecular weight, isoelectric pH,

carbohydrate content and composition, electrophoretic mobility and amino acid composition in human, rat, rabbit and hamster (Musto *et al.*, 1982, Danzo and Black 1990, Cates and Damassa 19997). The heterogeneity of ABP could be attributed to variations in glycosylation, phosphorylation, methylation, sulfation and acetylation pattern of ABP molecules (Larrea *et al*, 1981). The physiological significance of heterogeneity of ABP in the biology of reproduction is not clear. Using androgen affinity chromatography, ion-exchange chromatography, sephadex G200 gel filtration, analytical PAGE, HPLC, ultracentrifugation and isoelectric focusing techniques, ABP of various species has been isolated, purified and characterized (Musto *et al.*, 1977, 1982, Danzo and Bell 1988, Larrea *et al.*, 1981, Cheng and Musto, 1982).

Recent studies have indicated the implification of ABP in the process of epididymal maturation of spermatozoa, acquisition of sperm motility and fertilizing ability (Anthony *et al.*, 1984), in the regulation of function of epididymis including the synthesis and secretion of epididymal sperm maturation proteins (Hedge 1996), regulation of nuclear 5α reductase enzyme and 5α DHT, which mediate androgen action in the epididymis (Hinton *et al.*, 2000). Information on ABP status in cattle and buffalo bulls and their role in the bovine male reproduction is not available and need investigation.

Inhibin (INH)

Inhibin is a steroid free glycoprotein hormone of 15-30 kDa, present in seminal plasma and ovarian follicular fluid of various mammalian species (Miyamoto *et al.*, 1985, 1989). Inhibin is mainly synthesized by Sertoli cells in the male and granulosa cells in the female. Inhibin preferentially suppresses the FSH secretion and modulates reproductive functions in the male and female.

Structure of inhibin

Inhibin molecule is composed of two dissimilar disfulfide linked subunits which inhibit pituitary gonadotropin synthesis and/or secretion, preferntially FSH (Burger 1988). Two forms of inhibin known as inhibin A and inhibin B have been isolated and characterized. Inhibin A is a long peptide chain composed of α, βA and inhibin B is a short cian α, βB. The difference between A and B forms of inhibin is due to variations in the amino acid sequence of β-subunit. A 32 kDa inhibin molecule is composed of an β subunit (20 kDa) and one of the two related (14 kDa) beta subunits (βA and βB). both inhibin A (α-bA) and inhibin B (α-βB) suppress FSH release form the anterior pituitary.

Role of inhibin in the regulation of FSH secretion

Inhibin exerts its physiological effect at pituitary level by decreasing the basal FSH secretion, which in turn modulates the reproductive functions in the male and female. Two separate mechanisms of action of inhibin on the synthesis and secretion of FSH have been reported viz. (1) at low concentrations inhibin rapidly suppresses FSH synthesis and secretion and (2) at higher concentrations the content of both FSH and LH is affected by degradation of intracellular stores of these gonadotropins. Recent studies on the physiological role of inhibin in bulls, (Kaneko *et al.*, 1996, Bame *et al.*, 1999) heifers (Glencross *et al.*, 1992) rams (Mc Keowun *et al.*, 1997) ewes (D'Alssandro *et al.*, 1999) and goat (Arakai *et al.*, 2000) have established the involvement of inhibin in the regulation of FSH synthesis and secretion in both the male and female.

Role of inhibin in the regulation of spermatogenesis

The major hormones involved in the initiation and maintenance of spermatogenesis are FSH, LH, INH and testosterone. FSH binds Sertoli cells and stimulate the biosynthesis of androgen-binding protein (ABP) which is secreted in the lumen of the seminiferous tubule. Testosterone produced by Leydig cells is transported by ABP in very high concentration to the site of spermatogenesis. In response to FSH and androgen, Sertoli cells synthesize and secrete inhibin which in turn feeds back negatively to the hypothalamus inhibiting the synthesis and secretion of GnRH, which reduces the synthesis and secretion of FSH. LH binds to the receptors on the membrane of Lyedig cells and stimulate steroidogenesis and biosynthesis of testosterone. Testosterone by feed back mechanism controls the synthesis and secretion of LH.

Application of inhibin immunization biotechnology for augmentation of fertility in farm animals

Immunoneutralization of endogenous inhibin by active or passive immunization of the animal for increasing reproductive efficiency, fertility and productivity of farm animals is a recent

biotechnological development in animal production industry. Immunization against inhibin causes formation of antibodies againt inhibin by lymphoreticular system. These antibodies cause immunoneutralization of endogenous inhibin which alters the biosynthesis and secretion of pituitary and gonadal hormones which in turn modulate the reproductive processes viz, spermatogenesis, folliculogenesis and oogenesis. It is postulated that immunoneutralization of endogenous inhibin during the follicular phase of estrous cycle will lead to increase in the concentration of FSH, resulting in increased ovulation rate in the female and increased testicular sperm concentration in male. Recent studies on active/passive immunization of prepubertal bulls (Martin *et al.*, 1991, Bame *et al.*, 1990) prepubertal heifers (Gleneross *et al.*, 1992) lambs (al'Obaidi *et al.*, 1987) ewes (Kusina *et al.*, 1995, D'Alessandro *et al.*, 1999), cows (Williams *et al.*, 2000) against inhibin have demonstrated 1) significant increase in plasma FSH levels 2) increase in scrotal circumference, sperm output in the male and follicular development and ovulation rate in the female as compared with the control animals. Studies on inhibin immunization of cattle, buffalo, sheep and goats for increasing fertility have not been reported from India. Systematic studies on this aspect of reproductive biotechnology are suggested for the augumentation of fertility and productivity of Indian livestock.

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