Role of fluid from seminal vesicles and coagulating glands in sperm transport into the uterus and fertility in rats

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Summary. The relationship between the quantity of seminal vesicle secretion in the ejaculate, the percentage of spermatozoa reaching the uterus and fertility was studied in rats. Different portions of seminal vesicles were removed from male rats; 15 min after coitus (day 0), the numbers of spermatozoa in the uterus and vagina were counted and the vaginal plug characteristics were noted. Fertility was evaluated by the number of fetuses on day 14. A gradual decrease in the percentage of spermatozoa in the uterus was positively related to the reduction in seminal vesicle secretion, estimated by plug weight. This decline was not caused by a delay in sperm transport to the uterine lumen and the results suggested that the spermatozoa that fail to enter the uterus in the first minutes after coitus never enter. The vaginal plug weight, which is related to the seminal vesicle weight, and the position of the plug, which must be firmly lodged into the cervical opening, seem to be the most important conditions for promoting the rapid passage of spermatozoa into the uterus. When the seminal vesicles were partially removed, the plug was not tightly lodged and formed a 'cup' filled with spermatozoa. The number of fetuses did not show a close correlation with the quantity of seminal vesicle secretion. Studies of males in which the seminal vesicles had been removed indicated that a normal number of fetuses can be obtained despite low numbers of spermatozoa reaching the uterus. Ablation of the coagulating glands showed that, when there is no vaginal plug, no spermatozoa reach the uterus and fertility is suppressed. Nevertheless, the complete removal of coagulating glands is difficult; when small portions of these glands remain, the vaginal plug is formed and then fertility is achieved.

Keywords: coagulating gland; fertility; seminal vesicles; sperm transport; vaginal plug; rat

Introduction

Seminal vesicles and coagulating glands in rats are well-developed glands that produce a proteinaceous secretion. The proteins of the seminal vesicle secretion are substrates for the enzyme vesiculase, a transglutaminase present in the fluid of the coagulating glands (Bradshaw & Wolfe, 1977; Williams-Ashman, 1984; Fawell & Higgins, 1987). When ejaculation occurs, the two secretions are mixed and seminal vesicle fluid coagulates to form the copulatory plug.

A series of experiments were performed in which all or part of these glands were surgically removed to analyse the functions of seminal vesicles. When seminal vesicles were removed from fertile male rats (Queen *et al.*, 1981) or seminal vesicles and coagulating glands were removed (Blandau, 1945), fertility was completely suppressed. Similar results have been obtained in mice (Pang *et al.*, 1979; Peitz & Olds-Clarke, 1986) and guinea-pigs (Lawlah, 1930), but Chow *et al.* (1986) claimed that removal had no effect in hamsters. Apart from the participation of seminal vesicle secretion in the production of the vaginal plug, several other functions have been proposed, such as stimulating sperm motility (Morita & Chang, 1971; Peitz, 1988), serving as an energy source (Mann & Lutwak-Mann, 1981), providing immunosuppressive factors (Mann & Lutwak-Mann,

1981) and participating in embryonic development (O *et al.*, 1988). Other functions could be mediated by the copulatory plug, such as serving as a 'chastity belt', preventing insemination by a second male (Martan & Shepherd, 1976) or avoiding the outflow of the ejaculate from the vagina (Mann, 1949). Sperm transport through the cervix seems to be highly dependent on the formation and position of the copulatory plug, which must be firmly lodged in the cervical opening to allow the passage of spermatozoa into the uterine horns (Blandau, 1945; Matthews & Adler, 1977; Matthews & Adler, 1978).

In addition to playing an important role in semen coagulation, secretions of the coagulating glands seem to have other roles, such as modifying the seminal vesicle proteins to allow their binding to the spermatozoa surface (Paonessa *et al.*, 1984) or catalysing the gelation process that takes place in the uterus after copulation (Joshi *et al.*, 1972). Nevertheless, the real importance of coagulating glands in relation to fertility is not completely understood and ablation of coagulating glands in rodents has produced variable results. Thus, Lawson & Sorensen (1964) and Chow *et al.* (1986) reported that ablation of coagulating glands had no effect on fertility in rats or hamsters, but other authors (Walker, 1911; Pang *et al.*, 1979; Queen *et al.*, 1981) claimed that removal of coagulating glands gave rise to infertility in some rats and mice.

