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EFFECTS OF THE SPERMICIDE NONOXYNOL-9 ON THE PREGNANT UTERUS AND THE CONCEPTUS OF RAT

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SUMMARY

In a series of experiments the embryotoxic potential of nonoxynol-9 (N-9) was investigated in adult female rats given a single per vaginam application of 5 mg/100 g (0.1 ml/100 g) of this spermicide on day 3 (preimplantation period) or 7 (postimplantation period) of gestation. Control rats were given physiologic saline (0.1 ml/100 g) intravaginally. The vulvar labia were apposed for 24 h by metallic clips to prevent leakage of the solution. Groups of dams treated on pregnancy days 3 and 7 were killed by CO_2 inhalation on gestational days 6, 9, 12 and 15, and 8, 9, 10, 12 and 15, respectively. Lesions attributable to N-9 included sequestration, active resorption and total resorption of the conceptus, embryonal and placental necrosis, placentitis, endometritis, multicystic endometrium, and diffuse or segmental dilatation of the uterine horns. Generally, the incidence of these lesions varied with the length of time after N-9 was administered and it was consistently higher in the females treated on pregnancy day 3 than in those treated on day 7. Acute vaginitis waned with time after the application of N-9. It was concluded that under the conditions of this study, N-9 is embryocidal/fetocidal in the rat if administered during the first week of gestation. The impairment of embryonal/fetal development was attributed to the N-9-induced changes in the endometrium, the placenta and/or the embryo.

Key words: Nonoxynol-9; Vaginal spermicide; Embryotoxicity; Contraceptive-induced embryonal and fetal lesions

Abbreviations: N-9, nonoxynol-9.

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INTRODUCTION

Vaginal contraception has been a human practice for centuries. This form of contraception is perhaps the oldest means by which women have attempted to control their fertility. And, to this effect, an impressive array of "active" ingredients have been used by the early civilizations [1,2].

Among the most popular present-day vaginal contraceptives are detergents or surfactants which act by disintegrating the sperm cell wall and its subcellular membrane structures [3]. Nonoxynol-9 (N-9), a non-ionic surfactant mixture containing an average number of 9 ethyleneoxy units per molecule, is the active ingredient of a large number of vaginal contraceptive preparations sold over the counter in many countries, including Canada. The concentration of N-9 in these spermicidal formulations (cream, foam, jelly, or suppository) ranges from 2 to 12.5% [4], and the amount of N-9 varies from approximately 50 to 140 mg/vaginal application. A vaginal contraceptive sponge marketed in the United States contains 1 g of N-9 and while in the vaginal cavity, the sponge is supposed to retain its spermicidal effectiveness for 24 h.

Contraceptive failure rates have been variably estimated to range from 3 to 30 per 100 women years [5]. The relatively high failure rate has usually been attributed to user misapplication. However, similarly high failure rates have been found in primate breeding-experiments performed under controlled conditions with Delfen contraceptive cream [6]. Because vaginal contraception is practised by women of childbearing age, and since the failure rate with vaginal chemical contraceptives is fairly high, the possibility exists that in case of accidental pregnancy, the spermicidal agents may induce abortion or may pose an embryotoxic risk in humans. Jick et al. [7] have tentatively linked the high occurrence of spontaneous abortions and a number of congenital defects in children with the use of N-9 type vaginal spermicides prior to conception. Other studies, however, have suggested that the incidence of congenital malformations in the offspring of spermicide users does not exceed that observed with other methods of contraception [8—10].

Previous studies have shown that a single vaginal application of an aqueous solution of N-9 (2.5 mg/100 g) during early pregnancy produces embryo- and fetocidal but not teratogenic effects in rats and that the most vulnerable period lies between gestational days 3 and 9 [11]. The purpose of this investigation was to define the type and site of N-9-induced injury to the pregnant uterus and the conceptus using gross and histopathologic lesions as an index of embryotoxicity.

MATERIALS AND METHODS

Animals

Nulliparus female Wistar rats (180—200 g), obtained from Canadian Breeding Farms, Montreal, Québec, were acclimatized to the laboratory environment for 7—10 days. The females were paired overnight with proven

sires and the morning on which spermatozoa were detected in vaginal smears was counted as day 0 of pregnancy. The pregnant females were randomly assigned to treatment and control groups. The rats were housed in stainless steel cages in a room having a mean temperature of 22.5 ± 1.5°C and humidity 50 ± 10%, and were given tap water and rat chow diet ad lib. A 12-h light/dark cycle was provided throughout the study period.

