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Seminal plasma proteins: what roles do they play?

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Short title: Functions of seminal plasma proteins.

Key words: Ejaculate fractions, seminal plasma, proteomics, sperm function, immunomodulation, fertility.

Abstract

Problem: Semen is a heterogenous and complex cell suspension in a protein-rich fluid with different functions, some of them well known, others still obscure.

Method of study: This paper reviews, comparatively, our current knowledge on the growing field of proteomics of the SP and its relevance in relation to the *in vivo* situation, for the sake of reproductive biology, diagnostics and treatment.

Results: Ejaculated spermatozoa, primarily bathing in cauda epididymal fluid are (*in vitro*) bulky exposed to most, if not all secretions from the accessory sexual glands. *In vivo*, however, not all spermatozoa are necessarily exposed to all secretions from these glands, since sperm cohorts are delivered in differential order and bathe in seminal plasma (SP) with different concentrations of constituents, including peptides and proteins. Proteins are relevant for sperm function and relate to sperm interactions with the various environments along the female genital tract towards the oocyte vestments. Specific peptides and proteins act as signals for the female immune system to modulate sperm rejection or tolerance, perhaps even influencing the relative intrinsic fertility of the male and/or couple by attaining a status of maternal tolerance towards embryo and placental development.

Conclusions: Proteins of the seminal plasma have an ample panorama of action and some appear responsible for establishing fertility.

Introduction

Studies of the male reproductive organs pertaining their basic reproductive biology, for diagnostics of dysfunction or for treatment are often restricted to our capability to perform clinical examinations, alongside to collection of samples, especially in humans. A semen sample reflects the status of the testes, the excurrent ducts, and of the accessory sexual glands, being thus probably the most widely accessible material for most of the above purposes. Semen is classically defined as a fluid conglomerate, where spermatozoa and other cells (classically named round cells, either lining cells of the excurrent ducts, epididymis or accessory glands, migrating leukocytes, and even spermatogenic cells) and cell vesicles (epididymidosomes and prostasomes) are suspended in. Per definition, semen is thus divided in “cellular” and “acellular” components, the latter generically named seminal plasma (SP). The SP is built by the combined contribution of the fluids of the cauda epididymides and

accessory sexual glands. Species of mammals differ regarding presence and size of accessory sexual glands which obviously lead to variations in their relative contribution to semen composition and volume, particularly regarding SP. In some species SP represents up to 95-98% of total semen volume¹.

Methods for semen collection in human and other animals vary, including masturbation, digital collection, artificial vagina, electroejaculation. Semen can be collected into a single (bulk sample) or into consecutive vials (split sample). In many species (e.g. human, stallion, canine, pigs to name a few) the ejaculate is void in spurts (also called jets) with different composition, owing to the sequential emission and/or emptying of secretion of the sexual accessory glands². Therefore, semen composition –the SP in particular– also differs not only among species, among and within individuals but even within an ejaculate. Such differences are important when judging semen “quality”. Quality is classically screened in terms of number of spermatozoa present, their motility and morphological “normality”, the relative numbers of shed leukocytes (classically seen as signs of inflammatory changes), or of immature germ cells (as signs of defective spermatogenesis) etc. The SP of humans, but not of other species, is also examined, albeit not routinely, for specific markers (neutral α -glucuronidase for epididymis fluid, phosphatases or zinc levels for prostate fluid, or fructose for seminal vesicles)³. The reluctance in examining SP is often related to the classical view that SP is a vehicle for spermatozoa, and even regarded as deleterious for some purposes, such as storage. For that reason, the SP is largely removed and replaced by extenders for further handling or freezing⁴.

