On a matter of seminal importance:

The emerging influence of seminal plasma components on fertility and future progeny

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Abstract

Egg and sperm have, understandably, been the "stars" of mammalian fertilization biology, particularly because artificial reproductive technologies allow for fertilization to occur outside of the female reproductive tract without other apparent contributions from either sex. Yet, recent research, including an exciting new paper, reveals unexpected and important contributions of seminal plasma to fertility. For example, seminal plasma proteins play critical roles in modulating female reproductive physiology, and a new study in mice demonstrates that effects of some of these proteins on the female can even affect the health of her progeny. Furthermore, although several actions of seminal plasma have been conserved across taxa, male accessory glands and their products are diverse — even among mammals. Taken together, these studies suggest that the actions of seminal plasma components are important to understand, and also to consider in future development of assisted reproductive technologies for humans, farm species, and endangered species of mammals.

Keywords

accessory sex glands; insect fertility; mammalian fertility; offspring health; reproduction; reproductive tract physiology; seminal proteins

Introduction

Years ago, one of us underwent an assisted reproductive technology procedure: intrauterine insemination with washed sperm. After the procedure, the physician told the patient and her spouse that they should have intercourse that evening because "that way you'll never know if your baby was conceived through this medical procedure or by more traditional means". The couple followed the physician's advice, and in the fullness of time a perfect and healthy baby was born.

The couple assumed that the physician's advice was given so as to help them avoid associating their child's conception with a clinical procedure. But in fact, that advice was

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scientifically prescient. Subsequent physiological, molecular, genetic, and 'omic studies, performed on a wide variety of species, have revealed that seminal plasma components play important roles in fertility beyond simply being a passive delivery vehicle or nutritive medium for sperm (reviewed in [1–4]) – yet in human intrauterine insemination, most seminal components would have been removed from the sperm by the washing step. Moreover, a new study in mice has now shown that seminal plasma contains components essential for embryo development and the health of male offspring [5]. These findings prompt us to suggest that the field think again about the roles of seminal proteins in fertility – including investigating whether addition or inclusion of some seminal proteins in assisted reproduction procedures (or even a post-procedure 'roll in the hay' [http://www.urbandictionary.com]) might be helpful in improving the <40% current success rates for assisted reproductive technologies (Society for Assisted Reproduction 2012 Report).

The development of assisted reproductive technologies (ART), such as intrauterine insemination and in vitro fertilization (IVF), has focused on the use of sperm that were separated from seminal plasma. This is usually done by using density gradient centrifugation of semen. Because such "washed" sperm produced pregnancies in these ART procedures, seminal plasma was considered by many to be unnecessary for fertilization. In fact, if anything, seminal plasma was thought to exert negative effects [6]. Seminal plasma does contain factors that inhibit the ability of sperm to undergo a process called "capacitation", which sperm must complete in order to be able to fertilize eggs in vitro and in vivo [7–9]. Other seminal plasma factors may inhibit sperm motility, such as platelet activating factor (PAFah), which is found in human seminal plasma [10]. Finally, human seminal plasma contains prostaglandins [11–13], and such molecules (e.g. PGE2) have been shown to stimulate contraction of nonpregnant human myometrium (the muscle layer of uterus) [14]. Thus there was concern that inclusion of seminal plasma could be deleterious to retention of sperm or embryos in the uterus after ART procedures; and, apparently, seminal plasma was not needed for successful ART. Consequently, interest in research on the functions of seminal plasma waned, particularly with regard to applications in reproductive medicine. Since the first success of human IVF over 30 years ago, fertilization research became focused on the biology of sperm and eggs.

