Fate and distribution of seminal plasma proteins in the genital tract of the female rat after natural mating

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This paper describes the distribution and fate of seminal plasma proteins in the rat female genital tract after insemination, using immunological detection in tissue sections and in fluids collected from different regions. The localization of seminal plasma proteins in the uterus and the vagina correlated with that of spermatozoa, suggesting that passive transport mechanisms operate in these regions. No seminal plasma proteins were detected in the oviduct, indicating that their presence is probably restricted to the uterine environment. Possible mechanisms for eliminating seminal plasma molecules after copulation include leakage from the uterus after relaxation of the cervical muscles and endocytosis by the endometrial cells. Large amounts of both vesicular and coagulating gland proteins were detected in the vagina of females at the time of cervical relaxation, indicating that the first mechanism of leakage from the uterus after cervical relaxation operates. Immunocytochemical procedures were used and seminal vesicle antigens were detected inside uterine epithelial cells, which indicates that endocytosis is also a mechanism for elimination of these molecules after copulation. Western blot results suggest proteolytic cleavage as a third mechanism. However, coagulating gland antigens are neither endocytosed nor cleaved, and their elimination takes place only by backflow to the vagina. The seminal plasma distribution in experimental situations in which sperm transport is altered was also studied. The implications of our findings for mechanisms of sperm transport in the female are discussed.

Introduction

Seminal plasma constitutes the acellular fraction of semen and is derived from the admixture of secretions from various male accessory glands. Seminal plasma composition in several species has been thoroughly analysed (Mann and Lutwak-Mann, 1981; Setchell *et al.*, 1994) but its role in reproduction is still largely a matter of speculation. In humans, the main role of seminal plasma may be to maintain the spermatozoa in a decapacitated state (Cross, 1996). Furthermore, adding seminal plasma to a suspension of previously capacitated spermatozoa appears to reverse capacitation in a variety of species (Chang, 1957; Florman and First, 1988; Cross, 1993). This is mediated by the binding of seminal plasma components to the sperm membrane (Davis and Davis, 1983; Dostalova *et al.*, 1994). Other functions do not require physical association between the seminal compounds and the spermatozoa. Seminal oestrogens and prostaglandins stimulate smooth muscle contractions in the uterus, promoting passive sperm transport (Claus, 1990; Clavert *et al.*, 1990). In addition, since seminal components display immunosuppressive activity they could regulate the maternal immune functions during insemination (Thaler, 1989). The immunosuppressive factors characterized are polyamines (Quan *et al.*, 1990) and proteins (Metafora *et al.*, 1989; Claus, 1990; Dostal *et al.*, 1995), many of them of vesicular origin

(Metafora *et al.*, 1989; Porta *et al.*, 1993; Peluso *et al.*, 1994; Dostal *et al.*, 1995).

Many rodents, including those of the family Muridae, have a complex system of accessory glands. In addition to a well-developed pair of seminal vesicles, they also have a prostatic complex that consists of several lobes, each with distinct anatomy and secretory function (Setchell *et al.*, 1994). In these species, the seminal vesicle proteins present in the ejaculate are clotted by transglutaminases secreted by the cranial lobes of the prostate (also called coagulating glands), forming the structure known as the copulatory plug (William-Ashman, 1984), which plays a crucial role in reproduction (Blandau, 1945; Carballada and Esponda, 1992, 1993; Sofikitis *et al.*, 1992). However, many other roles have been shown for the seminal vesicle and prostatic antigens, such as the promotion of sperm motility (Peitz, 1988) and immunosuppressive activities (Galdiero *et al.*, 1989; Metafora *et al.*, 1989; Persico *et al.*, 1990).