However, growing evidence demonstrates the SP plays other roles, including modulation of sperm function, of their ability to interact with the epithelia and the secretions of the female genital tract and also as a carrier of signals for the female, its immune system in particular⁵⁻⁷. Simple components of the SP seem to play important roles for sperm viability. Bicarbonate modulates sperm motility and destabilizes the plasmalemma during capacitation⁸⁻⁹ while zinc modulates chromatin stability¹⁰. Most peptides and proteins of the SP, which often make up to 40-60 g/L per ejaculate (human 25-55 g/L; boar 30-60 g/L) play major other roles. Interestingly, the roles of seminal fluid protein appear to be highly conserved. In insects, transfer of seminal fluid, its proteins in particular, induces numerous physiological postmating changes, ranging from enhancement of egg production, modulation of sperm storage and competition, mating plug-formation and the expression of antimicrobial peptides. Moreover,

seminal fluids appear to induce behavioural changes, including decreased receptivity to remating and modified feeding behaviour, with clear changes in female gene expression postmating for mating-dependent genes with predicted functions in metabolism, immune defense and protein modification¹¹. Despite our filogenetical distance, mammals -including humans- also seem to ascribe exposure to SP-proteins other roles than serving as a nutrient and vehicle for spermatozoa, such as the induction of both innate and adaptative immunological responses by the female. These phenomena include the cleansing of eventually introduced pathogens and redundant allogeneic spermatozoa, while calling for immunotolerance towards tubal spermatozoa, developing embryos and feto-placental tissues, i.e. all components essential for reproductive success¹².

Proteomics (e.g. the study of protein products expressed by the genome) has dramatically expanded over the past decade, owing to multidisciplinary methodological and instrumental developments, but also due to the central role of protein interactions in cell function¹³⁻¹⁴. Attempting to unravel these interactions requires direct studies of protein function, sometimes even bypassing genomic studies, considering the number of proteins present in mammals is 10-20 times higher than the number of protein-encoding genes (20-25,000). Presence of alternative splicing or post-translational modifications in proteins (such as glycosylation, phosphorylation, proteolytic processing, lipid modification, etc) explains these basic numerical differences. Interestingly, fluids such as semen appear, in the context of protein identification and relation to function, really complex, ranging from few relevant proteins in spermatozoa towards hundreds in SP¹⁵. Moreover, the fact that ejaculation is in many species fractionated adds a new dimension to the action of SP-proteins (and their interaction) on sperm function and on the female reactivity.

The present paper attempts to review aspects of the composition of the seminal plasma of mammals, with a particular focus on its proteomics and the differential functions this fluid would play in relation to sperm function and signalling to the female, with an ultimate focus on its role in modulating fertility.

Ejaculation: deposition of a heterogenous ejaculate in the female

As already mentioned, collection of a naturally fractionated ejaculate (as in humans, pigs or horses) into a single vial represents a non-physiological situation, since such bulk ejaculate,

where all fluids mix at a single time does not exist *in vivo*. During coitus, individuals from these exemplified species deliver spurts of fluid in a sequential manner and to a specific location in the female. In primates and some artiodactyla, sperm deposition is done deep in the vagina, in front of the cervical opening or in the vaginal fornix while in other species of ungulates, sperm deposition occurs intra cervically or even intrauterine². The first secretion (pre-ejaculate) presented to the urethra is that of the urethral and/or bulbourethral glands (Littré and Cowper for human, a secretion containing mainly mucin, sialic acid, galactose and salts in a slightly viscous, clearly aqueous fluid). This is followed by the emission of spermatozoa from the caudae epididymides to the urethra accompanied by secretion from the prostate, followed by ejaculation proper (e.g. expulsion of semen into the female) in a series of spurts. The initial spurts are usually called the sperm-rich fraction of the ejaculate, since most spermatozoa are present there¹⁶, with a blend of the acidic cauda epididymides and ampullar fluids together with the slightly acidic citrate and zinc-rich prostate fluid, which also contains specific peptides and proteins (as acid phosphatase and PSA in humans). In the following spurts, there is a gradual dominance of secretion from the seminal vesicles (rich in fructose, peptides, proteins, prostaglandins (PGs) etc, which is clearly basic in nature)^{2,17} as well as gradual diminution of sperm numbers⁶. Presence of PGs or hormones such as estrogens in the post sperm-rich fractions (vesicular-derived) is well documented, with large species variation². Some species (for instance boars and stallions) have a noticeable gel-rich secretion from the bulbourethral glands which can virtually coagulate the entire ejaculate if placed together, thus this component is deliberately removed during semen collection. *In vivo*, this gelifying fraction enters the cervical canal in these species by the end of ejaculation, a process also seen in other species¹⁸. In humans, at or immediately after ejaculation, a sample of semen collected in a single vial coagulates to form a gelatinous mass that immobilizes the spermatozoa. If an ejaculate is collected using a split procedure (i.e. several vessels for collection of different fractions), as it presumably occur *in vivo*, the first spurts (prostate-dominated) do not coagulate, while the last ones (vesicular-dominated) do¹⁹. Such coagulum is rapidly (*in vivo*, within minutes) or more lengthy (15-30 min *in vitro*) liquefied by prostatic-derived proteolytic enzymes²⁰. Interestingly, most human spermatozoa are, as described, present in the first (non-coagulating) fractions, so a certain proportion of them can well rapidly enter the cervical canal, as extrapolated from studies which recorded sperm present in the Fallopian tubes as early as few minutes after coitus²¹, transport sustained by the myometrial and myosalpingeal contractions that characterize this period. Such phenomena seem clearly conserved among mammals²², suggesting that there might be a numerically