In all the studies in which the seminal vesicles were removed, the whole gland was removed, and no quantitative analyses were performed to determine the numbers of spermatozoa in the uterus and vagina of the females mated with the operated males. In the present study, by removing different portions of the seminal vesicles and quantifying sperm transport, we hoped to establish the relations between the size of the seminal vesicles (estimated by weight), the quantity of seminal vesicle secretion in the ejaculate (estimated by plug weight), the characteristics of the vaginal plug, sperm transport through the cervix and the number of fetuses produced. Since previous experiments involving removal of the coagulating glands examined fertility exclusively, we analysed the effects of removal of the coagulating glands on the numbers of sperm reaching the uterus.

Materials and Methods

Animals and treatments

Male Wistar rats 3–4 months old were maintained in a light:dark regimen (lights off at 19:00 h and on at 07:00 h). Males were anaesthetized using intraperitoneal injections of a mixture of ketamine (50 mg Ketolar; Parke Davis, Spain), valium (Valium 10; Roche, Spain) and atropine (Atropin 1 mg ml⁻¹; Lafsa, Spain), at 5:4:1. The doses used were 0.25 ml of the mixture per 100 g body weight.

Surgery

An abdominal incision was made and seminal vesicles and coagulating glands were exposed. Surgical removal of different portions of the seminal vesicles was performed as follows: using fine forceps, the seminal vesicles were isolated and two ligatures placed; a cut was made between ligatures and the region was discarded. The different regions considered are shown (Fig. 1). The males were classified into seven groups according to the region removed (see Table 1). For sham operation, after an abdominal incision, seminal vesicles and coagulating glands were separated and then the abdominal wall and skin were sutured. All the males used in the experiments had completely recovered 2 weeks after surgery, exhibited normal copulatory behaviour and ejaculated a normal number of spermatozoa (about 60 million), which was assessed by counting spermatozoa using a haemocytometer. This number of spermatozoa per ejaculation is in agreement with the results of Blandau & Odor (1949) for adult albino rats.

Mating and counting spermatozoa in females

The males were mated with 3-month-old oestrous females (selected by their vaginal smears) and coitus was observed under red light. In all cases, a minimum of two females was mated for each male. After the orgasm was observed, the female was isolated and killed 10 min later. The uterine cornua were exposed through a midventral incision and clamps were placed between vagina and uterus to block sperm transport through the cervix (about 15 min after orgasm). The pubic bones were cut and the vagina was opened ventrally. The vaginal plug was carefully observed to check its position in relation to the cervical opening. It was then removed and rinsed in phosphate-buffered saline.

Fig. 1. Diagram showing the regions of rat seminal vesicles that were surgically removed. Coagulating glands are indicated as dotted areas. (A) includes the upper third of the seminal vesicle, (B) the middle third and (C) half of the gland.

Table 1. Numbers of spermatozoa in the vagina and uterus and percentages reaching the uterus 15 min after coitus for intact rats and males from which parts of the seminal vesicles had been removed. Two or three females were mated to each male to calculate the numbers of spermatozoa

Group	Number of males	Number of females	Number of spermatozoa in vagina ($\times 10^{-6}$)	Number of spermatozoa in uterus ($\times 10^{-6}$)	Percentage of spermatozoa in uterus
0: Intact males	5	11	1.49 ± 0.72	60.68 ± 7.83	96.93 ± 1.39
1: Sham operated males	5	11	2.27 ± 1.06	77.14 ± 5.85	97.39 ± 1.15
2: Males without region A† of each seminal vesicle	6	14	$12.27 \pm 3.85^*$	64.23 ± 6.83	$83.83 \pm 4.72^*$
3: Males without one of the seminal vesicles	5	10	$9.73 \pm 3.19^*$	62.25 ± 4.92	$87.03 \pm 3.84^*$
4: Males without region C of each seminal vesicle	5	12	$27.82 \pm 3.84^*$	$21.93 \pm 4.80^*$	$40.46 \pm 9.11^*$
5: Males without regions A and B of each seminal vesicle	5	11	$51.79 \pm 6.42^*$	$12.67 \pm 6.64^*$	$17.10 \pm 8.17^*$
6: Males without one seminal vesicle and regions A and B of the other	5	10	$48.89 \pm 3.50^*$	$3.02 \pm 2.19^*$	$5.04 \pm 3.67^*$
7: Males without seminal vesicles	6	12	$45.73 \pm 3.75^*$	$0.00 \pm 0.00^*$	$0.00 \pm 0.00^*$

*Value significantly different from controls ($P < 0.01$).