The distribution and fate of seminal plasma proteins in the female genital tract has received scarce attention and data are often contradictory (Fox and Fox, 1971; Harper, 1994). In general, there is variation among species, which reflects different mechanisms of sperm transport. Thus, in rabbits and humans, the seminal plasma is restricted to the vagina (Asch *et al.*, 1977), although a small fraction might be propelled by the vaginal and uterine contractions and could reach the Fallopian tubes (Egli and Newton, 1961; Overstreet and Tom,

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1982; Jappel, 1992). In these species, the entry of spermatozoa into the uterus is largely achieved by sperm motility. In other species in which the sperm cervical passage takes place mainly by passive transport (that is, in the pig and most laboratory rodents), seminal antigens have been detected in the uterus after coitus in large amounts (Mann *et al.*, 1956; Dravland and Joshi, 1981; Nicholson *et al.*, 1983). With reference to the elimination of seminal plasma molecules after coitus, the species that has received most attention is the pig (Hunter *et al.*, 1972) but there are also some data for humans because this information is of interest in forensic medicine (Gohara, 1980).

Here we describe the distribution and persistence of seminal plasma proteins in the female rat genital tract and the mechanisms for their elimination. Immunofluorescence in tissue sections was used to determine the spatial and temporal distribution of the antigens, and western blot localization was used to identify the proteins and confirm their distribution. We have also studied their localization in situations in which the sperm transport is abnormal (after partial or total surgical removal of the seminal vesicles or the coagulating glands or after vasectomy, in which there are no spermatozoa in the ejaculate). The results are discussed with respect to the mechanisms of sperm transport in rodents.

Materials and Methods

Animals

Male and female Wistar rats (Ico: WI (IOPS AF/Han) bred in our colony were used in all the experiments. All the animals were sexually mature and all the males were of proven fertility. Animals were maintained in a dark:light cycle of 12 h dark:12 h light.

Antibodies

Two rabbit polyclonal antibodies were used. The first was obtained by immunization with whole seminal vesicle secretion (SVS), as described by Carballada and Esponda (1991). The second was obtained using the whole coagulating gland secretion (CGS) as immunogen; this fluid was obtained by isolating the coagulating glands and then gently squeezing them. The fluid drops were collected in PBS. The immunization procedures are the same as those described for the seminal vesicle fluid. The specificity of both antibodies was tested on fluids from different male genital tract regions by western blot.

Immunofluorescence in tissue sections

Oestrous females were selected at 21:00 h based on the cell types present in their vaginal smears (Inglis, 1980). They were mated to vigorous stud males and copulation was observed under red light. After ejaculation, the male was removed from the cage and the females were killed by an overdose of ether 15 min, 4 h, 8 h or 12 h after coitus; 3–6 females were analysed for each time point. The genital tract was carefully isolated and fixed by immersion in Bouin's fixative for 8–12 h at 4°C. The fixed tissue was embedded in paraffin wax and thin sections (5–8 µm) were deparaffined, hydrated and then used for immunofluorescence.

The immunofluorescence protocol used was as follows. Sections were blocked for 1 h in 3% (w/v) BSA in PBS containing 0.05% sodium azide and then incubated with the primary antibody for 1 h (anti-SVS diluted 1:200 or anti-CGS diluted 1:50, both diluted in 1% BSA in PBS containing sodium azide). After three consecutive washes, the second antibody (a commercial anti-rabbit IgG conjugated to fluorescein diluted 1:200 in 1% BSA in PBS containing sodium azide; Pharmacia, Uppsala) was added and the sections were incubated for 45–60 min in the dark. The sections were then mounted on slides, observed and photographed. At least three different slides per animal and per region of the genital tract were scored. Controls included immunofluorescence using pre-immune sera and also adding the antibodies to sections from oestrous females that had not been mated.

