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Seminal Vesicle and Preputial Gland Response to Steroids in Adult Male Mice Is Influenced by Prior Intrauterine Position

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EVEN, M. D. AND F. S. VOM SAAL. *Seminal vesicle and preputial gland response to steroids in adult male mice is influenced by prior intrauterine position.* PHYSIOL BEHAV **51**(1) 11–16, 1992.—There are differences in serum steroid concentrations during fetal life between male mice that develop between two male fetuses (2M males, with elevated testosterone) and between two female fetuses (0M males, with elevated estradiol). The present studies were undertaken to determine whether prior intrauterine position would influence the weight of seminal vesicles and preputial glands in adult male mice. To eliminate any potential differences between 2M and 0M males in circulating gonadal steroids, all males were castrated in adulthood and implanted with silastic capsules containing testosterone (T), dihydrotestosterone (DHT) or a combination of T and estradiol-17β (E_2) or DHT and E_2. Three weeks later, preputial glands were significantly heavier in 2M than 0M males after treatment with T but not DHT. Seminal vesicles were also significantly heavier (blotted wet weight) in 2M than 0M males after treatment with T. For 2M males, seminal vesicles weighed the same in response to treatment with T or DHT. However, relative to the effect of T, DHT significantly increased seminal vesicle weight in 0M males such that they were equivalent to weights in 2M males treated with T or DHT. This finding suggests that seminal vesicles in 0M males have lower concentrations of 5α-reductase and, thus, a lower capacity to metabolize T to DHT which is required for normal seminal vesicle function. There were no significant effects of E_2 (in combination with T or DHT) on seminal vesicle or preputial gland weight.

Intrauterine position Seminal vesicles Preputial glands Sexual differentiation Testosterone Estradiol 5α-Reductase

THE seminal vesicles are prominent in mice and contribute the bulk of the seminal fluid (a fructose-rich milky fluid) into the ejaculatory ducts during an ejaculation. Removal of the seminal vesicles reduces fertility in mice, which does not appear to be due to the absence of the formation of the copulatory plug (27,29). Instead, fertility may be influenced via effects of seminal fluid constituents on sperm motility (28).

The preputial glands are thought to be modified sebaceous glands (31,32). The preputial glands secrete lipids via ducts which empty into the prepuce; the structure of the prepuce in male rodents has been related to marking behavior (22). Preputial gland secretions may be involved in communicating information about reproductive state and social status in rats and mice (6, 9, 31) and may secrete an aggression-promoting pheromone in male mice (19,24). The preputial glands also appear to be involved in maternal identification of sex of newborns in rats (23).

Near the end of the second week of gestation in mice, the testes in male embryos differentiate and begin secreting testosterone (T) (4). Morphogenesis of the seminal vesicles begins on Day 15 of fetal life in mice with dilation of the lower region of the Wolffian ducts. After Day 17 of fetal life, tips growing into the mesenchyme are identifiable, and growth continues after birth (21).

Differentiation of the accessory reproductive organs that derive from Wolffian ducts in males is mediated by T binding to androgen receptors. In contrast, in urogenital sinus tissue, which differentiates into the prostate, scrotal tissue and penis, T serves as a prohormone which is reduced to 5α-dihydrotestosterone (DHT) by the enzyme 5α-reductase prior to binding to androgen receptors. However, by adulthood, organs which differentiated from both urogenital sinus and Wolffian ducts metabolize T to DHT prior to binding with androgen receptors (37). In the seminal vesicles, 5α-reductase is thus synthesized subsequent to the fetal-neonatal period of differentiation (Fig. 1).

The preputial glands also respond to T (18). It appears that reduction to DHT is not involved in the development of the preputial glands prior to puberty in rats (17). However, the preputial glands are similar to the seminal vesicles in that T is reduced to DHT within the gland cells of adult males by the action of 5α-reductase, and it is DHT which serves as the ligand for binding to androgen receptors (5).

Male mouse fetuses positioned in utero between two other male fetuses (2M males) have significantly higher serum concentrations of T than do male fetuses positioned between two female fetuses (0M males) which are exposed to significantly higher serum concentrations of 17β-estradiol (E_2) (35,36).