†See Fig. 1.

Data are means $\pm$ SEM.

Using a Pasteur pipette, the inner walls of the vagina, especially the cervical regions, were thoroughly washed with phosphate-buffered saline. The contents of both washes were mixed, vigorously agitated in an Eppendorf tube and the spermatozoa were counted using a haemocytometer. Vaginal plugs were fixed in 70% ethanol and stored at 4°C; they were then dried and weighed. Uterine sperm were checked by placing two ligatures in each uterine cornu, one at the level of the uterotubal junction and other in the cervix. Using a syringe, 0.1 ml of phosphate-buffered saline was instilled in each cornu, then retrieved by aspiration with uterine contents and spermatozoa were counted. Using the values of numbers of spermatozoa in the uterus and vagina, the percentage of spermatozoa reaching the uterus was calculated. In addition to these analyses of sperm transport, a female was introduced in the cage of each male and allowed to mate until ejaculation occurred. The female was removed and killed 2 weeks later, the uterus was exposed and the number of fetuses counted to permit analysis of fertility.

After the experiments, all the males were killed, their genital tracts were exposed and the remnants of the seminal vesicles were ligated at their bases and cut. They were then blotted in a filter paper and weighed. At the same time, the other portions of the genital tract were examined carefully to verify that no damage had occurred.

Statistical analysis

An analysis of variance (one-way ANOVA; Scheffe, 1959) was performed to test differences between groups of rats in which the seminal vesicles had been removed and control males. Several tests were done to analyse seminal vesicle and plug weights, the number of spermatozoa in the uterus and vagina, the percentage of spermatozoa in the uterus and the number of fetuses. The mean of each group was compared with the mean of the control groups using Student's *t* test. Simple regression analyses were carried out to study the correlations between the percentage of spermatozoa in the uterus and plug weight, between plug weight and seminal vesicle weight and between the number of fetuses and seminal vesicle weight. Analyses were carried out on all the animals, as well as on each group. The correlation coefficient was calculated in each case assuming a linear model.

Delay of sperm transport

Another series of experiments was carried out to elucidate whether the different percentages of spermatozoa found in the uterus reflected a delay in gamete transport. One male belonging to group 4 (see Table 1) was randomly chosen and mated to a total of 12 females over several nights. These were divided into three groups, according to the time when clamps were placed between the vagina and uterus, blocking sperm transport; clamps were placed 15 min, 1 h and 2 h after orgasm. The percentages of spermatozoa in the uterus were calculated for each female and the mean was calculated for each group.

Coagulating gland experiments

Surgical ablation of the coagulating glands was performed as follows: the coagulating glands were freed of the seminal vesicles using fine forceps and clamps were placed at the base of each coagulating gland. Two ligatures were placed, one above and one below the clamps; then the clamps were removed and the coagulating glands cut between the ligatures. Fifteen males were operated on and used in a similar way to those in which the seminal vesicles had been removed to study sperm transport and fertility. Two females per male were used for the calculation of the percentages of spermatozoa in the uterus 15 min after coitus, and one female was used to study the number of fetuses. As for experiments on seminal vesicles, special attention was paid to the presence and characteristics of plugs.

As for the males in which the seminal vesicles had been removed, all the males from which the coagulating glands had been removed were killed after use and their genital tracts were examined to assess the consequence of surgery and to ensure that no damage had occurred in the genital tract.