Western blot detection of seminal plasma proteins in fluids collected from the female genital tract

Oestrous females were allowed to mate with vigorous stud males. Coitus (which took place from 22:00 h) was observed under red light, and after ejaculation the time was recorded and the male was removed from the cage. The females were killed by an overdose of ether 15 min, 2 h, 4 h, 8 h or 12 h after ejaculation; at least five females were considered for each time. After death, each female was bled until exhaustion, by cutting the femoral arteries to minimize blood contamination. Then the genital tract was exposed through a mid-ventral incision and clamps were placed at the cervix and at the two uterotubal junctions. Two regions were considered in the vagina: distal (between the clitoris and the vaginal plug) and proximal (between the plug and the cervix). Samples were collected from the vagina using a Pasteur pipette. PBS, 0.1 ml, was injected from a tuberculin syringe into each uterine cornu and then retrieved by aspiration, together with the uterine contents. Finally, to recover samples from the oviduct they were flushed with 0.3 ml PBS using a tuberculin syringe with a blunted needle of 30G 1/2 gauge. The samples were centrifuged at 10 000 g for 5 min and stored at –20°C until use. Uterine samples had a high protein concentration and hence did not need any further processing. However, vaginal and oviductal fluids had low protein concentrations and for this reason fluids from ten females were pooled and concentrated using the commercial concentration system Minicon (Amicon, Danvers, MA). SDS-PAGE was carried out according to Laemli (1970) in 10% gels. The same amount of protein (either 50 µg or 100 µg) was loaded on every lane of a gel. The proteins were subsequently transferred to nitrocellulose filters and used for western blot procedures described by Carballada and Esponda (1991). At least three separate experiments were done per time point. Controls using preimmune sera or adding antibodies to fluids collected from virgin oestrous females were done in parallel.

Examination of seminal plasma antigens in situations of abnormal sperm transport

For these experiments, we used healthy males that had undergone surgical removal of (1) two-thirds of the seminal

vesicles (SV*) or (2) both seminal vesicles (–SV) or (3) both coagulating glands (–CG), as described by Carballada and Esponda (1992). A fourth group comprised vasectomized males (V). Vasectomy was carried out by the abdominal route and the males were mated two or three times before they were used in the experiments to make sure that no spermatozoa were left. A minimum of three females were mated per male and fluid collection was carried out 15 min after coitus. Western blot analysis was done as described above.

Results

Characterization of the antibodies against seminal vesicle and coagulating gland secretions

The specificity of the sera obtained was tested by western blot (Fig. 1). The anti-SVS antibodies recognize a variety of proteins in the seminal vesicle fluid between 18 and 45 kDa in size, with molecules of 18, 32, 37 and 45 kDa staining very intensely on the gel. However, they did not recognize proteins in any of the other male secretions tested (Fig. 1a). The serum anti-CGS recognized three proteins of 62, 65 and 115 kDa in the coagulant gland fluid (Fig. 1b); the only crossreactivity detected was with proteins from the dorsal prostate.

Detection of seminal plasma antigens in tissue sections from the female genital tract

Seminal vesicle proteins were detected in the vagina (copulatory plug), uterine lumen and inside some of the endometrial cells 15 min after insemination (Fig. 2a, b). After 4 h, the pattern changed and some spermatozoa in the vagina became strongly stained (data not shown). The increase of positive staining in the endometrial cells was obvious at this time (Fig. 2c). Some females had lost the copulatory plug 8 h after coitus, and did not display reactivity in the vagina, or show a weak staining in the uterus (Fig. 2d). In contrast, positive staining in both the uterine lumen and uterine epithelial cells was detected in females that still had the copulatory plug. The intensity of staining in the lumen was considerably less than that detected 4 h after coitus. (Fig. 2e). Reactivity was restricted to the plug 12 h after coitus (Fig. 2f). No positive staining was detected in the oviducts at any of the times studied.

Shortly after insemination, we observed positive reactivity for CGS in the vaginal plug and in the uterine lumen (Fig. 3a, b). This pattern remained 8 h after coitus but was of weaker intensity if the female still had a plug (Fig. 3c). It is noteworthy that, in contrast to the situation described for the seminal vesicle proteins, the coagulating gland antigens were detected on the apical surface of the endometrium but never inside the cells. When the plug was absent, no positive staining was detected 8 h (Fig. 3d) or 12 h after coitus. In the oviducts, we detected positive staining in epithelial cells of some regions of the isthmus; however, this staining pattern was the same in virgin females and reflects crossreactivity of the antibody with proteins present in the oviductal cells (data not shown).