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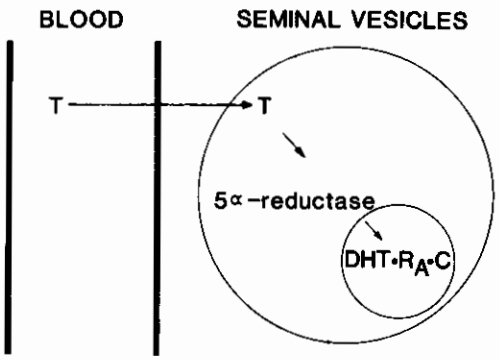

FIG. 1. A schematic diagram depicting the movement of testosterone (T) from the blood into a cell in the seminal vesicles and the reduction of T to 5α-dihydrotestosterone (DHT) by the action of 5α-reductase. Within the nucleus, DHT binds to androgen receptors (RA), which leads to transcription of specific genes on chromosomes (C).

In the studies described here, we examined whether the seminal vesicles and preputial glands in adult, castrated 2M and 0M male mice would respond similarly to treatment with T or DHT, since prior studies have revealed that differences in exposure to gonadal steroids during fetal life due to intrauterine position influences sensitivity to steroids in both male and female mice [(36); reviewed in (35)]. A difference in the response of accessory reproductive organs in 2M and 0M males to DHT would suggest a difference in intracellular androgen binding, while a difference in response to T coupled with no difference in response to DHT would suggest a difference in the capacity to form DHT within target cells.

Accessory reproductive organs in male rodents also show the capacity to aromatize androgen to estrogen as well as specific binding of estrogen (12, 13, 30). In the present studies we, thus, examined whether the seminal vesicles and preputial glands in adult, castrated 2M and 0M male mice would respond similarly to treatment with a combination of T + E_2 or DHT + E_2 in order to compare the sensitivity of these organs in 2M and 0M males to a combination of androgen and estrogen treatment.

GENERAL METHOD

Animals and Housing

CF-1 house mice (*Mus domesticus*) were housed in 18 × 29 × 13 cm polypropylene cages and maintained at 25 ± 2°C on a 12:12 light:dark cycle, with lights on at 1200 h. Mouse breeder chow (Purina 5008) and water were available in excess.

Mating and Intrauterine Position Classification Procedures

To obtain mouse fetuses from known intrauterine positions, adult CF-1 female mice were time mated by being placed daily with a stud male beginning at 0800 h. Removal of the male and examination for vaginal plugs occurred 4 h later. Inseminated females were housed three per cage and not disturbed until Day 10 of pregnancy, at which time the cages were changed. One day before the fetuses were to be removed from the uterus, pregnant females were housed individually.

Beginning at 0900 h on Day 19 of pregnancy (approximately 5 h prior to parturition), females were killed by cervical dislocation, and the pups were removed sequentially from each uterine horn. Sex was determined by examining anogenital distance, which is about two-times longer in males than in females. The intratuerine position of each male was classified at cesarean delivery according to the sex of directly adjacent fetuses: 2M males developed between 2 male fetuses, 1M males developed between a male and a female fetus, and 0M males developed between 2 female fetuses in utero. Intrauterine positioning is a random developmental event (10,34), and using this classification scheme we find, on average, 1-0M, 2-1M and 1-2M male fetuses per litter. Average litter size in CF-1 mice is 12 pups; 33% of the pups (at the ends of the two uterine horns) cannot be classified using this scheme.

Males were reared by foster mothers which had delivered naturally within the prior 24 h. Foster litters consisted of either 5-2M males and 5-1M males or 5-0M males and 5-1M males; 1M males were toe clipped for identification. Weaning occurred at 23 days of age, at which time the males were housed in groups of 5 with other foster littermates from the same intrauterine position. At 35 days of age, each male was housed individually to avoid any effects that fighting and becoming dominant or subordinate might have on accessory reproductive organ function.

Data Analysis

There are different strategies used for presenting data for reproductive organs. Typically, organ weight is presented after being adjusted for body weight (mg organ weight/g body weight). One problem with this approach is that a treatment might have independent effects on reproductive organs and body weight, and presenting the data as a ratio could obscure this. Also, an effect of treatment only on body weight could be misinterpreted as being an effect on the reproductive organ, even if the treatment, in fact, had no measurable effect on reproductive organs. The approach of matching animals for weight prior to treatment and then independently examining treatment effects on body weight is one method of addressing this problem. Alternatively, rather than explicitly assuming a relationship between different variables, such as seminal vesicle weight and body weight, and presenting the data in the form of a ratio of these possibly unrelated variables, analysis of covariance can be conducted. Analysis of covariance tests the proportion of variance in organ weight which is accounted for by body weight, and this method of analysis is, thus, the appropriate method for analyzing data on organ weights (14).