Results

Sperm transport and fertility in males without seminal vesicles

There was a relation between the portion of seminal vesicle removed and the efficiency of sperm transport (Table 1). Although the numbers of spermatozoa in the uterus were similar in groups 0, 1, 2 and 3, the numbers in the vagina were greater in groups 2 and 3 than in groups 0 and 1. Thus, the percentage of spermatozoa in the uterus calculated from numbers in the uterus and vagina seems to be a better estimate of sperm transport than the number of spermatozoa in the uterus. A clearer relation between the percentage of spermatozoa in the uterus and seminal vesicles can be seen by comparing the mean of the weights of the plug in each group (see Table 2) with the mean percentage of spermatozoa in the uterus. However the number of fetuses does not seem as closely related to the seminal vesicle volume, and only groups 4, 5, 6 and 7 showed a reduced number of fetuses (Table 3). However, the values shown in Table 1 are means and must be analysed carefully. In groups 4 and 5 (Table 4) there are two types of male: those that were sterile and produced no pregnancy and males that caused a normal pregnancy. The variability between males in a group increased particularly in groups 4 and 5, as is shown by the SE values (Table 1), which reflect variations not only among different males, but also in a male in several ejaculations. This situation was observed in some fertile males in groups 4 and 5, which showed a striking variability in their ejaculations with percentages of spermatozoa in the uterus as different as 15.89 and 0.20 (male OVS60), 20 and 0 (male OVS53) or 37.48 and 5.44 (male OVS64).

Table 2. Weights (mg) of seminal vesicles and copulatory plugs for the groups of rats (see Table 1)

Group	Seminal vesicles	Plug
0	960.00 $\pm$ 100.58	57.72 $\pm$ 4.81
1	1155.00 $\pm$ 112.88	58.52 $\pm$ 3.68
2	575.00 $\pm$ 22.17*	38.70 $\pm$ 1.97*
3	526.00 $\pm$ 32.34*	36.37 $\pm$ 2.15*
4	530.00 $\pm$ 57.74*	25.78 $\pm$ 3.22*
5	291.67 $\pm$ 24.89*	19.32 $\pm$ 2.68*
6	171.67 $\pm$ 32.19*	16.98 $\pm$ 2.46*
7	26.5 $\pm$ 5.21*	0.00 $\pm$ 0.00*

Data are means $\pm$ SEM.*Value significantly different from controls ($P < 0.01$).**Table 3.** Number of fetuses sired by the different groups of male rats with seminal vesicles removed (see Table 1)

Group	Number of males	Number of females	Number of fetuses
0	5	5	13.00 $\pm$ 0.71
1	5	5	14.80 $\pm$ 0.58
2	6	6	12.67 $\pm$ 0.84
3	5	5	11.00 $\pm$ 2.15
4	5	5	7.40 $\pm$ 3.19*
5	5	5	5.20 $\pm$ 3.43*
6	5	5	1.00 $\pm$ 1.00*
7	6	6	0.00 $\pm$ 0.00*

Data are means $\pm$ SEM.*Value significantly different from controls ($P < 0.01$).

Statistical analysis

The results of ANOVA showed differences between groups for all the parameters considered ($P < 0.00001$). The experimental groups were also compared individually with the intact males using Student's *t* test. Regarding the regression analyses, when the values of the percentages of spermatozoa in the uterus were considered in relation to the values for plug weights, the correlation coefficient calculated was 0.844 (r^2 71.20%). When the percentage of spermatozoa in the uterus and plug weight values for individual groups were analysed, the correlation coefficients calculated were lower than the previous one, group 4 having the highest value (0.754; r^2 56.86%) and group 2 the lowest (0.086; r^2 0.47%). The other values were about 0.4, which indicates little, if any, correlation between the two variables. The correlation studies involving seminal vesicle weight and plug weight showed a correlation coefficient of 0.87 (r^2 76.47%), indicating that it is the size of the seminal vesicles that determines plug weight, as expected. However, the relation is not so simple, as individual regression analyses undertaken for each group showed lower values (except for group 0, which exhibited the highest value, 0.98). Males in group 3 ejaculated plugs larger than those of group 4, in spite of having seminal vesicles of similar size.

The correlation coefficient for the number of fetuses with seminal vesicle weight was 0.757 (r^2 57.34%). Individual analyses in the different groups showed low correlation between these variables.