Nevertheless, for decades it has been known that in many insect species, seminal proteins and other seminal components cause important effects on mated females that can enhance fertility (reviewed in [1, 4]). For example, mating initiates or increases egg production in insects. Furthermore, in some cases, the increased egg production continues for a long period of time and the females utilize sperm efficiently for fertilization by storing part of the inseminate and gradually releasing sperm to fertilize their eggs. Behaviors of female insects also often change after mating: mated females can become unreceptive (or less receptive) to re-mating. In some species mated females stop the pheromone production and "calling" behaviors that attract males, or in some cases their feeding and locomotory behaviors change after mating. Early experiments had shown that implantation of male reproductive secretory glands (or injection of extracts of those glands) into females could induce such responses in the absence of mating (e.g. [15, 16]). Subsequent genetic experiments in Drosophila indicated that seminal proteins produced by the aforementioned glands are responsible for such changes. Over the years, seminal proteins were identified in numerous insects, and in

some cases individual proteins were identified as the agents that cause specific post-mating changes in females. For example, genetic studies have shown that sex peptide, one of the more than 200 proteins detected in *D. melanogaster* semen, induces egg production, decreases re-mating receptivity [17–19], and increases feeding [20]. Other proteins increase ovulation rate by inducing neuromodulatory machinery in the female (the seminal protein "ovulin" [21, 22]), or promote the storage of sperm by inducing uterine contractions (the seminal protein "Acp36DE"; [23, 24]). In *Anopheles gambiae* mosquitoes, a transglutaminase that is transferred in the seminal fluid was shown to be essential for storage of sperm by females [25], and prostaglandin-synthetic machinery transferred in seminal fluid stimulates egg release in mated female crickets [26]. Even now, insect seminal proteins are being studied as a model for the action of seminal proteins in all animals (including the mammals described below), and also as potential agents for use in controlling the reproduction of insect vectors of disease [25, 27–29].

Seminal plasma proteins contribute to fertility

At first blush, it may have seemed a leap to suggest that studies of insect seminal proteins can inform our understanding of yet undescribed roles for human seminal fluids. Yet, about 15 years after insect studies indicated important roles for seminal proteins in fertility [15, 16], studies in mammals also detected positive effects of seminal plasma on reproduction [30]; a recent study even showed that one mammalian seminal plasma protein that had previously been documented to have negative effects as a decapacitation factor in vitro [31] actually exerts strong positive effects on fertility *in vivo*: it is required for sperm to survive in the uterus [32]. In another parallel to insect seminal fluid, mammalian seminal plasma is a highly complex biological fluid containing proteins, amino acids, enzymes, carbohydrates, lipids, and other trace elements, including mRNA and microRNAs (miRNAs) (reviewed in [33]). The identities and functions of mammalian seminal plasma molecules that support sperm transport, survival, and fertilization have recently been reviewed elsewhere [33]. While large scale proteomic studies have identified upwards of 2,000 seminal plasma proteins in humans [34–36], the identities of seminal plasma proteins in other mammals have not been comprehensively revealed [2, 37–39] and the functions of the majority of these proteins are poorly understood. In mammals, seminal fluids are derived from the epididymis and male accessory organs, including the seminal vesicles, prostate, and bulbourethral glands (Fig. 1). These tissues produce unique, non-cellular components of the seminal fluid that are transferred to the female sequentially during ejaculation, with the transfer of sperm. The variety of seminal plasma components – particularly the large numbers of seminal plasma proteins – suggest that seminal plasma plays greater roles in the processes of fertilization that occur naturally in the female, than simply to nourish and protect sperm.

Seminal fluid components and male reproductive physiology evolve fast and vary greatly across species

Interestingly, and similar to insects, there is tremendous variation across mammalian species with respect to the major proteins present in the seminal plasma as well as with respect to the anatomy (Fig.1) and secretory activities of the organs of the male reproductive tract [2].