Detection of seminal plasma proteins in fluids collected from the female genital tract after normal coitus

Vesicular proteins were detected in the uterus of the females 15 min after copulation (Fig. 4). The proteins appeared as sharp bands and the overall pattern resembles a ladder. There are several protein bands with molecular masses ranging from 45 to 90 kDa that were not detected in the seminal vesicle fluid (compare Fig. 1a, lane 2 with Fig. 4, gel for proteins detected 15 min after copulation). The patterns in the anterior and posterior vagina were similar but fainter in the anterior region; a gradient in intensity from the distal vagina to the uterus was visible. This pattern began to change 2 h after coitus, but the changes were more evident 4 h after coitus. The ladder pattern in the uterine fluid began to change with the appearance of new bands of proteins of intermediate molecular mass (between 18 and 37 kDa), which could be detected 2 h after coitus. However, the intensity of staining increased in the vaginal fluids, and the intensity in the proximal vagina and the uterus was very similar 2 h after coitus. The females could be classified into two populations 8 h after coitus, according to the presence (+p) or absence (–p) of a vaginal plug in their vagina. In those without a plug, no reactivity was detected. For the females that still had a plug, the situation resembled that obtained 4 h after coitus, but some of the proteins with low molecular masses (of 15–30 kDa) had been eliminated, especially from the vagina. The only reactivity present 12 h after coitus was in the vagina, provided that the plug was present. However, there were two exceptional cases in which the females displayed the plug firmly attached to the cervix, and large numbers of spermatozoa after coitus. In these cases, a high reactivity was still detectable in the uterine fluid, although the proteins of high molecular mass (60–90 kDa) were absent. The oviductal fluid was always negative.

The distribution of antigens 15 min after coitus was similar in the vagina and uterus (Fig. 5). The three protein bands of 62, 65 and 115 kDa observed in the Coomassie blue staining pattern of CGS are clearly seen in fluids collected 15 min, 2 h and 4 h after coitus (compare Fig. 1b, lanes 2 and 5 with Fig. 5, gel for antigens detected 15 min after coitus). As for seminal vesicle proteins, only those females with a vaginal plug displayed any antigens in their genital tract fluids; 12 h after coitus, no reactivity was detected even if a plug was still present. This does not include the two females mentioned above, in which positive staining could be detected in the uterine fluid. No reactivity for CGS in the oviductal fluids was detected. Results also show that the pattern of CGS staining did not change with time.

Detection of seminal plasma proteins in fluids collected from the female genital tract in cases of abnormal sperm transport

Fluids were collected 15 min after coitus from females that were mated to males in which two-thirds of the seminal vesicles had been surgically removed (SV*). In these females, different vesicular antigens (protein bands of 15–80 kDa) and coagulating gland proteins (protein bands of 62, 65 and 115 kDa) were detected (Fig. 6a, b); the situation closely resembled that obtained in females mated with normal males, the only difference between them being that the intensity in

Fig. 1. Specificity of the antibodies, anti-SVS (seminal vesicle secretion) and anti-CGS (coagulating gland secretion), against seminal fluids in the rat female tract. Protein (100 μ g) was loaded per lane in 10% acrylamide gels and then used for western blot. (a) SVS antibodies. Lane 1: Coomassie blue staining pattern of seminal vesicle fluid. Lanes 2–10 show the results of western blotting using serum or fluids from different regions of the male genital tract as antigens: lane 2, seminal vesicle fluid; lane 3, rat serum; lane 4, ventral prostate fluid; lane 5, dorsal prostate fluid; lane 6, coagulant gland fluid; lane 7, testicular fluid; lane 8, caput epididymal fluid; lane 9, corpus epididymal fluid; lane 10, cauda epididymal fluid. The SVS antibody recognizes several major and minor proteins in seminal vesicle fluid. A non-specific positive band appeared in the serum sample and is indicated on the gel by an asterisk. (b) CGS antibodies. Lane 1: Coomassie blue staining pattern of coagulating gland fluid. Lanes 2–10 show the results of western blotting using serum or fluids from different regions of the male genital tract as antigens: lane 2, coagulant gland fluid; lane 3, rat serum; lane 4, ventral prostate fluid; lane 5, dorsal prostate fluid; lane 6, seminal vesicle fluid; lane 7, testicular fluid; lane 8, caput epididymal fluid; lane 9, corpus epididymal fluid; lane 10, cauda epididymal fluid. The CGS antibody recognizes three proteins in the coagulant gland fluid and has crossreactivity with proteins from the dorsal prostate (lane 5).