In the study reported here, the data for weights of reproductive organs are presented as the mean ± SEM for each group after being adjusted for body weight by analysis of covariance rather than as a ratio of organ weight/body weight. Analysis of covariance was conducted using the Statistical Analysis System (SAS) program. Planned comparisons of group means were made using the LS means program on SAS if the overall analysis was statistically significant. The criterion for rejection of the null hypothesis was $p<0.05$.

EXPERIMENT I

The objective of this study was to determine the dose of T, DHT and E_2 to use in the next experiment. Specifically, we wanted to determine the dose of T and DHT which maintained organ weights at about 75% of the weight of intact males. Barkley and Goldman (2) provided information concerning different doses of T on seminal vesicle weight and intermale aggression in mice, and we based the doses chosen for study in this experiment on their findings.

Method

Different doses of T, DHT or E_2 were administered to young

TABLE 1

	Treatment		
	T	DHT	E_2
High	106	72	20
Medium	82	58	20
Low	26	26	18

The mean blotted wet weight (in mg) of seminal vesicles from male mice which were castrated and administered 3 doses (high, medium and low) of steroids: testosterone (T; 5.0, 0.5 or 0.05 mg/capsule), dihydrotestosterone (DHT; 5.0, 0.5 or 0.05 mg/capsule) or estradiol (E_2; 1000, 100, or 1 μg/capsule) via silastic capsule for 21 days. Standard errors are not presented and statistical comparisons not made since means are based on only 3–5 observations. Other males (n = 5) were castrated and not administered any hormone for 21 days; the mean weight of blotted paired seminal vesicles was 12 ± 2 mg. Blotted paired seminal vesicle weights of between 60–120 mg are typically observed in adult gonadally intact CF-1 mice.

adult (90–100 days old) 1M male mice at the time of castration. At the time of castration and implantation of hormones, males were matched for body weight and then assigned to treatment groups.

Three different doses (0.05, 0.5 and 5.0 mg) of T were dissolved in 0.02 cc sesame oil; the same three doses of DHT were also dissolved in 0.02 cc sesame oil. Three doses (1, 100 and 1000 μg) of E_2 were also dissolved in 0.02 cc sesame oil. Each solution was loaded into a separate silastic capsule (0.062 in i.d. 0.125 in o.d.; Dow 602-285) measuring 1 cm between the capped ends of the capsule (a 1 cm length of this capsule holds a 0.02 cc volume).

Adult 1M males were anesthetized with an IP injection of Nembutal (90 mg/kg) and castrated. The males were then implanted SC with a silastic capsule at the base of the neck. There were 3–5 males implanted for each treatment group: T (0.05, 0.5 and 5.0 mg), DHT (0.05, 0.5 and 5.0 mg), E_2 (1.0, 100 and 1000 mg).

The males were killed 21 days later via an overdose of nembutol followed by cervical dislocation, and the seminal vesicles and preputial glands were removed. The coagulating glands were stripped off of the seminal vesicles, and the two seminal vesicles containing seminal fluid were weighed on a Mettler AE-163 balance. The seminal vesicles were then blotted on moist towels to remove luminal fluid and reweighed. The two preputial glands were gently blotted and weighed together.

Results

The data for the blotted seminal vesicles are presented in Table 1. The high (5.0 mg) dose of T resulted in seminal vesicle weight which was similar to that observed in most intact male mice (blotted seminal vesicle weights vary in intact males from 60 to 120 mg/paired glands). The medium (0.5 mg) dose of T resulted in seminal vesicles which were 78% of seminal vesicle weight in males receiving the 5.0 mg dose. Similarly, the 0.5 mg dose of DHT resulted in glands which were 80% of weights recorded in males receiving the 5.0 mg dose of DHT, although the response to DHT was somewhat less than for an equivalent capsule dose of T. The seminal vesicles in males administered the low (0.05 mg) dose of either T or DHT were twice as heavy as the seminal vesicles in castrated males (n = 5) not treated with androgen (12.2 ± 2 mg). There was no difference in the weight of seminal vesicles in response to the different doses of E_2 without simultaneous treatment with androgen.