restricted cohort of vanguard spermatozoa that can be relevant in establishing a sperm reservoir either in the cervical crypts and/or in the Fallopian tubes to warrant eventual fertilization²³⁻²⁵. The other spermatozoa, including those trapped in a coagulum might well still be fertilizing, but time might play against them, since most spermatozoa are, together with the liquefied semen coagulum, flowbacked from the site of deposition via vagina, within minutes, *in vivo*²⁶. Those spermatozoa not included in the female sperm reservoirs but yet having ascended to the uterus are considered foreign and thus phagocytosed by invading leukocytes, mostly in the form of polymorph-nuclear neutrophil granulocytes (PMNs)²⁷.

Proteomics of the ejaculate

Proteomic studies of spermatozoa are limited. This situation is due to difficulties in separating spermatozoa from the round cells that might follow preparation of samples for analyses, something that can be easily solved by use of density separation or swim-up preparation techniques²⁸. Spermatozoa are, by being so highly differentiated, advantageous cells to study proteomics of specific compartments such as the membrane, which basically is the area of major importance for its role in interacting with the surroundings and the oocyte. Comprehensive sperm protein databases had been established since the late 1990's²⁹ with above 1,000 spots listed; a number that had increased over time¹⁵. Proteins identified thus far cover the expected spectrum of function (from energy-production to cell-recognition), but few, too few, are accurately linked to (in)fertility; thus calling for other methods of isolation of the membranes so that specific areas, relevant for capacitation or binding (either to the ZP or the oolema) are more closely examined¹⁴.

As for the SP, availability of seminal material has not been an issue since volumes are sufficient for analyses for either human or animal studies. Moreover, sampling methods can be refined for examination of other portions than the bulk ejaculate, such as specific fractions or even specific accessory glands (for instance after massage expression of prostate, seminal vesicles, etc). Neither does the protein content matter, since proteins are a major component, throughout species. Major SP-proteins belong to one of three main groups: proteins carrying fibronectin type II (Fn-2) modules, spermadhesins or cysteine-rich secretory proteins (CRISPs)³⁰. However, differences in type and source of proteins are present among species, owing to the already named differences in glands and/or the sequence they are emptied; or the

type of ejaculate they have. In most species, proteins are mainly of vesicular gland origin, and in ungulate mammals (boar, stallion, bull, buck) most proteins are Fn2 and/or spermadhesins³⁰⁻³¹. Spermadhesins have been most thoroughly studied in pig SP, as a family built by three members; the Alanine-Glutamine-Asparagine proteins AQN (-1 and -3), the Alanine-Tryptophan-Asparagine proteins [AWNs] and the porcine seminal plasma proteins I and II [PSP-I and PSP-II]³².