the uterine fluid was weaker. The fluids obtained from females mated with males without seminal vesicles (–SV) were negative for vesicular proteins, as expected (Fig. 6a), and the CGS antigens were detectable only in the vagina (Fig. 6b). In

those females mated with males without coagulating glands (–CG), all the antigens (coagulating gland and vesicular) were restricted to the vagina. In females mated with vasectomized males (V), the pattern of vesicular proteins resembled that

Fig. 2. Immunocytochemical localization of the antibody, anti-SVS (seminal vesicle secretion), in tissue sections of the female rat genital tract at different times after coitus: (a) vagina (15 min); (b) uterus (15 min); (c) uterus (4 h); (d) uterus (2 h, plus vaginal plug); (e) vagina (12 h, plus vaginal plug); (f) vagina (12 h, plus vaginal plug). E, ejaculate; e, endometrium epithelial cells; p, vaginal plug; ve, vaginal epithelium. Scale bars in (a), (e) and (f) represent 200 μm and in (b), (c) and (d) represent 100 μm.

Fig. 3. Immunocytochemical localization of the antibody, anti-CGS (coagulating gland secretion), in tissue sections of the female rat genital tract at different times after coitus: (a) vagina (15 min); (b) uterus (15 min); (c) uterus (8 h, plus vaginal plug); (d) uterus (8 h, minus vaginal plug). E, ejaculate; e, endometrial epithelial cells, p, vaginal plug. Scale bars in (a) and (c) represent 200 μ m and in (b) and (d) represent 100 μ m.

obtained in females mated with normal males but the gradients in intensity were opposite: maximum in the distal vagina and minimum in the uterine fluid (Fig. 6a). The distribution of coagulating gland proteins did not undergo noticeable changes (Fig. 6b).

Discussion

In this study, the fate of seminal plasma in the rat after copulation was analysed using two antibodies that recognized a broad spectrum of proteins in the coagulating gland and vesicular secretion. Both antibodies were specific, as demonstrated by western blot. The anti-SVS recognized a broad spectrum of seminal vesicle proteins, as has been

previously shown (Carballada and Esponda, 1991), while the anti-CGS only recognizes three proteins of 62, 65 and 115 kDa that are probably related to the transglutaminases present in the secretion (Williams-Ashman, 1980). In this regard, a transglutaminase of 65 kDa has been detected in the rat coagulating gland and dorsal prostate gland (Steinhoff *et al.*, 1994). However, this antibody also recognizes homologous proteins in the dorsal prostate, which is in agreement with previous reports (Williams-Ashman, 1980). Our results using these antibodies have shown that all the seminal plasma proteins analysed enter the uterus and can be detected 15 min after coitus. They are also present in the vaginal lumen, forming part of the plug, and also occur in solution, but they do not appear to enter the Oviducts. This

Fig. 4. Detection of the antibody, anti-SVS (seminal vesicle secretion), by western blotting in fluids collected from the rat female tract during oestrus and at different times after coitus: 100 µg proteins were loaded per lane of the gel. The times given above the gel pictures indicate the time of fluid collection after coitus. Letters indicate the place of fluid collection: a, anterior vagina; b, posterior vagina; c, uterus; d, oviduct; + p, presence of vaginal plug; - p, absence of vaginal plug; c*, uterine fluid of one of the two females with a firmly attached plug.

