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= Mini Review =

Critical roles of seminal plasma on sperm migration in the female reproductive tract

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ABSTRACT

In mammals, ejaculated sperm have to travel a long distance through the female reproductive tract, from the vagina to the oviduct. A sperm's journey often exceeds the sperm's length by 1000-fold, and includes varied hurdles. However, our understanding of the state of the female reproductive tract after copulation has been highly limited due to its complexities. Gene-engineered animal are at last being used to reveal the *in vivo* mechanisms that sperm require the help by the seminal plasma, including a huge number of factors. One of them is assumed to function as a sperm guardian against the enemy hidden inside the female reproductive tract, efficiently achieving sperm guidance to oocytes. This review focuses on the regulatory mechanisms of the seminal plasma, so as to provide more insights into sperm's journey through the female reproductive tract.

Key words: seminal plasma, sperm migration, sperm selection, female reproductive tract

INTRODUCTION

In mammals, the female reproductive tract is a complex organ system that requires proper function and coordination in order to reproduce life within a body. The female reproductive tract is divided into three main parts: the vagina, the uterus, and the oviduct [49]. These organs have distinctive roles in reproduction: the vagina as the passage between outside of the body and the uterus, the uterus as the site of the development of the fetus, and the oviduct as the place of fertilization. Although internal fertilization is an effective and reasonable tool for achieving high fecundity, transmission of pathogens from semen during sexual behavior between unspecified partners is a critical problem for female health. On the other hand, the maternal immune system is able to strictly exclude xenoantigens to protect the mother and her fetus, while this system allows allogeneic sperm and semiallogeneic fetuses for the purpose of reproduction, leading to biological diversity [55]. The incidence of these adverse events suggests that the balance between exclusion and tolerance in the female reproductive tract is important for successful reproduction via internal fertilization.

Successful internal fertilization of a female requires only two components from a male: sperm and seminal plasma. The seminal plasma consists of secretions from several accessory sex glands (the prostate, seminal vesicles, epididymis, and bulbourethral glands), which show nutritive and protective effects for ejaculated sperm [32,60]. On the other hand, it has been reported that

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seminal plasma from rabbits contains factor(s) bringing great detriment to sperm fertility *in vitro* [7] a phenomenon known as sperm "decapacitation" [4]. The seminal plasma of other species decreased the fertility of rabbit sperm in the same way as rabbit seminal plasma [7], but the decapacitation factor(s) conserved among species have remained obscure. Moreover, this inhibitory effect of seminal plasma *in vitro* is not observed within female reproductive tracts. Even today, the physiological importance of seminal plasma in the female reproductive tract remains unclear.

The time periods of sperm's journey from ejaculation to arrival at the oviduct have been reported in various species [20,35,51]. Because of its occurrence inside of female bodies, it is difficult to accurately determine the period of this journey within the female reproductive tract. Overstreet and Cooper (1978) reported that rabbit sperm were found at the ampulla and fimbria of oviducts within 1–15 minutes after copulation, yet almost all of the sperm were dead; motile sperm that had migrated from the uterus into the oviduct were first detected at 90 minutes after copulation [42]. This famous article implies that rabbit sperm is physically able to reach the oviduct within 15 minutes, but another 75 minutes is needed for vigorous sperm capable of fertilization to arrive at the oviduct; raising the possibility that the sperm are selected by unknown processes in the female reproductive tract. In this review, we discuss the regulatory mechanisms of sperm migration, survival, and selection within the female reproductive tract.

PENETRATION OF EJACULATED SPERM INTO FEMALE REPRODUCTIVE TRACT

At copulation, human semen ejaculated into the vagina is spontaneously coagulated into a semisolid gelatinous mass, which then liquefies within 5–20 minutes [1,30]. The ejaculated sperm is first immobilized by semen coagulum, and then the liquefaction of the semen leads to the ejaculated sperm swimming toward the uterine cervix [44]. In rodents, the ejaculated semen separates in two phases: the solid phase, which forms the copulatory plug that fills the vagina, and the liquid phase, within which enriched sperm are deposited directly into the uterus [28]. These semen coagulations, found commonly in primates and rodents, are caused by a conserved system including transglutaminase IV (Tgm4) secreted from the prostate (the coagulating gland in rodents) [11], and its substrates, identified as the seminal vesicle proteins semenogelin I/II (SemgI/II) in primates and its mouse homolog seminal vesicle secretion 2 (SVS2) [33,43,44,57]. It is well known that *SEMG1/2* and *Svs2* genes are expected to evolve rapidly in their accumulation of amino acid replacement [25,33]. The molecular evolution of SemgII in primates correlates to the hardness of semen coagulum, and to the degree of female promiscuity [14]. This evidence proposes the possibility that the seminal protein adapts to the copulating style of the species in order to maximize fecundity. Recently, it was reported that the lack of Tgm4 or SVS2 causes a failure of copulatory plug formation, a reduction of sperm number in the uterus, and subfertility in mice [11,26]. This evidence indicates that semen coagulation is important for maintaining sperm number in female mice. That mouse seminal vesicle protein SVS2 is homologous with SemgI/II in primates [33] raises the possibility that Semg I/II also regulate the maintenance of sperm number in female primates. Although the precise biological function of coagulum in primates is still unclear, there is a high possibility that physical properties of semen adapt to the copulating style and morphology of the female reproductive tract in order to prevent from backflow of the semen.