Spermadhesins are multifunctional 12-16 kDa glycoproteins whose biological activities depend on their sequence, grade of glycosylation or aggregation state, as well as on their ability to bind heparin (AQN-1, AQN-3 and AWN, grouped as heparin-binding proteins [HBPs]) or not (PSPs), as they attach in varying degree, to the sperm plasma membrane, from the testis to the ejaculate. Collectively, they have been related to multiple effects on spermatozoa including membrane stabilization, capacitation, and interplay between sperm-oviductal lining or sperm-ZP. The HBPs seem to stabilize the plasma membrane over the acrosome prior to capacitation³³. Detection of AWN-epitopes on boar spermatozoa bound *in vivo* to the ZP strongly suggests the protein mediates sperm-ZP interaction³⁴. While HBPs do not seem to promote sperm survival, at least *in vitro*³⁵, the non-heparin-binding PSP-I and PSP-II^{36,37}, which account for >50% of all SP-proteins and forms a glycosylated heterodimer³⁸, which binds to the sperm surface and displays protective action on highly-extended and processed spermatozoa^{39,40}. The PSPs depict, moreover, clear immunostimulatory activities *in vitro* and *in vivo*, presumably in relation to specific cytokines^{6,7}.

Within a boar ejaculate, protein amounts increase 4-fold alongside the secretion of the vesicular glands, so that relative protein concentrations are low in the pre-sperm fraction and the first portion of the sperm-rich fraction (called sperm-peak portion), to increase (most HBPs and, particularly the PSPs) towards the end of the ejaculate (See **Figure 1**). In the sperm-peak portion (first-fraction), where most spermatozoa are present, other proteins, presumably of epididymal origin, such as Lipocalins and inhibitor of acrosin/trypsin are detected⁶. In other species, such as the stallion, protein amounts follow a similar disposition and main SP-proteins are equivalent; Fn-2, CRISPs and spermadhesins. These proteins, initially described as horse seminal protein (HSP)-1 to HSP-8, are mostly of low molecular weight (14-30 kDa) forming multi-protein aggregates, which -with the exception of HSP-4- attach to the sperm surface⁴¹. The two major proteins, the heparin-binding HSP-1 and HSP-2,

accounted for 70-80% of the total protein, and were considered modulators of capacitation. Both HSP-1 and HSP-2 (also called SP-1 and SP-2) are short Fn-2 type proteins, similar to the major bovine heparin-binding proteins (BSP), also associated with capacitation⁴². These Fn-2 type proteins bind to phosphatidylcholine or sphingomyelin phospholipids of the ejaculated sperm membrane, causing changes in the membrane structure^{43,44}. The HSP-3 (or equine CRISP-3) is associated to fertility⁴⁵ perhaps via its role as selective protector against PMN-cell binding⁴⁶. Examining fractions of the equine ejaculate, the first fractions contained acrosine inhibitor and prostate specific antigen (PSA), or kallikrein-like proteins (as HSP-6 and HSP-8 representing isoforms), yet with all HSPs being present in the rest of the fractions and HSP-1 being the major protein present in all ejaculate fractions⁴⁷. HSP-7 is the only member of the spermadhesin family, and like its porcine homologue AWN-1, shows ZP-binding activity⁴⁸.

Human SP is also a rich source of proteins and phosphatases, aminopeptidases, glycosidases, hyaluronidase, mucin etc have been detected more than 50 years ago¹⁵. Since then, more and more spots have been identified, and SP-proteins corresponding to the same parent protein appear in multiple spots and bands, implying that there is a clear multiplicity of isoforms present, independently of the SP-source (expressed prostate^{49,50}), or the bulk ejaculate⁵¹. Thousands of unique proteins have over time been identified, of which ~25% were secretory^{52,53}. The major accessory glands of men contribute differentially to the SP-protein pool. The major protein constituents of the seminal vesicle fluid are mainly semenogelin I but also semenogelin II, involved in the gelification of the latter spurts of the ejaculate (coagulum) and, following liquefaction, yielding products with clear biological functions such as inhibition of sperm motility, antibacterial activity etc alongside with other seminal vesicle proteins that include lactoferrin, fibronectin and protein C-inhibitor¹⁵. Unfortunately, seminal vesicle secretions are rarely examined alone, unless split ejaculates are used, in contrast to secretions from the human prostate, which are more commonly studied via gland expression *per rectum*. Prostate secretions, albeit only representing 20-30% of the total SP-volume, are in direct and immediate contact with the major numbers of spermatozoa, and are the first SP-portion to confront the cervical canal. The protein contents consists of three major proteins, all under hormone regulation: Prostate-Specific Antigen (PSA, Zinc-binder, Kallikrein family, mainly released in prostasomes but also produced by the Littre glands), prostatic acid phosphatase and the cysteine-rich prostate-specific protein-94 (PSP-94, β -inhibin- β -microseminoprotein)^{54,55}. PSA primary function is the liquefaction of the coagulum by