The weight of preputial glands in young adult, gonadally intact male mice averages about 120 mg. As was true for the seminal vesicles, the 0.5 mg dose of T alone resulted in preputial gland weight which was heavier (101 mg) than weights in response to the 0.5 mg dose of DHT (65 mg). However, E_2 exposure alone had a different effect on preputial weight than on seminal vesicle weight in castrated males. The weight of preputial glands in castrated, nontreated males (n = 5) was 45 ± 5 mg. As the dose of E_2 increased, the weight of the preputial glands decreased (1, 100 and 1000 μg E_2 resulted in 55 ± 5, 42 ± 11, and 21 ± 3 mg preputial glands, respectively). The animals were matched for body weight prior to assignment to groups, and there was no effect, $F = 1.0$, $p > 0.1$, of hormone treatment on body weight at the time of organ collection.

EXPERIMENT II

The results of Experiment I showed that the medium (0.5) mg dose of T and DHT led to maintenance of the seminal vesicle and preputial glands at a less than maximum weight. In this experiment we used the 0.5 mg dose of T and DHT to increase the likelihood of observing individual differences in seminal vesicle and preputial gland weight in response to stimulation by androgen or androgen + estrogen.

The main objective of this experiment was to examine whether a difference in seminal vesicle weight due to prior intrauterine position would be observed in males that were castrated and administered either T or DHT. The reason for administering DHT was to bypass any effect that prenatal differences in exposure to T might have on 5α-reductase activity, since in adult males T is reduced to DHT in seminal vesicle tissue prior to binding to androgen receptors (1).

Both T and DHT were administered alone, as well as in combination with E_2, in adulthood. The rationale for including E_2 is that within accessory reproductive organs in mice and rats there are tissues that can aromatize testosterone to estradiol and bind estrogen (3).

The preputial glands also respond to testosterone (18), and T is reduced to DHT in the preputial glands of adult males (5). We, thus, also examined the effects of these treatments on preputial gland weight in 2M and 0M males.

Method

Four hormone-treatment groups of adult (90–120 days old) 0M males and 2M males (n = 7/Group) were matched for weight and castrated. The males were administered one of the following hormone regimes in silastic capsules: 1) T (0.5 mg), 2) DHT (0.5 mg), 3) T (0.5 mg) and E_2 (100 μg), or 4) DHT (0.5 mg) and E_2 (100 μg). When two hormones were administered, they were contained in separate silastic capsules. The seminal vesicle and preputial glands were removed and weighed 21 days later using procedures described in Experiment I. Animal cages were coded so that dissection and weighing were performed without knowledge of the treatment condition or prior intrauterine position of the animals.

Results

The data presented in Table 2 show that treatment of castrated males with T resulted in the blotted seminal vesicles in 2M males being 30% heavier than the blotted seminal vesicles

TABLE 2

	Treatment			
	T	DHT	T+E_2	DHT+E_2
2M Males Seminal Vesicles	78.8 ± 8 †	73.2 ± 7	71.9 ± 5	76.5 ± 4
0M Males	61.5 ± 6 *	81.5 ± 8	78.1 ± 6	78.4 ± 8
2M Males Preputial Glands	93.2 ± 6 *	75.6 ± 7	97.3 ± 5 *	77.3 ± 10
0M Males	72.5 ± 7	67.2 ± 8	65.3 ± 9	68.4 ± 8

*$p<0.05$; †$p=0.07$.

The mean blotted wet weight (in mg) of the seminal vesicles and preputial glands of 2M and 0M adult male mice which were castrated and administered different steroids via silastic capsule: 0.5 mg testosterone (T), 0.5 mg dihydrotestosterone (DHT), a combination of 0.5 mg T and 100 μg estradiol (E_2), and a combination of 0.5 mg DHT and 100 μg E_2 (n = 7/Group). Twenty-one days after castration and hormone implant, the animals were killed.

in 0M males ($p=0.07$). Treatment with DHT eliminated the difference between 0M and 2M males in blotted seminal vesicle weight observed in T-treated animals, and seminal vesicle weight in DHT-treated 0M males was significantly greater ($p<0.05$) than in T-treated 0M males; T- and DHT-treated 2M males showed no significant difference in blotted seminal vesicle weight.