Table 4. Percentages of spermatozoa reaching the uterus 15 min after coitus and number of fetuses for the five male rats in each of groups 4 and 5 (see Table 1)

Males	Percentage of spermatozoa in uterus	Number of fetuses
Group 4		
OVS46	68.96, 46.91, 69.62	10
OVS57	34.61, 74.62	16
OVS60	0.2, 15.89	0
OVS61	90.9, 41.85	11
OVS62	0, 42.31, 0	0
Group 5		
OVS51	0, 0	0
OVS53	0, 20	9
OVS63	0, 0	0
OVS64	5.44, 37.48	17
OVS65	0, 83.72, 41.42	0

Percentages for each male represent the values estimated in different ejaculations. The numbers of fetuses were obtained from other females that were mated to these males and killed on day 14.

Percentages of spermatozoa in the uterus in females killed at different times after coitus

The percentages of spermatozoa in the uterus, reflecting gamete transport, were: 95.98% $\pm$ 0.17 (15 min after coitus), 92.22% $\pm$ 3.88 (1 h after coitus) and 81.67% $\pm$ 3.74 (2 h after coitus).

Vaginal plugs

All the males analysed, except those in group 7, produced copulatory plugs of different sizes (see Fig. 2a). The intact and sham-operated animals achieved the largest plugs, which were positioned such that the anterior portion of the plug was closely apposed to the cervical opening. The size of the vaginal plugs in the experimental groups (groups 2 to 6) decreased, reflecting the volume of seminal vesicles present. Moreover, all the animals from groups 2 to 6 produced plugs in which the anterior end was not tightly lodged in the cervical opening. Such plugs presented their proximal end in the form of a cup filled with spermatozoa (Fig. 2b). In the females in which vaginal spermatozoa were found (all of them, except some animals in group 0), most spermatozoa were located between the plug and the cervical opening.

Sperm transport, vaginal plugs and fertility after ablation of coagulating glands

Of the fifteen operated males, eight did not form copulatory plugs after coitus; there was no transport of any spermatozoa to the uterus and thus the males were completely sterile. The other seven males showed variable degrees of fertility: four produced plugs of moderate size (Fig. 2a), percentages of spermatozoa in the uterus up to 45% and were fertile. The remaining three males produced very small plugs (Fig. 2a) and only one was fertile (see Table 5). In all males producing plugs, these were not firmly attached to the cervical opening and, as happened in males without part of the seminal vesicles, they displayed a cup-like structure filled with spermatozoa in their proximal ends.

The *post mortem* examination of the genital tracts of all these operated males revealed no obvious differences between the sterile and fertile animals.

Fig. 2. (a) Vaginal plugs produced by normal male rats, and by rats in which seminal vesicles or coagulating glands had been partially removed. Plugs are orientated with their proximal ends at the top. Numbers indicate the group of males to which vaginal plugs belong (see Table 1). A gradual decrease in the plug size is clearly seen in the second row. The last row shows three plugs produced by males from which the coagulating glands had been removed; they are (from left to right) from males OGC3, OGC10 and OGC2 (see Table 5); to scale.

(b) Vaginal plug from a male belonging to group 4 (see Table 1); the proximal end of the plug (arrow) displays a plug cup. $\times 4$.

Table 5. Percentages of spermatozoa reaching the uterus, vaginal plug weight (mg) and number of fetuses for male rats from which coagulating glands had been removed. Each male was mated to two females and these were killed to calculate the percentages of spermatozoa in the uterus and to check the vaginal plug. A third female was mated to each male and killed on day 14 to count the number of fetuses

Males	Weight of vaginal plug (mg)	Percentage of spermatozoa in uterus	Number of fetuses
OCG1, 4, 5, 7, 8, 9, 11 and 15	—	0, 0	0
OCG2	12.5, 8.1	0, 0	0
OCG14	13.6, 16.8	0, 0.4	0
OCG10	12.2, 19.7	0, 3.7	13
OCG6	32, 37.4	0, 38.77	15
OCG3	41.2, 42.2	29.97, 14.55	5
OCG12	52.6, 54.3	44.07, 36.08	16
OCG13	48.3, 33.7	34.78, 1.71	16