hydrolyzing semenogelins while prostatic acid phosphatase and the PSP-94 have enzymatic respectively growth factor action. As per the Cowper's gland (which is difficult to sample isolated) it contains an extremely abundant protein: mucin². As well, peptides are a major component of the SP albeit most of them are either fragment products of SP-proteins or sperm-associated peptide hormones¹⁵. Other enzymes are also present in the SP, such as glycosidases (β -glucuronidase (BG), α -glucosidase, β -glucosidase, α -galactosidase, β -galactosidase and β -N-acetylglucosaminidase (NAG), etc)². Lipocalin-type prostaglandin D2 synthase, an enzyme present in the stallion and boar SP, is of epididymal origin^{6,56}, and related to male fertility⁵⁷⁻⁵⁹. Other enzymes, such as lipases⁶⁰ or matrix metalloproteinases (MMPs) relate to semen quality^{61,62}.

In addition to enzymes, the SP of most species contains protein compounds similar to those present in blood plasma, such as pro-albumin, albumin, α -, β - and γ -globulins, transferrin, some immunoglobulins, complement factors and differential amounts of cytokines and chemokines⁶³⁻⁶⁶, as studied in thawed SP derived from individual or pooled whole ejaculates post-licquefaction. Whether these cytokines are related to inflammation in the male genital tract (i.e. prostatitis⁶⁷) or are in direct relation to presence and amounts of shed leukocytes⁶⁸⁻⁶⁹ remains to be fully studied. Besides, there are specific amounts of pro- and anti(or tolerance-related)-cytokines (see below)^{70,71}. Moreover, there are differences regarding their source, which calls for differential studies of ejaculate fractions. In that direction, we have studied SP of different categories of human samples grouped as (i) whole ejaculates (control) (ii) samples with low-zinc levels, eg vesicular vesicle-dominated samples, (iii) ejaculates from men with agenesis of the seminal vesicles, eg prostata-dominated secretion and, (iv) ejaculates post-vasectomy, eg without sperm-, testicular or epididymal fluid exposure, and detected a rather large number of cytokines and chemokines. The pro-inflammatory CXCL8 (ex-IL-8) and GRO (CXCL1/Th17); as well as the anti-inflammatory or tolerating monocyte-recruiting protein 1 (MCP-1/CCL2), IP-10 (CXCL10/Th1), M-CSF (M2 inducer) or the immune-deviating transforming growth factor- β (TGF β) isoforms (1-3) were among those in highest concentrations (0.5-300 ng/mL), thus being most reliably measurable. Both pro-inflammatory (TNF, IFN- γ , IL-6, IL-8, GM-CSF), and anti-inflammatory cytokines (TARC, M-CSF) were highest in vesicular-dominated fractions. Not surprising, leukocyte (PMN)-counts correlated with the relative levels of TNF, IL-6 and CXCL8 (ex-IL-8) but not with those of TGF β 1-3. Consequently, anti-inflammatory and tolerance-related cytokines (IL-10, LIF, M-CSF), but not of TGF β 1-3, dominated in samples with few leukocytes, being their relative concentration

lowest in leukocytic samples (>1 million/mL). These preliminary results suggest differences in cytokine/chemokine levels among fractions of the human ejaculate which might be related to specific signaling properties *in vivo*.

Functions of SP-proteins

The suggested functions of SP proteins include their involvement in several essential steps preceding fertilization, such as regulating capacitation, establishment of the oviductal sperm reservoir, modulation of the uterine immune response, and sperm transport in the female genital tract, as well as in gamete interaction and fusion⁴². Interestingly, individual proteins from the same family appear to function in a species-specific manner. Differences in their structure, relative abundance and patterns of expression appear to determine species-specific effects of homologous proteins³¹. SP-proteins differ somewhat in functionality related to their source, more clearly seen when fractionated ejaculates are examined.