SPERM TRANSPORT BY UTERINE PERISTALSIS

The famous inducers of uterine contraction in labor and delivery are two representative hormone groups, prostaglandins and oxytocin, which are dramatically increased and function in the uterine decidua during the late pregnancy term [16,22,41,56]. For effective parturition, the number of oxytocin receptors is significantly increased about 100-fold in uterine smooth muscle cells [17]. One of these hormones, prostaglandin F2α, is secreted from human endometrial epithelial cells even in the menstrual cycle [46]. The receptor of prostaglandin F2α, prostaglandin F receptor (FP), is also predominantly expressed in the human endometrial epithelium, and more highly expressed during the proliferative stage of the menstrual cycle [5,36].

In some vaginal semen depositors, such as primates, cows, and ewes, it is reasonable to assume that uterine contraction helps the sperm migration from the uterine cervix to the oviduct because a large portion of the seminal

plasma flows away from the vaginal vault. It was reported that the uterine smooth muscle moves and contracts strongly during the estrus period in humans [29,34], cows and ewes [21]. In domesticated cats, the patterns of the uterine contraction during estrus involve both directions, ascending and descending, resulting in uterine contents that flow back and forth [8]. Sperm migration throughout the uterus is improved by prostaglandins in rabbits [48], suggesting that the uterine contraction during estrus is elicited by prostaglandins or oxytocin.

Prostaglandins were first discovered and isolated from human semen in the 1930s, and named for the discovery source, the prostate gland [15]. Today, it is well known that large amounts of prostaglandins in the seminal plasma are produced mainly in the seminal vesicle [12]. In uterine semen depositors, such as rodents, pigs, and horses, prostaglandins in the seminal plasma directly change the uterine contraction. In swine, the contractive movements begin to be observed in the corpus uterus after direct administration of prostaglandins [38]. In mated female rats, uterine contractions are weakened by a treatment of indomethacin, an inhibitor of the production of prostaglandins [9]. Besides uterine contractions, many studies focused on the phenotypes of mice lacking an enzyme involved in prostaglandin biosynthesis, cyclooxygenase-2 [13,58], because prostaglandins play myriad roles as local mediators of inflammation and as modulators of physiologic functions [39]. Shortage of prostaglandins is implicated to associate with female infertility by causing multiple failures in ovulation, fertilization, and implantation [31,58]. Although the lack of endogenous prostaglandins in the female reproductive tract has been demonstrated in many reports, there are very few studies on the phenotype of prostaglandins in the semen; except for the sentence that "*Ptgs2*-deficient male mice are fertile" [58]. Further work needs to be done to determine whether prostaglandins of the seminal plasma have a role in sperm transport throughout the uterus.

SPERM SELECTION IN THE UTERUS

In some vaginal semen depositors, such as primates and cows, the restriction of sperm entry and sperm selection, such as the rapid selection of sperm with high DNA integrity, largely occurs at the uterine cervix between the vagina and the uterine body [54]. The cervix opens only during the estrus period and produces mucus whose biological function is determined by its macromolecular architecture [49,61]. The cervical mucus consisting of glycoproteins is highly hydrated at estrus, which leads to low viscosity and allows sperm migration into the uterine body [37].

In the cynomolgus monkey, a single epididymis-derived protein, beta-defensin 126 (DEFB126), coats the sperm surface during epididymal maturation, and then is detached from the sperm surface during *in vitro* capacitation [62]. DEFB126 is crucial for sperm to penetrate and move efficiently in the peri-ovulatory cervical mucus [54]. *In vitro* assays reveal that if DEFB126 has a highly negative charge it changes sperm capability for penetrating the cervical mucus. A common mutation of *DEFB126* in humans significantly reduces sperm penetration of even a viscous hyaluronic acid gel, used experimentally instead of the cervical mucus [53]. In a prospective cohort study, husbands with the *DEFB126 del/del* genotype actually showed a statistically significant decrease in fertility compared to those with *DEFB126 wt/wt* or *wt/del* genotypes [53]. In total, these evidences suggest the the mechanism that the uterine cervix selects mature sperm in epididymides, and consequently eliminates immature sperm.