is in agreement with the report that seminal plasma molecules do not pass the uterotubal junction (Asch *et al.*, 1977; Nicholson *et al.*, 1983). Although other authors have used radiolabelled compounds as tracers and have detected seminal plasma proteins in the oviducts (Gunn and Gould, 1958; Einarsson *et al.*, 1980; Viring *et al.*, 1980), these experiments used artificial rather than natural insemination, and the tracers used were often small or medium-sized and had neither the viscosity nor many other of the characteristics that seminal proteins have. We cannot exclude the possibility that small amounts of seminal plasma undetectable by the methods we have used in this study could pass the uterotubal junction by what is known as rapid transport (Overstreet and Tom, 1982;

Harper, 1994). Nevertheless, we believe that the bulk of seminal plasma is restricted to the vaginal and uterine environments, and that these antigens play their physiological role there. Seminal plasma molecules are detectable in the uterus for at least 4 h after coitus. Two situations occur 8 h after coitus: in females that retain the vaginal plug, seminal plasma proteins remain in the tract; in those females with no plug, none were left. When no plug remained, no uterine spermatozoa were found.

In rats, most of the soluble antigens are located in the uterus after normal copulation, as shown by the western blot. Once in the uterus, coagulating gland and vesicular proteins undergo different fates. Vesicular proteins are modified inside the uterus

Fig. 5. Detection of the antibody, anti-CGS (coagulating gland secretion), by western blotting in fluids collected from the female rat genital tract during oestrus and at different periods after coitus: 100 μ g protein were loaded per lane of the gel. The times given above the gel pictures indicate the time of fluid collection after coitus. Letters indicate the place of fluid collection: a, anterior vagina; b, posterior vagina; c, uterus; d, oviduct; + p, presence of vaginal plug; - p, absence of vaginal plug; c*, uterine fluid of one of the two females with a firmly attached plug.

because after coitus we detect a ladder pattern of proteins different from that obtained from the fluid, which probably reflects oligomerization caused by transglutaminases. Transglutaminases are responsible for the clotting of the rodent semen (Williams-Ashman, 1984) and covalently modify the rat seminal vesicle proteins (Paonessa *et al.*, 1984), which could be important for their physiological role (Porta *et al.*, 1993; Peluso *et al.*, 1994). In the uterus, the proteins are modified again. Our western blot analysis shows the appearance with time of many proteins that were not present in the fluid recovered after ejaculation. The pattern displayed seems to indicate that proteolysis is taking place. This is not surprising since the

prostate secretes proteases (Ban *et al.*, 1984; Wilson *et al.*, 1991), many of which cleave seminal vesicle proteins (Lilja, 1985; McGee and Herr, 1988).

In contrast, the proteins of the coagulating gland and dorsal prostate secretion do not appear to undergo any major modification, or if so it does not significantly affect their molecular masses. Another important difference between vesicular and coagulating gland proteins is that vesicular proteins seem to be endocytosed by the uterine epithelium. This starts briefly after ejaculation and progresses for 4 h after coitus. The antigens must be processed quickly by the cells because 8 h or 12 h after coitus, depending of the presence or not of a plug,

Fig. 6. Detection of the antibodies (a) anti-SVS (seminal vesicle secretion) and (b) anti-CGS (coagulating gland secretion) by western blotting in fluids collected 15 min after coitus from female rats mated with males in which two-thirds of the seminal vesicles have been removed (SV*), males that had undergone seminal vesicle removal (–SV) or coagulating glands removal (–CG) or males that had been vasectomized (V). Letters below the gel pictures indicate the place of fluid collection: a, proximal vagina; b, distal vagina; c, uterus. 100 µg protein were loaded per lane of the gel.

they are no longer detectable. However, coagulating gland antigens were never detected inside the cells.