For example, in mice, the seminal vesicles are by far the largest accessory sex gland; however, the most prominent accessory sex glands in boars are the bulbourethral glands. Furthermore, dogs and cats lack seminal vesicles altogether. In another example, the homologs to BSP proteins produced by the seminal vesicles in bulls (described below) are produced by the epididymis in mice and humans [40]. The amount of seminal plasma transferred and its fate within the female reproductive tract also vary considerably across species. For example, although bull semen contains high concentrations of sperm, and those sperm are accompanied by very small volumes of seminal plasma, stallion and boar ejaculates contain large volumes of seminal plasma. Human and cattle ejaculates are deposited into the vagina, but in horses and pigs, all semen is ejaculated directly into the uterus. Further, many genes encoding seminal proteins display signatures of an extremely high rate of adaptive evolution (e.g. [27, 29, 41–48]), suggesting that sexual selection is driving the rapid evolution of seminal fluid proteomes in insects and mammals. Why would sexual selection cause such rapid evolution of seminal plasma components if the components served only minor functions in nourishing and protecting sperm? As discussed below, recent research has revealed a variety of effects of mammalian seminal plasma, including regulating interaction of sperm with the female tract and stimulating physiological changes in the female.

Mammalian seminal plasma proteins have diverse effects on the mated female

As recent findings indicate, mammalian seminal plasma molecules, like those of insect seminal plasma, mediate a variety of processes within the mated female. Several lines of recent research have demonstrated roles of seminal plasma in mediating additional post-mating responses in the female, embryo development, and offspring health. We highlight a few of these roles below.

Seminal plasma proteins mediate interactions between sperm and the female reproductive tract

This function of seminal plasma proteins has been conserved from insects to mammals. BSP proteins, the major proteins produced by the seminal vesicles of bulls, coat bull sperm and mediate binding between sperm and the epithelial lining of the oviduct (fallopian tube). Binding of sperm to the oviductal epithelium forms a storage reservoir, which serves to maintain fertility of sperm until ovulation (reviewed in [49]). Sperm storage reservoirs have now been identified in a broad range of mammals [49, 50].

Seminal fluid modulates the immune response in mammals

This phenomenon was identified long ago in mammals as well as in insects. In mammals, inflammatory cells are drawn to the site of ejaculate deposition within the female reproductive tract (reviewed in [1, 51]). The inflammatory response in mammals may aid in clearing the reproductive tract of microorganisms or in remodeling the reproductive tract tissue after fertilization in preparation for implantation. For example, in humans and other primates, β-defensin 126, a glycosylated polypeptide produced in the epididymis and adsorbed onto sperm, facilitates sperm penetration of cervical mucus and protects sperm

from immune surveillance in the female tract [52]. In a population cohort of newly married Chinese couples, wives of men who were homozygous for a deletion variant of the β-defensin 126 gene were only 60% as likely as other wives to become pregnant during the time period of the study [53], suggesting that the action of ß-defensin is important for full fertility.

Seminal plasma proteins influence post-fertilization reproductive fitness

Although it has long been known that seminal plasma proteins are the primary components of copulatory plugs that form at the vaginal opening in a variety of taxa, a recent study suggests that the copulatory plug also affects processes after fertilization, within the female, such as implantation or some aspects of gestation. Mouse seminal vesicles (and the human prostate gland) produce a transglutaminase found in the seminal fluid. Loss of this transglutaminase (Tgm4) in mice prevents copulatory plug formation, and reduces male fertility. Interestingly, more than fertilization *per se* is impaired in these matings; although sperm from Tgm4- males could fertilize eggs, females were less likely to produce progeny after mating with these males than after mating with normal males [54].

In addition, recent studies in Drosophila show that seminal proteins can also trigger neuroendocrine changes in females that can induce important reproductive tract processes such as ovulation [22, 55]. Effects of seminal proteins on neuroendocrine or endocrine modulators are also seen in mammals. For example, some mammals, including rabbits, cats, voles, Sumatran rhinos, camels, and llamas, are categorized as induced ovulators, because their ovulation is triggered by mating. Only eight years ago, a comprehensive 44-page review on mechanisms of induced ovulation devoted only a single paragraph to the possibility that seminal plasma plays a role in inducing ovulation in these species [56]. Yet, just two years ago, a nerve growth factor (ß-NGF) found in camelid seminal plasma was identified as primarily responsible for inducing ovulation by stimulating pituitary secretion of luteinizing hormone (LH) [57]. Other species of mammals, such as cattle, pigs and humans, are categorized as spontaneous ovulators, because ovulation occurs in the absence of coitus. Nevertheless, although ovulation does not depend on mating in these species, seminal plasma has been shown to advance the time of ovulation in at least one of them [58, 59].