Treatment

N-9, Lot No. 11A 30RR, (Rougier Inc., Chambly, Quèbec) was dissolved in physiological saline (0.9% NaCl, w/v) to give a total concentration of 50 mg/ml. This dose is approximately 18—50 times higher than that recommended for human use. Prior to dosing, the females (5/group) were lightly anesthetized with halothane and a single pulse of 5 mg N-9/100 g (0.1 ml/100 g) was administered intravaginally on gestational days 3 and 7, using the technique described previously [12]. The concurrent control rats (5/group) received a per vaginam application of physiological saline (0.1 ml/100 g). Metallic wound clips were applied for 24 h on the vulval labia to prevent leakage of the solution. Groups of dams treated on gestational days 3 and 7 were killed by CO_2 inhalation on pregnancy days 6, 9, 12 and 15, and 8, 9, 10, 12 and 15, respectively.

Pathology

Following complete gross examination, the whole reproductive tract was removed and the implants and resorption sites were counted externally and histologically. Tissues were fixed in Bouin's fluid for 4 h followed by several washings in 70% ethanol for 6 h and storing in 70% ethanol before processing. Paraffin sections were cut 6 μm thick and stained with hematoxylin and eosin (H.E.).

To facilitate the histologic evaluation of lesions encountered in the pregnant uterus, the resorptions were grouped into sequestration, active resorption and complete resorption. Sequestration was defined to mean an increased cell loss in the decidua with incipient separation of the decidual mass from the surrounding endometrium, loss of the implantation recess, and embryonal necrosis or combinations thereof. Active resorption, a rather broader term, was applied to implantation sites which in addition to sequestration had any of discrete areas of coagulation and liquefactive necrosis in the decidua, the placenta and/or the fetus; polymorphonuclear infiltration of the same; and disarray of embryonal and decidual cells. The term complete resorption was reserved for implantation sites in which the conceptus was replaced by a small amount of serous or serosanguineous material and the uterine epithelium was tall cuboidal, progesteronic or low papillary.

Statistics

Total number of implantation sites, frequency of normal implants, and number of resorptions/uterus in the treated and respective control dams were compared using the two tailed student's *t*-test. Differences were considered significant at $P \leq 0.05$.

RESULTS

Gross findings

Treated dams, especially those killed during the preimplantation period, had varying amounts of intravaginal fluid which in some cases was cloudy, flocculent and/or inspissated. An additional finding was perivaginal edema which occasionally extended to the rectal wall and the pelvic connective and adipose tissues. As the time interval between the dosing and killing dates increased, the severity of vaginal and perivaginal lesions decreased. The vaginas of rats killed on day 15 were unremarkable.

A decrease in the number of embrya and a concomitant increase in the number of resorption sites were common findings in the treated dams. The frequency of these alterations was indirectly proportional to the duration of pregnancy at which N-9 was administered. Microscopic examination confirmed that, for dams treated on day 3 of pregnancy, the mean number of normal implantation sites was reduced to 1 or less/uterus (Fig. 1) and resorption sites were increased to 11.5/uterus. For dams treated on day 7 of pregnancy the figures were 9.2 normal implantation sites/uterus and 4.8 resorption sites/uterus. Correspondingly, the control dams administered physiological saline on gestational days 3 and 7 had 12.5 and 13.8 normal implantation sites/uterus and 0.72 and 0.32 resorption sites/uterus, respectively. These differences between treated and control dams were statistically significant ($P \leq 0.01$).

Histopathologic findings

Acute diffuse ulcerative vaginitis and perivaginal edema were present in all treated females examined 3 days after the treatment (Fig. 2). After this period the incidence of acute vaginitis abated with time becoming 0 by post-treatment day 12 in females treated with N-9 on pregnancy day 3 and 1/5 by post-treatment day 8 in those treated on day 7. Control rats were unremarkable. In a few control females, however, estrus related polymorphonuclear leukocytes were usually confined to the vaginal lumen and were intermixed with desquamated keratinized epithelial cells or cellular debris.