Discussion

The role of the seminal vesicles and copulatory plug in gamete transport in rats has been pointed out in earlier studies (Blandau, 1945; Matthews & Adler, 1978; Overstreet, 1983). However, since these studies were carried out after the complete ablation of the gland (Blandau, 1945) or with the seminal vesicles intact (Matthews & Adler, 1978), a relationship between the seminal vesicle content

and sperm cervical transport could not be demonstrated. By the removal of various defined portions of seminal vesicles and using the percentage of spermatozoa in the uterus as a good estimation of gamete transport, we showed that there is a close relation between seminal vesicle size, the quantity of seminal vesicle secretion in the ejaculate and sperm transport through the cervix. There was a decrease in the percentage of spermatozoa in the uterus which correlated with a reduction in weight of the plugs (related to seminal vesicle weights), as shown in Tables 1 and 2. This correlation was supported by the results of the ANOVA and Student's *t* test, and specially by the regression studies that demonstrated a strong correlation between the weight of the plug and the percentage of spermatozoa in the uterus when analysed as a whole. The most conspicuous fall in the percentages of spermatozoa in the uterus was evident when at least region C (see Fig. 1) of the seminal vesicles was removed, in which the plug weight was reduced from 58.52 ± 3.68 to 25.78 ± 3.22 mg. The fall in plug weight in group 4 was not explained by seminal vesicle weight. Males in group 3 possessed seminal vesicles of similar size to those of group 4, but ejaculates from the former produced bigger plugs than the latter. An explanation for this could be different behaviour of the distal and proximal regions of the seminal vesicles during ejaculation. In this case, only one seminal vesicle maintaining its distal portion intact could produce (in an ejaculation) more secretion than the pair of seminal vesicles with their distal regions removed.

However, the quantity of seminal vesicle secretion is not enough to explain the percentages of spermatozoa observed in the uterus. Males in the same group, and therefore with seminal vesicles of similar size, produced different percentages of spermatozoa in the uterus. This also occurred with a male mated with different females (Tables 4 and 5). The weights of the plugs do not vary significantly in different ejaculations, so there must be another factor affecting the percentage of spermatozoa in the uterus; this appears to be related to mating behaviour.

Regarding the analyses undertaken to establish whether the decrease of the percentage of spermatozoa in the uterus could be caused by a delay in sperm transport to the uterus, our findings demonstrate that no delayed transport occurs because the percentages of spermatozoa in the uterus were not significantly greater when evaluations were performed up to 2 h after coitus. This agrees with the conclusion of Blandau (1945), who stated that the spermatozoa that fail to enter the uterus in the first minutes after coitus will never enter.

The results show a direct correlation between the volume of seminal vesicles in the male, plug weight and the percentage of spermatozoa in the uterus. However, apart from size of the copulatory plug, it seems that the position reached by the plug in the proximal opening of the vagina is the most important feature to affect the percentage of spermatozoa in the uterus. In this regard, the work of Matthews & Adler (1978) deserves some comment. Using normal rats, they demonstrated that, when the plug is firmly lodged in the vaginal cervical junction, many spermatozoa reach the uterus, but when the plug is not so tightly attached, fewer spermatozoa reach the uterus and the plug displays in its proximal end a 'plug cup' containing many spermatozoa. This seems to account for some results in the present study; when seminal vesicle secretion was reduced, as in males from groups 2 to 6, plugs were defective in lodging in the cervical opening and the spermatozoa remaining in the vagina were preferentially located between the plug and the cervix. In these circumstances, the plug formed a 'cup' lodging these spermatozoa, as described by Matthews & Adler (1978).

Some males in groups 4 and 5 showed conspicuous variation in percentages of spermatozoa in the uterus, probably owing to the capacity for forming vaginal plugs in different positions. We observed that when the plug was lodged in the cervical opening, there was passage of spermatozoa into the uterus, but plugs ejaculated by the same male (and, thus, of similar weight) that did not reach the cervix were associated with no or low percentages of spermatozoa in the uterus. This variability could be a reflection of mating behaviour. The co-ordination of male and female orgasms in rats seems to be mediated by different factors. It has been suggested that anatomical and positional factors (i.e. male immobile posture and prolonged penile penetration) might aid the penetration of the accessory gland fluids into the vaginal cervical junctions (Pollack & Sachs,