There was no significant difference in blotted seminal vesicle weight between 2M males treated only with T and 2M males implanted with a capsule containing E_2 along with the capsule containing T. The 0M males treated with both T and E_2 had a somewhat heavier blotted seminal vesicle than did 0M males only treated with T ($p=0.1$). The combination of E_2 and DHT had no effect on blotted seminal vesicle weight in either 0M or 2M males relative to treatment with DHT alone. There were no significant effects ($p>0.1$) of hormone treatment or prior intrauterine position in terms of the weight of the unblotted seminal vesicles containing seminal fluid (Table 3).

The weight of paired preputial glands differed significantly as a function of prior intrauterine position. However, while preputials in 2M males treated with either T alone or T + E_2 were significantly heavier than preputials from similarly treated 0M males, the difference between 2M and 0M males treated with DHT or DHT + E_2 in preputial weight was not statistically significant ($p>0.1$).

Prior to castration, animals were assigned to groups matched for weight, and after 21 days of treatment there were no significant differences in body weight between males in any of the groups, $F=0.3$, $p>0.1$. Body weight was not significantly correlated ($p>0.1$) with the weight of any organ measured, whether the correlation was performed for each treatment group independently or using all animals, without regard to treatment condition. Also, body weight did not account for a significant portion of the variance (based on analysis of covariance) between groups in seminal vesicle (blotted or containing fluid) weight or preputial gland weight; specifically, F values for body weight from analysis of covariance on blotted seminal vesicle weight, fluid-containing seminal vesicle weight and blotted preputial gland weight were 1.6, 0.5, 0.8, respectively (all p's>0.1).

GENERAL DISCUSSION

We found that when 2M and 0M male mice were castrated in adulthood and treated for three weeks with equal concentrations of T, 2M males had heavier seminal vesicles (blotted wet weight) than 0M males. In contrast, no difference between 2M and 0M males in seminal vesicle weight was observed when they were castrated and treated with DHT. When compared to treatment with T, DHT significantly increased the weight of seminal vesicles in 0M males but had the same effect as T in 2M males.

TABLE 3

	Seminal Vesicles Containing Seminal Fluid			
	T	DHT	T+E_2	DHT+E_2
2M Males	332 ± 34	258 ± 26	276 ± 12	256 ± 10
0M Males	304 ± 38	284 ± 44	310 ± 36	248 ± 22

The mean weight (in mg) of the unblotted seminal vesicles containing seminal fluid from 2M and 0M adult male mice which were castrated and administered different steroids via silastic capsule: 0.5 mg testosterone (T), 0.5 mg dihydrotestosterone (DHT), a combination of 0.5 mg T and 100 μg E_2, and a combination of 0.5 mg DHT and 100 μg E_2 (n = 7/Group). Twenty-one days after castration and hormone implant, the animals were killed.

Testosterone acts as a prohormone for DHT synthesis in the seminal vesicles after the perinatal period of sexual differentiation (Fig. 1), while reduction of T to DHT is not required for differentiation of the seminal vesicles during fetal life (37). The findings from Experiment II suggest that the lower weight of the seminal vesicles in 0M males when castrated and treated with T was due to a deficit in the activity of 5α-reductase. This hypothesis has now been confirmed by the finding that gonadally intact as well as gonadectomized and testosterone-treated 0M males had significantly lower seminal vesicle 5α-reductase activity than did 2M males (25). Thus, seminal vesicle 5α-reductase activity in adulthood appears to be modulated by the concentrations of T to which the seminal vesicles are exposed during the fetal period of organ differentiation (1); 0M males have significantly lower serum concentrations of T than 2M males during fetal life. However, this finding could also be mediated, at least in part, by the higher levels of E_2 in 0M male fetuses (35), since E_2 inhibits 5α-reductase activity (26). Experiments are underway in which we are manipulating serum concentrations of T vs. E_2 during fetal life and examining the capacity for the seminal vesicles to metabolize T to DHT as well as bind androgen in adulthood.