Acute endometritis, encountered only in N-9-treated dams, was usually focal and was characterized by moderate to heavy polymorphonuclear cell infiltration of the uterine lumen, epithelium, and lamina propria; by desquamation of the uterine epithelium; and, occasionally, by epithelial cell necrosis (Fig. 3). The incidence of endometritis was lower than that of vaginitis and waned with time.

Embryonal and fetal resorptions were seen in both the treated and control dams. However, the incidence of resorptions was highest among dams treated during the preimplantation period, i.e. pregnancy day 3. Depending on its stage of development and the age of the conceptus at the time of the histologic examination, resorption of implantation sites appeared as sequestration, active resorption, or complete resorption. The commonest manifestations of sequestration were decidual cell loss and incipient separation of the decidua

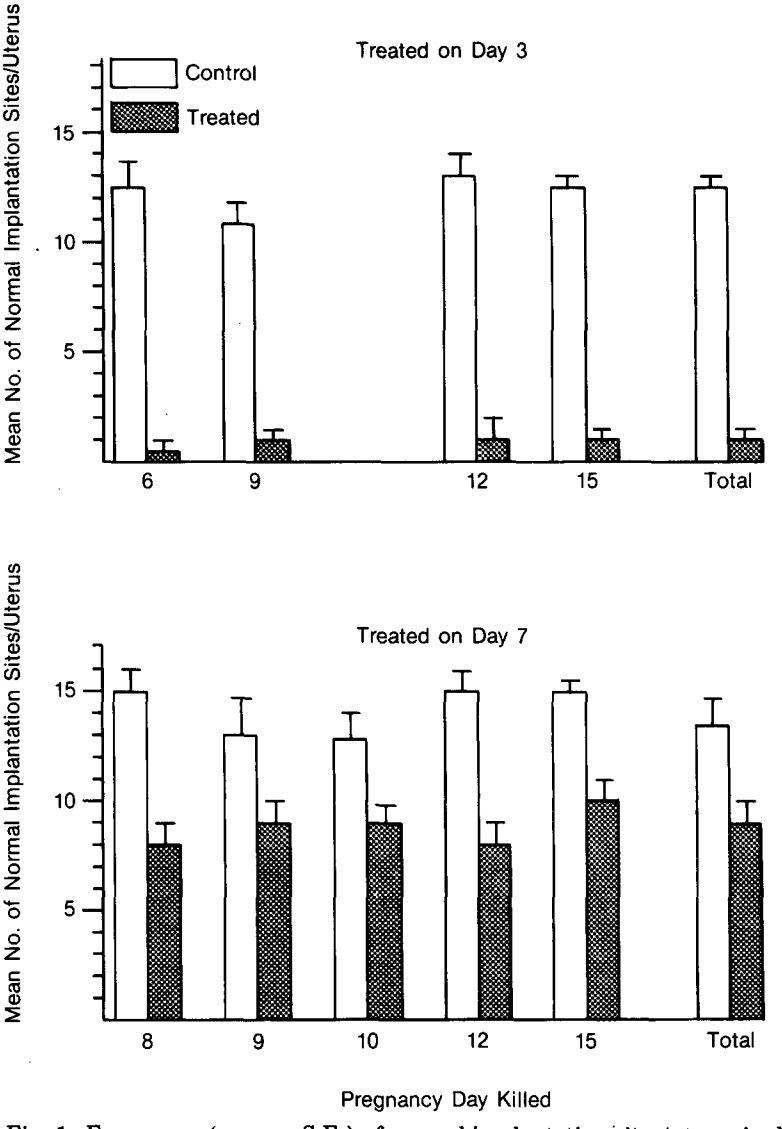


Fig. 1. Frequency (mean ± S.E.) of normal implantation sites/uterus in dams treated with N-9 or physiologic saline on pregnancy day 3 (upper) or on day 7 (lower) and killed at varying intervals afterward. A severe drop in the number of normal implantation sites was seen in dams treated on day 3 as compared to that seen in dams treated on day 7 ($P \leq 0.01$). Differences between treated and concurrent controls killed at specified days of pregnancy also were statistically significant ($P \leq 0.01$).