1976; Matthews & Adler, 1978). Toner *et al.* (1987) found that the number of pre-ejaculatory intromissions was related to the ability to produce good-fitting copulatory plugs and to cervical sperm transport. Thus, there would appear to be two factors determining the percentage of spermatozoa reaching the uterus. The first would be the seminal vesicle secretion ejaculated, which reflects seminal vesicle weight. It is clear that a male with well-developed and intact seminal vesicles has a high probability of producing a high percentage of spermatozoa in the uterus. A second factor, mating behaviour, would modulate the percentage of spermatozoa in the uterus. A prolonged immobile stance of the pair with prolonged seminal emission could produce a high percentage of spermatozoa in the uterus, even with seminal vesicle contents reduced, and then the male would be fertile. In animals with seminal vesicles of similar size, which produce plugs of similar weight, it is probably mating behaviour that determines the differences in plug position and in the percentages of spermatozoa in the uterus (Pollack & Sachs, 1976; Matthews & Adler, 1978; Toner *et al.*, 1987). In such situations, no correlation would be expected between plug weight and percentage of spermatozoa in the uterus, a prediction confirmed by our regression analyses in the individual groups.

However, the number of fetuses does not seem to be as closely correlated with seminal vesicle weight, but with the ability of spermatozoa to reach the uterine lumen. All sterile males showed a low percentage of spermatozoa in the uterus in at least one of the ejaculations (Table 4). As it is impossible to know the percentages of spermatozoa in the uterus associated with matings where females were killed only after 2 weeks, one can assume that they were zero or low. On the other hand, males producing low percentages of spermatozoa in the uterus are sometimes capable of siring a normal number of fetuses (males OVS53 or OVS64, Table 4). This can be explained in two ways. A male that ejaculated few spermatozoa into the uterus in the two females used for calculating the percentage of spermatozoa in the uterus could ejaculate a higher number in the third female used for counting fetuses. The second point to consider is that fertility can be reached even when few spermatozoa are used for insemination, as demonstrated in several species (Austin, 1948; Betteridge, 1986). In rats only when the number of spermatozoa is $< 5-8 \times 10^6$ is the number of embryos reduced (Toner & Adler, 1985).

It seems that the main effect of removal of seminal vesicles on fertility is failure of cervical transport; this does not exclude other possible functions of seminal vesicle secretion when spermatozoa reach the uterus or the oviduct. Components of the seminal vesicle secretion may act as stimuli for sperm motility (Peitz & Olds-Clarke, 1986; Peitz, 1988) and may be involved in embryonic development (O *et al.*, 1988).

Coagulating gland secretion appears to be essential for formation of the vaginal plug; it would thus be expected that, after the complete ablation of these glands, no vaginal plug would be formed and no spermatozoa would reach the uterus. When the males were successfully operated on (as judged by no plug formation), the percentages of spermatozoa in the uterus were zero and the males were sterile. Some males lacking coagulating glands could form plugs and were fertile, probably because of a defect in the surgical methods. Any tiny piece of coagulating gland that remained could be responsible for the plug formation, because, as stated by Samuel & Flickinger (1987), very small quantities of vesiculase can induce clotting of seminal vesicle proteins. A failure to remove these glands completely is probably the explanation for the variable results obtained in other studies involving ablation of coagulating glands. In previous reports (Pang *et al.*, 1979; Queen *et al.*, 1981), the females were not generally examined for plug structures and, when they were, as in the case described by Chow & O (1989), one-third of them produced plugs. In most of the experiments the absence of the plug after removal of seminal vesicles or coagulating glands was assumed but not checked, and so we recommend a strategy based on observing the mating, assessing the orgasm and taking away the female for immediate analysis. The vagina must also be carefully examined for the presence of small vaginal plugs. Regarding the spermatozoa that reach the uterus, it seems important to indicate that the use of percentages, instead of numbers, may give a clearer picture of gamete transport, as the normal variation in numbers of spermatozoa among different

males could mask a small effect in sperm transport (see Table 1). In any case, it is clear that similar data from other rodents are needed to clarify the roles of seminal vesicles and coagulating glands.

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