Seminal plasma has effects on embryos and offspring

Perhaps even more astonishing than the notion that components of the seminal fluid can modify the female's neuroendocrine system, a study published earlier this year has shown that seminal fluid can also affect fetal development and the health of offspring long after sperm meets egg [5]. Bromfield et al. surgically removed the seminal vesicles, the major source of seminal plasma components in mice, and allowed these males to mate with females. Only approximately 35% of females who did not receive seminal vesicle secretions from their mates were able to conceive (compared with 85% of control females who received seminal vesicle secretions). Furthermore, the experimental females who were able to conceive had enlarged placentas and their embryos exhibited developmental delays. Most striking, however, was the observation that male offspring of the females who did not receive seminal vesicle secretions from their mates exhibited symptoms of metabolic

syndrome, including obesity and high blood pressure. Bromfield et al. further demonstrated that the metabolic phenotypes of offspring correlated with decreased levels of several oviductal cytokines that are beneficial to embryonic development and with decreased mRNA levels of a cytokine, TRAIL, which is harmful to development. Until now, the roles of seminal plasma proteins were viewed as "accessory": improving the efficiency or perhaps the likelihood of fertilization. But now, Bromfield et al. have shown that seminal-vesicle derived molecules in seminal plasma play an additional critical role in mice: they are needed to modify the biological processes of the female reproductive tract to support the development of the embryo, potentially including stimulation of epigenetic changes that lead to healthy offspring. It should be noted that, in mice, the seminal vesicles are the chief contributors to the seminal plasma; however, the epididymis also contributes a numbers of proteins and other factors. Furthermore, as discussed above, the size, activity, and specific secretions of the epididymis and the various accessory glands vary considerably among mammalian species, so there may be many more effects of seminal plasma waiting to be discovered (Fig. 1).

Conclusions and outlook

Since the development of artificial uterine insemination and IVF for treating human infertility, interest in potential roles of seminal plasma waned. [30, 60] However, recent studies compiled from a variety of disciplines including insect physiology, mammalian reproductive physiology, genetics and genomics, and even evolutionary biology, have begun to paint a more comprehensive picture, which is that seminal plasma is much more than a mere medium for sperm delivery. Instead, seminal plasma acts collectively with the female reproductive tract to create a post-copulatory environment that not only ensures fertilization, but also contributes to modifications that ensure embryo implantation and development, and even to the health of the offspring.

As research increasingly uncovers the identities and reproductive functions of individual seminal plasma molecules, it will be important to think again about the important contribution of seminal plasma to reproduction. Given recent results, we believe that it is time to adopt a broader approach to determine the full extent by which seminal fluid proteins affect fertility and to investigate their roles in directly modulating the physiology of the female, of the embryo, and even that of her offspring. Importantly, while assisted reproductive technologies have slowly been improving over the years across all age groups, implantation rates and overall success rates are still below 40% (Society for Assisted Reproduction 2012 Report). Although a variety of factors contribute to unsuccessful implantations, the absence of seminal plasma in these technologies may, in fact, be the largest missing piece of the puzzle.