Sperm transport and survival

Following mating or intercourse, mammalian spermatozoa are transported from the site of deposition towards the oviduct within minutes, owing to the concerted motility of the female tract muscle⁷². These spermatozoa bathe, in individuals with fractionated ejaculation, in different fluids, such as the epididymal cauda fluid and the accessory gland secretion that is verted at the time the corresponding spurt of ejaculation is issued. As mentioned before, the secretion of the first spurts of the sperm-rich fraction is acidic, and sperm proteins demonstrated to link themselves to acidic polysaccharides such as those in the secretion of the cervix, uterus and even oviduct⁸. On the other hand, binding of some SP-proteins, at least in the bull and stallion, inhibits such interaction of sperm proteins with acidic polysaccharides⁷³. SP-proteins affect differentially sperm survival post ejaculation, and those present in the the last ejaculate fractions (seminal vesicle origin) have a more pronounced negative effect, perhaps in relation to the extensive presence of several proteins. For instance, cleavage products of the human ejaculate coagulum (basically vesicular secretion) inhibit sperm motility (see above), which indicates those spermatozoa might be in disadvantage *in vivo*. The primary secretion in the first spurts, however, where spermatozoa are present, promotes longer sperm survival in humans¹⁶ and boars^{5,6}. Remarkably, both these species contains prostasomes, known by their ability to fuse with spermatozoa and provide a mechanism for

protein and lipid transfer⁵⁵, thus enhancing sperm motility and stabilizing the sperm membrane. Comparative studies in pigs have shown porcine SP-proteins influences sperm physiology⁵. As such, HBPs play a major role during sperm transport³³ while the heterodimer PSP-I/PSP-II maintains fertilising capacity^{40,74}. This beneficial effect depends on the PSP-II moiety, active at doses as low as 0.75 mg/mL³⁹, concentrations present in the first spurts of the boar ejaculate.

Protein interaction with the female genital tract and the oocyte

As listed above, porcine HBPs spermadhesins coat the sperm membrane during ejaculation, producing structural changes to the sperm plasma membrane in relation to capacitation, ZP recognition and fertilization. AWN follows, for instance, the spermatozoa up to the ZP³⁴, perhaps due to their role in inhibiting sperm capacitation^{75,76}, an effect that is lost when this protein is removed from the sperm surface³³. At the same time, such initial layer of proteins might provide an anchor for aggregated spermadhesins to further coat the sperm surface⁷⁷, further stabilizing the plasmalemma and preventing premature acrosomal exocytosis. The heparin binding AQN-3, the most prominent ZP-binding protein in boar spermatozoa remains -for instance- attached to the sperm surface after capacitation (*in vitro*) and can only be recovered from the aggregating raft area of the apical ridge of the sperm head^{78,79}. The non-heparin binding protein PSP-I prevents premature capacitation and acrosome exocytosis (see above)⁸⁰. Whether these proteins are also involved in the interaction between spermatozoa and oviductal epithelium during sperm capacitation remains to be explored.

Increasing evidence exist that SP-proteins are able to interact with the vaginal, cervical and, particularly, the uterine epithelium to elicit a series of changes in the immune responsiveness of the female, apparently modulated by pro- and anti-inflammatory SP-proteins⁸¹. This is not surprising, since the ejaculate (spermatozoa and SP) is to be considered foreign by the female and thus prompt to rejection. Deposition of semen into the vagina or the uterine cavity elicits a massive invasion of PMNs toward the lumen, followed by formation of neutrophil extracellular traps (NETs) and sperm phagocytosis. Although PMN presence and infiltration are estrogen-dependent⁸², PMN migration to the surface epithelium and lumen can be elicited by SP-glycoproteins (spermadhesins⁷) and pro-inflammatory soluble cytokines⁸³, see **Figure 2**. This primary inflammatory reaction cleanses the intra-uterine lumen from foreign cells, proteins and eventual pathogens, in preparation for the descending embryo. On the other

hand, it does not occur in the oviduct, where spermatozoa find a haven until fertilization^{8,9,27}. Induction of PMN invasion is, evidently, not the only effect of the SP on the female.