In adulthood, treatment with a silastic capsule containing a 100 μg dose of E_2 in conjunction with either T or DHT did not have a significant effect on seminal vesicle weight. This finding is consistent with our observation that seminal vesicles in young adult CF-1 male mice do not bind estrogen; whereas we do observe estrogen binding activity in prostate (25). In addition, preliminary findings also suggest that treatment with lower doses of E_2 (in combination with the same 0.5 mg dose of either T or DHT used in the present study) has different effects on androgen binding activity and accessory reproductive organ weight (seminal vesicles, preputial glands and prostate) than does the 100 μg dose of E_2 (along with T or DHT) used in the present study (unpublished observation). The implication of this finding is that the interaction between T and E_2 in regulating the functioning of accessory reproductive organs is complex and specific results are highly dependent on doses which are used.

Seminal vesicle weight has also been examined in 2M and 0M male mice that were gonadectomized within 1 hour of cesarean delivery to eliminate any postnatal effects of gonadal steroids. In adulthood, after 5 weeks of T treatment (via silastic

capsules), 2M males were found to have significantly heavier seminal vesicles than 0M males (36). This finding, as well as the results described here, shows that the difference in seminal vesicle weight between 2M and 0M males is not due to differences in postnatal testicular function. However, we cannot assume that this will hold for all other differences between 2M and 0M males, since a comparison of adult 0M and 2M male gerbils revealed that 2M males have almost twice the serum concentrations of T as do 0M males (11).

The findings here provide additional evidence that, unlike the situation in some mammals, differentiation of the seminal vesicles in mice is influenced by circulating T. In humans, the absence of one testis during embryonic development only leads to the absence of ipsilateral Wolffian duct derivatives, while contralateral Wolffian duct structures develop normally (16). This finding has been interpreted as indicating that differentiation of Wolffian duct organs is not mediated by circulating T. Instead, diffusion of T (and/or some other factor) from the ipsilateral testis appears to be required. However, female mice exposed to exogenous T both during fetal life (via treatment of the mother) and again in adulthood, had seminal vesicles that were similar to those of intact male mice on gross examination (33). Exposure to supplemental T during fetal life (20) or shortly after birth (8) increased adult seminal vesicle weight in male offspring; RNA and DNA content of seminal vesicles were also increased by neonatal T treatment (8). Thus, in mice T in the systemic circulation influences differentiation of the seminal vesicles.

When treated with either T alone or T and E_2, 2M males had heavier preputial glands than 0M males. However, 2M and 0M males did not differ in preputial gland weight when treated with DHT or DHT + E_2. The absence of a difference between 2M and 0M males in preputial gland weight was not similar to the effect of DHT on seminal vesicle weight, since in 0M males DHT did not lead to an increase in preputial gland weight relative to treatment with T.

In contrast to seminal vesicles, weight of preputial glands was unaffected by neonatal androgen exposure (8). Also, the onset of differentiation of the preputial glands in mice appears to be later (after birth) than for the seminal vesicles (G. Cunha, personal communication). Based on these findings, one would not have predicted that preputial gland weight would differ in 2M and 0M males. However, our findings show that in adult male mice, weight (and presumably functioning) of the preputial glands is correlated with the serum concentration of gonadal steroids during prenatal sexual differentiation. Even though the preputial glands may begin differentiating after birth, it thus appears that the steroidal milieu during fetal life can somehow permanently affect the subsequent course of development of the undifferentiated tissue into the adult preputial glands.

We are currently exploring the possibility that differences between 0M and 2M males in accessory reproductive organ function will influence the likelihood of developing pathologic lesions associated with aging. For example, old male mice with infected and enlarged seminal vesicles were the least likely to successfully inseminate females (7,14). We have also observed that the prostate doubles in size during aging in male mice (unpublished observation), although the consequence of this increase in size for fertility or longevity is unknown in mice. Preliminary findings suggest that the difference in seminal vesicle weight in young adult 2M and 0M males is still present in 1.5-year-old males; young adult 0M and 2M males also differ in prostate weight, 5α-reductase activity and androgen receptor numbers (25), and age-related changes in prostate function are being examined. Thus, the difference in circulating T and E_2 during the last week of pregnancy between 2M and 0M male mouse fetuses might influence fertility as well as pathologic changes in accessory reproductive organs during aging.

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