from the surrounding endometrium (Fig. 4). Loss of the implantation recess and presumably the embryo were less commonly seen. Active resorption, representing the stages between sequestration and complete resorption, was characterized by discrete areas of coagulation and liquefactive necrosis of the decidua, placenta or fetus, polymorphonuclear cell infiltration of same,

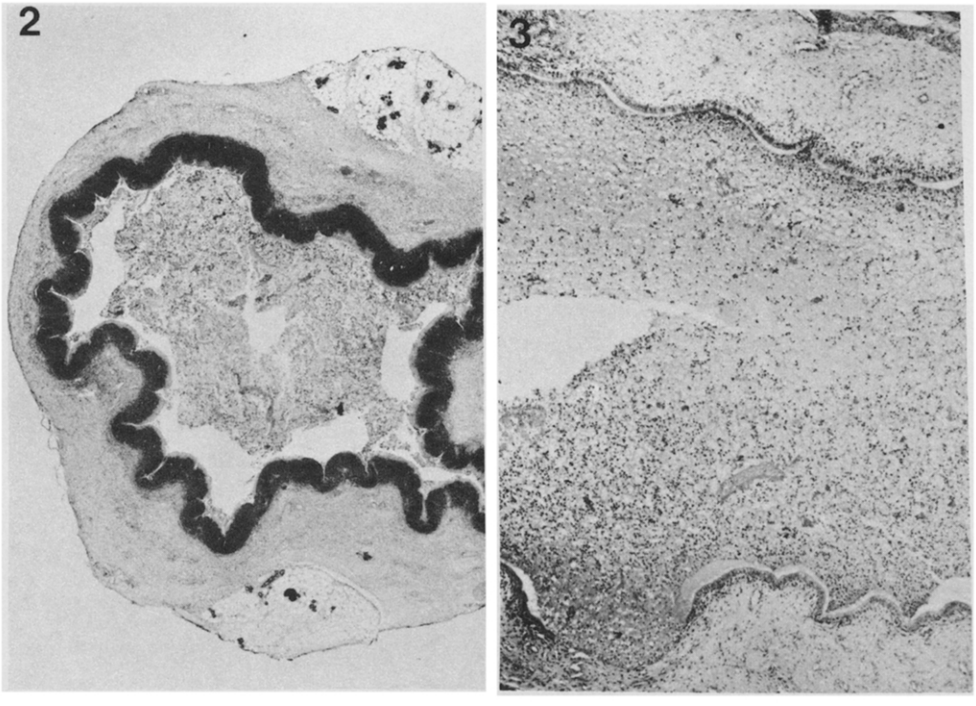


Fig. 2. Severe acute vaginitis and edema of the vaginal wall 3 days following the intravaginal application of N-9 (5 mg N-9/100 g body wt.) (H.E. × 16).

Fig. 3. Pronounced acute, focally erosive endometritis two days following the intravaginal application of N-9 (5 mg N-9/100 g body wt) (H.E. × 85).