In uterine semen depositors, such as rodents, pigs and horses, the seminal plasma directly enters the uterus and remains there, which induces more remarkable reactions in the uterus compared with those in the vaginal semen depositors. As described above, mouse SVS2 secreted from the seminal vesicles coagulates the semen (or forms a copulatory plug) in order to prevent the sperm from leaking out of the uterus [26]. On the other hand, SVS2 is partially degraded and enters the uterus along with ejaculated sperm as liquid semen [28]. To determine whether SVS2 functions as the decapacitation factor in female reproduction tracts, we produced mice lacking the *Svs2* gene. *Svs2*$^{-/-}$ male mice displayed strongly reduced fertility in natural mating, because of the ectopic acrosome reaction of the uterine sperm [26]. In the presence of SVS2, most of the uterine sperm did not alter acrosome intactness, but about 70% of the uterine sperm without SVS2 induced an acrosome reaction. This result brought us the idea that the ectopic acrosome reaction in the uterus might occur due to the induced deficiency in the decapacitation factor SVS2. Contrary to our idea, analyses of immunohistochemical distribution of the sperm-specific protein IZUMO-1 and observation of sperm configuration using transmission electron microscopy revealed that the

ectopic acrosome reaction of the uterine sperm does not occur; instead, what happens is simply sperm death caused by membrane disruption [26]. Importantly, this spermicidal effect was observed under *in vitro* culture conditions by the addition of uterine fluid collected from female mice, but not by the addition of oviductal fluid. These findings imply that the uterus selects the sperm by its status: sperm coated with SVS2, or those without.

The SVS2 receptor on the sperm membrane is identified as ganglioside GM1, and their interaction is regulated by an electrostatic difference [27]. For sperm selection by the uterus, there are at least two results of the binding of SVS2 to the sperm surface: one is the exclusion of the capacitated sperm, induced by detaching SVS2; and another is a stealth capability against uterine spermicide(s), engendered by coating with SVS2. Recently, it was reported that SVS2 maintains cholesterol level in the sperm membrane [2]. In the absence of SVS2, the cholesterol level in the sperm membrane is significantly decreased in the uterus. It is well known that the decrease of membrane cholesterol induces sperm capacitation [10]. Based on these evidences, it remains a possibility that the ejaculated sperm from *Svs2*$^{-/-}$ male mice are capacitated in the uterus, and then damaged by uterine spermicide(s). In artificial insemination of hamster sperm into the uterus, capacitated sperm neither enter the oviduct well, nor survive in the uterus well, compared with the incapacitated sperm [45]. On the other hand, in our report about the phenotypes of *Svs2*$^{-/-}$ male mice [26], transmission electron microscopic analysis demonstrated that a “thick wall” surrounds the uterine sperm from *Svs2*$^{+/+}$ male mice. This wall consists basically of seminal vesicle proteins including SVS2 and SVS4, suggesting a possibility that the wall physically blocks the interaction between uterine spermicide(s) and the sperm membrane.

SPERM TRANSPORT BY UTEROTUBAL JUNCTION BETWEEN UTERUS AND OVIDUCT

In uterine semen depositors, not only ejaculated sperm but also seminal plasma and pathogens enter the lumen of the uterus. Seminal plasma proteins are found abundantly in the lumen of the uterus, but never found in the oviduct [2,6,28]. Because the oviduct, the place wherein sperm fertilizes the oocyte, opens into the abdominal cavity, it is assumed that the uterotubal junction (UTJ) between uterus and oviduct functions as a barrier that only fertile sperm can pass through. Although anatomical morphology of the UTJ differs remarkably among species, there is a common characteristic that the lumen of UTJ is drastically narrower than that of the uterus, and filled with acidic mucus [24,49,50]. Although the barrier mechanism remains unclear, neither immotile, capacitated sperm nor pathogens can pass through the UTJ [3,45,47]. DEFB126 is required for ejaculated sperm to pass through the cervical mucus in vaginal semen depositors such as humans and monkeys [53,54,62]. The similar systems involving DEFB126 probably exist in the passage of sperm through the UTJ mucus. Several studies, using genetically modified mouse models, provide a hint to reveal the molecular mechanism of passage through the UTJ. Sperm without A Disintegrin And Metalloprotease 3 (ADAM3) cannot migrate to the oviduct, even if they are motile and morphologically normal [18,19,23,40,52,59]. ADAM3, as well as chaperone proteins and serine proteases such as calmegin, calsperin, protein disulfide isomerase homolog, and Prss37, is primarily present on the sperm head. Further studies on ADAM3 are needed to elucidate the mechanism of sperm transport through the UTJ.

CONCLUSION

Internal fertilization is indispensable to natural reproduction in mammals. To overcome infertility in humans, and the severely increasing issue of infertility in domestic animals, full understanding of this phenomenon is an attractive and extremely urgent theme for us. To unveil the overall configuration of internal fertilization, we wish to create a novel research field, traversing beyond given fields such as endocrinology, anatomy, cell biology, and immunology. It should not be forgotten that full competence of internal fertilization requires unique extracellular factors secreted from male genital organs. Sperms’ journey within the female reproductive tract is difficult to understand, and the production of genetically manipulated animals is only a royal road to reveal its molecular mechanisms. Among experimental subjects to detect phenotypes of gene-engineered animals, the judgement of fertility is relatively easier than the exploring phenotypes based on further internal mechanisms. Since it is burdensome to determine the cause of infertility in mice, classical methods are still needed on the research field. Today, the development and applications of CRISPR/Cas9 for genome engineering is

accelerating the discovery of novel factors, and presumably factors concerning internal fertilization in animals other than mice. Basic science targeted at internal fertilization in primates will shed light on the human reproduction system, leading, in the near future, to the identification of causes underlying human infertility.

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