Acknowledgements

Abbreviations

ART   assisted reproductive technologies

IVF   in vitro fertilization

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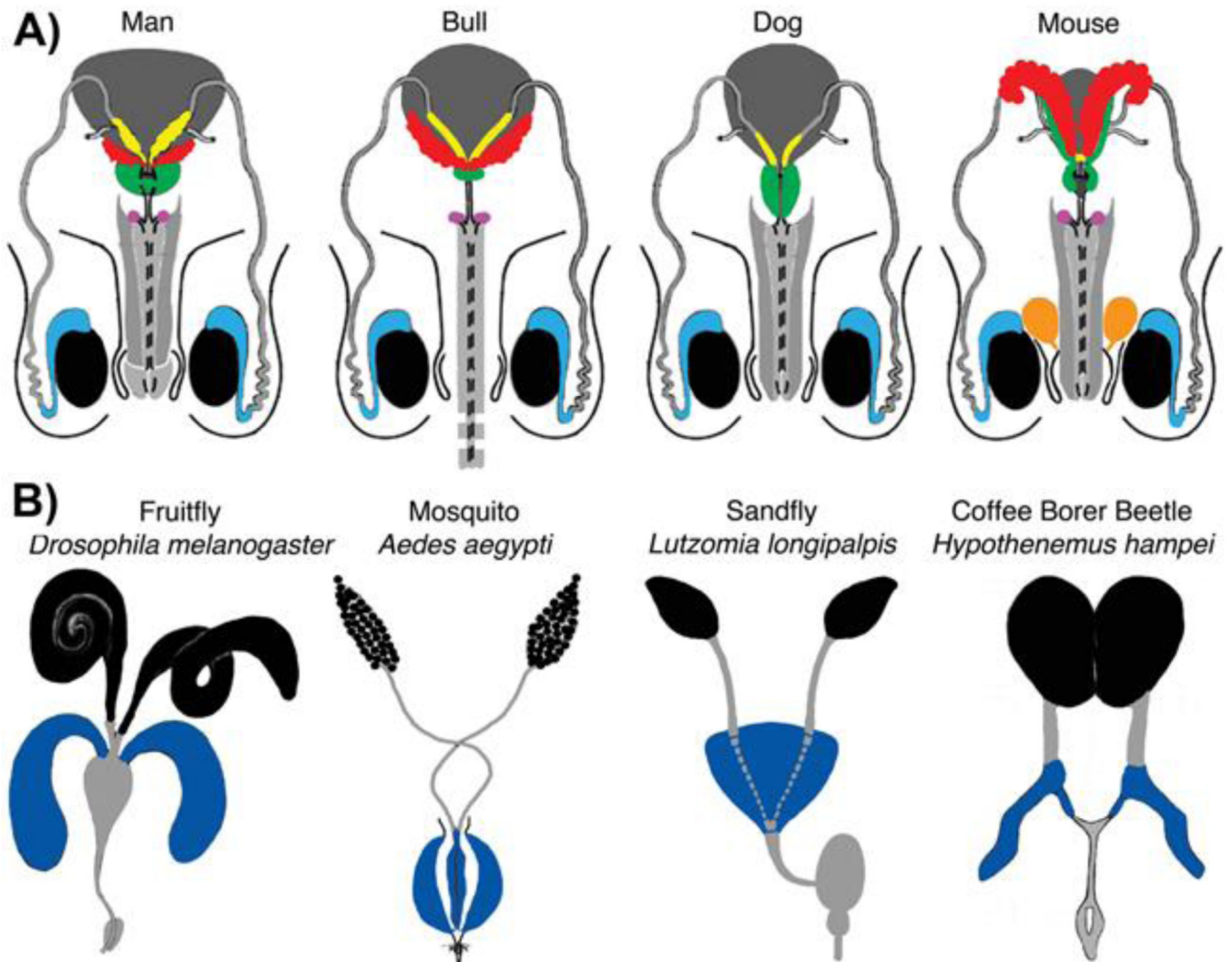
On a matter of seminal importance

Figure 1.

A: A diagrammatic frontal view the male reproductive tract of man and a dorsal view of three additional mammals, illustrating the variations in glands that contribute to the seminal plasma (yellow, ampullary glands; red, seminal vesicles; green, prostate glands; magenta, bulbourethral glands; orange, preputial gland). The epididymis (blue) also contributes to the seminal plasma. The testes are shown in black, the bladder in dark gray, and the vas deferens and penis in lighter shades of gray.

B: Diagrammatic view of the male reproductive tract of four insect species (modified from images in [61–64]). Unlike the situation in mammals, the accessory glands of the distantly-related insects are not homologous to the same extent as are the glands in mammals, so they are all indicated in dark blue (black, testes; gray, ducts and intromittent organ).