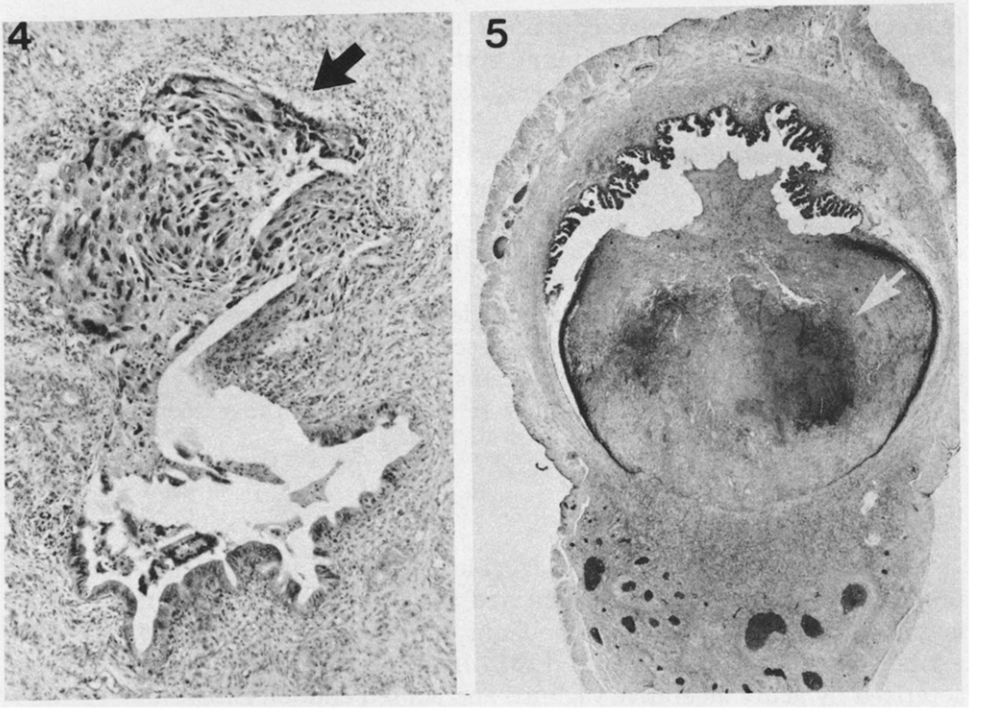
and disarray and degeneration of embryonal and decidual or placental cells (Fig. 5). Although the border line between sequestration and active resorption was not always clear, sequestration was predominantly present in implantation sites that were up to 9 days old. Complete resorption, exclusively seen in dams treated during the preimplantation period, was characterized by replacement of the conceptus by a small amount of serous or serosanguineous material which occasionally contained a few polymorphonuclear

Fig. 4. Sequestration of implantation site (arrow) on pregnancy day 12 in a dam treated on day 3. Notice disarray of decidual cells and separation of the decidua from the surrounding endometrium (H.E. × 92).

Fig. 5. Active resorption (arrow) on pregnancy day 12 in a dam treated on day 3. Notice discrete areas of coagulation and liquefactive necrosis of the decidua and polymorphonuclear cell infiltration of same (H.E. × 14).

Fig. 6. Complete resorption on pregnancy day 15 in a dam treated on day 3. There is focal distention of the uterine wall with accumulation of necrotic debris and fluid. The lining epithelium is progesteronic in character (H.E. × 14).

Fig. 7. Multicystic change (hyperplasia) in the endometrium of a female rat treated on pregnancy day 3 and killed on day 12 (H.E. × 12).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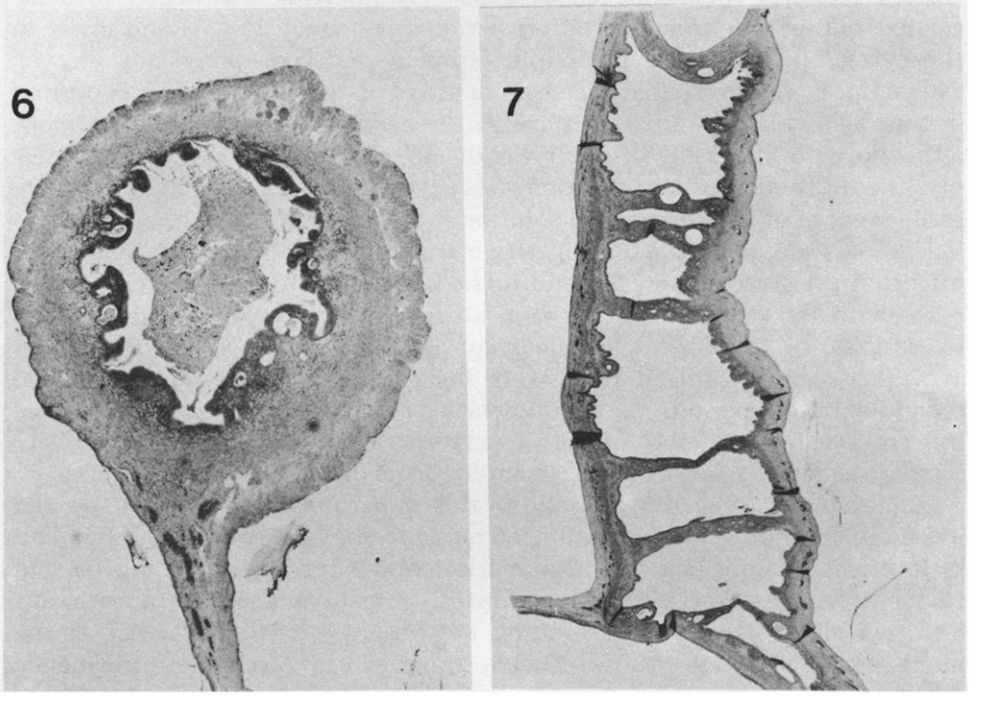
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leukocytes. This fluid was contained in a cavity lined by tail cuboidal, progesteronic, low papillary uterine epithelium (Fig. 6). At times complete resorption was associated with a cystic hyperplasia of the endometrium (Fig. 7).