The SP also mitigates the female immune responses to paternal antigen-bearing spermatozoa or early embryos in the oviduct (immuno-privileged area) or in the uterus (developing embryos/fetuses and their placentae), by eliciting a transient state of peripheral immune tolerance^{82,84,85}. The SP of boars and humans contains immune-regulatory molecules, including high concentrations of the potent immune-deviating TGF- β (particularly TGF- β 1, but also TGF β 2- and 3 isoforms) a member of the multifunctional cytokine TGF family^{86,87}. TGF β 1-concentrations are higher than in other body fluids, as blood plasma or breast milk, and similar to colostrum levels⁸⁸, reaching 120-150 ng/mL in boar semen⁸⁷ or even higher levels in human bulk ejaculates (~150-200 ng/mL) most of it being the latent (inactive) form and solely 1-2 ng/mL being the short-lived active form^{65,89}. The origin of the human TGF- β 1 latent form is yet discussed, while TGF- β 3 is apparently synthesized by the prostate as levels are highest in semen from men with agenesis of the seminal vesicles and lowest in samples where the seminal vesicle secretion dominates (Rodriguez-Martinez et al. unpublished). The latent forms can be converted to its active form under acidic conditions (as in the vagina) or by SP-acid enzymes upon ejaculation, and be then more firmly attached to the sperm post-acrosomal membrane^{87,90}. TGF- β seems to induce the differentiation and expansion of the bank of regulatory T (Treg) cells, a 5-10% sub-population of suppressor CD4⁺ T-cells, to reach a state of adaptive functional immune maternal tolerance to male antigens^{84,91-92}.

Males differ in their SP-contents of TGF- β , without straight relation to fertility^{86,89}. However, a female could express different levels of endogenous cytokines depending on the exposure to SP from different males, which might thus relate to the often-observed differences in embryo survival among sires (e.g. innate fertility), a real long-lasting effect of the SP on the female^{12,93}. Whether such mechanism is valid also for humans remains to be fully elucidated, but clinical evidence exists that fertility after ART is enhanced by accompanying unprotected intercourse or vaginal exposure to homologous SP¹². Interesting is the circumstantial evidence that the latent form of TGF- β -2 (as for TGF- β 1) could also have a preferential production by the epithelium of the prostate⁹⁴. Whether both are activated by PSA in relation to differences among males (or females) is yet to be tested.

Are SP-proteins related to fertility?

SP-proteomes have been assessed in relation to reproductive outcomes (either fertility levels or (in)fertility, in several species of mammals, particularly domestic animals but also human. SP-proteins have been identified as associated with high, respectively low fertility in bulls⁹⁵, isolated as osteopontin (OPN) and Lipocalin-Type Prostaglandin D synthase^{96,97}. The latter has been always present in the sperm-rich spurts of ejaculates in species (including humans) with fractionated ejaculation. OPN has been related to fertility in pigs (*in vitro* fertilization^{98,99}) and stallions¹⁰⁰. Three proteins (SP-2, SP-3 and SP-4) were found in higher concentrations in stallions with low fertility scores, while SP-1 was positively correlated with fertility and was suggested to be homologous to OPN⁹⁵. The spermadhesin PSP-I, common in pigs, seems negatively related to fertility⁵⁸ while other molecules, such as TGFβ, appear unrelated to overall fertility in relation to levels in semen⁸⁹. However, since the SP of a boar differs somehow from that of another boar, maybe it is not the amount of the cytokine that play the major role, but its capacity to differentially induce degrees of maternal tolerance by the female and thus attain differences in embryo survival, leading to variation in fertility. It is hoped that this line of research is followed.

Conclusions

Proteins of the seminal plasma are relevant for sperm function particularly for their interactions with the various environments of the tubular genital tract and the oocyte and its vestments. Moreover, specific peptides and proteins act as signals for the immune system of the female, ultimately modulating sperm rejection or tolerance, perhaps even influencing the relative intrinsic fertility of the male and/or couple.