Seminal plasma antigens remain in the uterine lumen 8–12 h after coitus. Since the decrease of these antigens in the uterus occurs in parallel to their increase in the vaginal fluid, this removal must be largely by backflow to the vagina, as previously suggested (Austin, 1957). There are conspicuous

differences among females depending on the presence or absence of a vaginal plug. It is important to note that in those females lacking a plug, there was no failure in sperm deposition since all of them had uterine and oviductal spermatozoa. The formation of a copulatory plug in close apposition to the cervix is essential to facilitate passive sperm transport and fertility in rats (Blandau, 1945; Carballada and Esponda, 1992). Once

formed, the high tonus of the cervical muscles is enough to retain the fluid inside the uterus (Blandau, 1945). However, when the cervical muscles start to relax (from 4 h after coitus) the vaginal plug could act mechanically to prevent or at least to slow down the uterine fluid backflow. This is not only indicated by the differences in sperm numbers and seminal plasma proteins among females with or without a plug but also by the situation found in the two females that had a plug in close apposition to the cervix 12 h after coitus. In these females, the uterine cornua were distended and full of fluid, with many spermatozoa still present (R. Carballada and P. Esponda, unpublished), and the vesicular and coagulating gland proteins were still detectable in the fluid collected from the uterus. This finding allows us to assign a secondary role to the vaginal plug in reproduction in rats: the prevention of the backflow of the semen when the cervical muscles have relaxed. Proteolytic cleavage and endocytosis also appear to play a role in the elimination of the vesicular proteins. The existence of secondary mechanisms to eliminate seminal plasma from the uterus has been reported in boars (Hunter *et al.*, 1972): after artificial intrauterine insemination, if the backflow of semen is prevented by ligation, several seminal components are still detectable up to 24 h after coitus (although the original amount is reduced, indicating that some uterine absorption has taken place).

We also studied seminal plasma distribution in cases in which sperm transport was experimentally altered. Although a certain number of spermatozoa reach the uterus from males from which part of the seminal vesicles has been ablated, there is also a percentage of spermatozoa that remains in the vagina (Carballada and Esponda, 1992, 1993). As expected, the distribution of the antigens examined matches the sperm distribution, and we detected an increase in the intensity of the proteins in the vaginal fluid compared with controls. In cases in which vaginal plug formation is prevented by surgical removal of the seminal vesicles or coagulating glands, seminal plasma molecules are detectable only in the vagina, again matching sperm distribution (Blandau, 1945; Carballada and Esponda, 1992). This supports the hypothesis that the sperm deposition in the rat is vaginal, and that uterine and vaginal contractions triggered by vaginal plug formation promote the transport of the bulk of semen into the uterine cornua in the first minutes after coitus. If, as some authors have proposed (Joshi *et al.*, 1972), the spermatozoa were deposited in the uterus and the plug prevented only their outflow, we would have detected some seminal plasma proteins in the uterine fluid in this experiment.

The vasectomized male offers another interesting model. Although seminal plasma can reach the uterus in the absence of spermatozoa, there are noticeable differences between vesicular and coagulating gland proteins with respect to spermatozoa. Seminal vesicle proteins seem to be transported largely in association with the spermatozoa; hence, when there are no spermatozoa, the intensity of staining in the uterine fluid decreases. However, there is no evidence of association of spermatozoa with the coagulating gland antigens; therefore, vasectomy does not affect the distribution of these antigens. This idea is supported by the fact that after normal coitus there is a gradient of intensity of vesicular proteins from the distal vagina to the uterus that matches the distribution of

spermatozoa, while the concentrations of coagulating gland proteins in the vagina and uterus are about the same.

In summary, seminal plasma in the rat clots after copulation to form the vaginal plug, which facilitates the passage of spermatozoa and large amounts of seminal plasma components into the uterus a few minutes after coitus. Seminal plasma molecules remain in the uterus and are eliminated between 8 h and 12 h after coitus, mainly by backflow to the vagina after relaxation of the cervix and dislodgement of the plug. Proteolytic and endocytic mechanisms also play a role in the elimination of vesicular proteins from the female tract. Finally, the vesicular and coagulating gland proteins studied do not reach the oviducts in significant amounts, so their putative roles in reproduction seem to be exerted mainly in the lower regions of the genital tract.

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