Other histopathologic lesions, which most commonly occurred in the uteri of treated dams, included periembryonal and perifetal accumulations of serous, hemorrhagic or inflammatory cell exudates. The presence of such exudates did not always appear to be related to regressive phenomena in the cónceptus associated with resorption.

In a small number of control dams, lesions of embryonal resorption were qualitatively similar to those just described in the treated females. The number of resorptions in control dams treated on pregnancy day 3 were slightly but not significantly higher ($P \leq 0.2$) than those seen in control dams treated on day 7.

DISCUSSION

The results show that N-9 is capable of causing genital tract lesions in pregnant rats similar to those reported previously in nulliparus rats treated either with single doses of an aqueous solution of N-9 [12] or with N-9-containing contraceptive cream [13]. In both cases the lesions were characterized by acute ulcerative vaginitis and acuted endometritis.

Interestingly, whereas acute ulcerative vaginitis was readily induced in all treated females, the incidence of acute endometritis was higher in dams treated during the preimplantation period (pregnancy day 3) and lower in those treated during the postimplantation period (i.e., pregnancy day 7). These findings suggest that a certain amount of the N-9 solution deposited in the vagina may have passed through the cervix even in pregnant animals, although with increasing difficulty as the age of pregnancy advanced. Probably, the more mature implantation sites act as a barrier impeding the anterograde seepage of the spermicidal solution into the uterine lumen.

The data also revealed that intravaginal application of N-9 has an adverse effect on pregnancy rates, the end point being resorption of the conceptus. Also, with the exception of endometritis, there was morphologically little to distinguish between spontaneously occurring and N-9-associated resorptions. The resorption rates were highest in dams treated during the preimplantation period. These observations suggest that the more mature the embrya/feti are at the time of exposure to N-9, the more effectively they are shielded against the detrimental effects of the spermicidal agent.

Experimental procedures used in this experiment, viz, dosing by the intravaginal route and application of metallic clips on the vulval labia, had little effect on pregnancy. The numerical values for implantation sites and frequency of resorptions in the control dams compared well with our historical control data. Furthermore, the observed small difference in the number of implantations/uterus between control rats treated on pregnancy day 3 and those treated on day 7, although suggestive of an increased susceptibility on day 3, were not statistically significant.

Although the underlying mechanisms leading to N-9-induced embryonal or fetal death remain to be elucidated, they may partially be related to interference with endometrial integrity [12] and/or to concurrent direct damage of fetal membranes (due to anterograde intrauterine spread of the intravaginally deposited N-9), either of which secondarily deprive the conceptus of an appropriate environment for growth. An alternative mechanism of embryonal/fetal death may perhaps be related to a direct embryotoxic action of N-9 via the circulation which may be brought to bear on the embryo concomitantly with or independently of the endometritis. Previous studies demonstrated that: (a) ^{14}C-labelled N-9 in aqueous solution is rapidly absorbed into the blood stream from the vaginas of non-pregnant [14] and pregnant [15] rats; (b) at 6 h the level of [^{14}C]N-9 in the uterus and placenta is in equilibrium with that of maternal plasma (approx. 1.3 μg/ml or g); and (c) the amounts of ^{14}C in the amniotic fluid and fetus are one-third of that observed in the mother's plasma [15]. Further, in vitro studies have indicated that the cleavage and hatchability of two-cell stage mouse embryos are adversely affected after exposure to Brinster's culture medium containing 0.5 μg/ml of N-9 [16].

The relevance of the rat model to the human gravida remains to be established. It would appear that anatomophysiologic differences between the genital system of the rat and that of the woman together with postural differences between the 2 species would tend to favor the development of a much more severe response in the rat [12].

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