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Figure texts:

Figure 1: Protein composition of the seminal plasma of the sperm-peak portion (FI, upper panels) and the rest of the ejaculate (FII, lower panels) in representative boars. Protein profiles were characterized by reverse-phase HPLC separation (left panels), SDS-PAGE (insert in lower left panel), and 2D-electrophoresis (right panels). Chromatographic peaks and electrophoretic protein spots were characterized by N-terminal sequencing and proteomic analysis, including MALDI-TOF tryptic peptide mass fingerprinting followed by collision-induced dissociation tandem mass spectrometric analysis of selected peptidic ions. Identified proteins are highlighted in the panels.

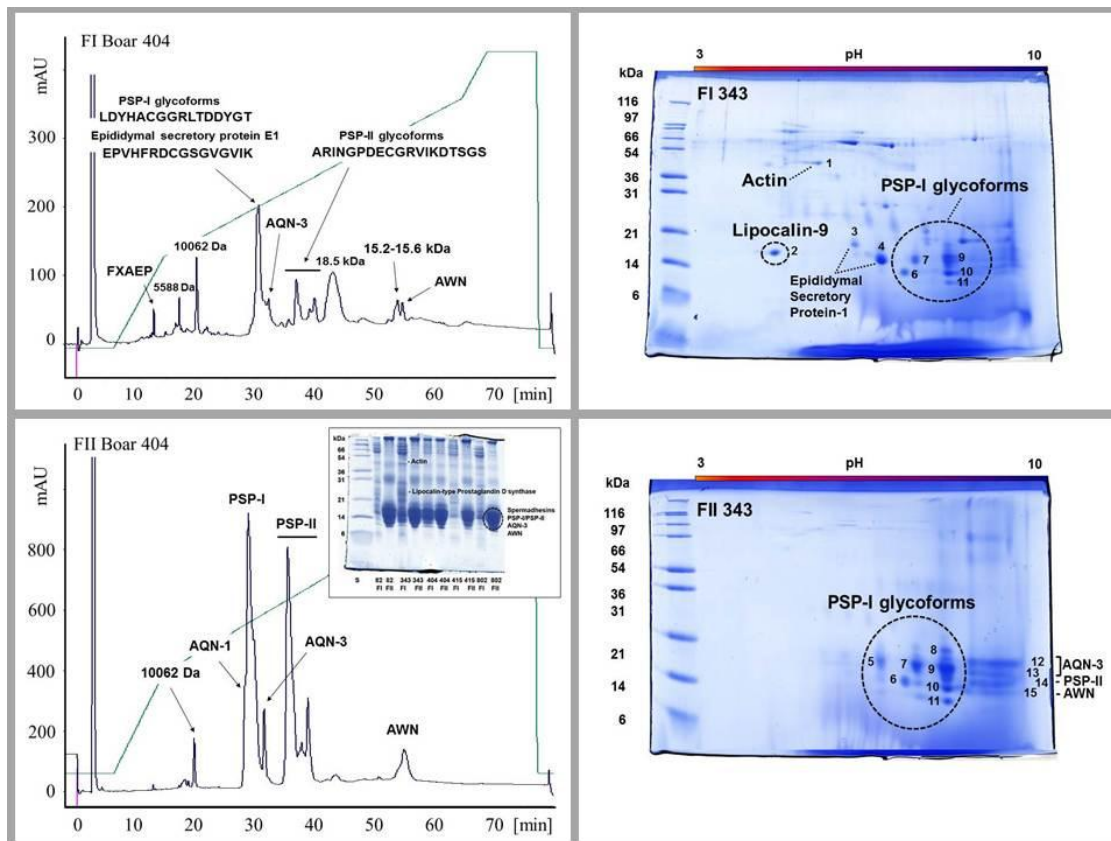


Figure 2: The emerging view is that the balance between cytokines released by macrophages ($\text{TNF}\alpha$) and mast cells (IL-4) controls the onset of neutrophil (PMN) migration in response to the inflammatory stimuli of PSP-I/PSP-II, thereby avoiding excessive PMN recruitment. This regulation seems crucial during situations where an excessive accumulation of PMNs would lead to tissue damage (i.e., in postmating endometritis)^{101,102}.

