Brief Definitive Report

EFFECT OF INGESTED SPERM ON FECUNDITY IN THE RAT

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The control of female fertility by alimentary tract vaccination with sperm antigens has remained a theoretical possibility connecting observations of sperm immunogenicity and consequent infertility in various species, including humans (for review see references 1–3), with the translation of gut mucosal immune reactivity to anatomically remote mucosal sites, including the female genital tract (4–6). Here one reports that the intragastric administration of live epididymal rat sperm to adult isohistogeneic female virgin rats resulted in short- to long-term infertility related to the appearance of antisperm antibodies in genital tract secretions. This finding supports the concept of a common mucosal immune system (7) in that an immune response to non-self tissue antigens generated in gut-associated lymphoid tissues may be expressed at other mucosal sites and that such a response to sperm antigens in the female genital tract may diminish fecundity.

Materials and Methods

Four-month old virgin inbred NZBW (Rt 1^1) female rats were divided into four test groups and caged according to their stage of estrus determined by vaginal smear stains (8); day 0 = estrus (7 rats), day +1 = metestrus (7 rats), day +2/3 = diestrus (10 rats), day −1 = proestrus (7 rats). Each was fed 1.5×10^7 washed NZBW epididymal sperm by gastric intubation (without anesthesia) within 20 min of each other on the same test group cycle day of two consecutive cycles. Sperm were obtained on each occasion from three mature syngeneic males by teasing the dissected epididymus in 10 ml of physiological phosphate-buffered saline (PBS) and washing three times in 40 ml PBS (centrifugation at 260 *g* for 10 min at room temperature) before resuspension of 1.5×10^7 sperm/ml. 10 age-matched NZBW control females were each similarly fed 1 ml of the PBS supernatant obtained from the final sperm wash (before resuspension of sperm for test rat feeding). All rats were rested for 20 d following the second intragastric dose of sperm. They were then douched, under halothane anesthetic, with 150 μl of PBS delivered and recovered by an automatic pipette through a soft silicone rubber tip introduced 5 mm into the vagina. The recovered genital tract secretory fluid was stored in sealed siliconized 300-μl glass test tubes at −20°C for subsequent antibody analysis.

Preliminary experiments indicated that although the vaginal deposition of epididymal sperm 8 d before mating was not, on its own, sufficient to influence fertility in untreated rats, it resulted in decreased fecundity in sperm-fed females during the initial 6 wk of male cohabitation ($P < 0.05$; Wilcoxin, Breslow). Thus, in an attempt to boost or "focus" any genital tract antisperm responses, without risking pregnancy, 4×10^6 immobile epididymal sperm (inspected after 45 min in PBS at 22°C) in 100 μl PBS were introduced into the vagina of each test and control rat. This was carried out using an automatic pipette and soft silicone rubber tip immediately after the first collection of genital tract

secretory fluid. 7 d later a second sample of genital tract secretory fluid was obtained from all rats and stored as before. On the next day seven NZBW males, of proven breeding ability, were introduced (one per cage) into cages containing five or seven test or control females, still grouped according to their original estrus stage determination. To minimize bias resulting from variations in individual male rat breeding performance, all males were rotated to the next cage of test or control females every 2 d for 180 d of breeding observation.

Antisperm antibodies present in genital tract secretory fluids of test and control rats were assessed by two procedures. Direct and indirect microagglutination was carried out and assessed according to the method of Friberg (9) with the additional inclusion of goat anti-rat IgG, IgM, and IgA antibodies (Miles Laboratories, Elkhart, IN) after washing epididymal sperm previously incubated in genital secretory fluids. Antisperm antibody titers were also measured by indirect immunofluorescence (10) in which microscope slides coated with air-dried methanol-fixed homologous strain sperm were first incubated with doubling dilutions of test or control genital tract secretory fluid. After washing, goat anti-rat IgG, IgM, or IgA antibodies (Miles Laboratories) were added to each slide. After again washing thoroughly, FITC-conjugated rabbit anti-goat IgG antibody (Miles-Yeda, Rehovot) was added. Titer determinations were read without knowledge of the source of secretory fluid on each slide using a Leitz Orthoplan microscope. Controls, including untreated virgin female rat genital tract fluid and the substitution of PBS for genital tract washings, were invariably negative.

Results

Results indicated that fecundity was reduced in test females following intragastric sperm administration. This appeared to be associated with early IgA antisperm antibodies present in genital tract secretory fluid following sperm ingestion. These antibodies preceded the intravaginal deposition of immobile sperm and the subsequent introduction of breeding males. Fig. 1 shows that 9 of 10 controls produced young by day 27 (one at day 40), whereas 14 of 24 rats fed sperm on estrus cycle days 0, +1, or +⅔ produced litters between breeding days 36 and 70. The remaining 10 females in these treated groups failed to bear young by day 180. Differences between controls and each experimental group taken singly or en masse were highly significant ($P < 0.0001$, Wilcoxin (Breslow)). The most striking reduction in fertility was noted in females fed sperm during proestrus. Of these seven rats, one delivered two pups on day 56 while the second produced three on the 104th day. However, owing to the limited size of the experimental test groups, this apparent trend was not significant when analyzed by the Wilcoxin (Breslow) nonparametric survival test that accommodates consideration of breeding status ($P > 0.05$, <0.10).

Assays of genital tract antisperm antibodies in test and control groups were compared and indicated the absence of IgM or IgG activity following two intragastric doses of sperm, vaginal deposition of immobile sperm, or mating. In contrast, high IgA antisperm activity was observed in treated females 20 d after intragastric sperm. IgA antibody gradually diminished, but was still readily detectable, in test rats 1 wk after the vaginal dose retaining antisperm activity 50 and 180 d after permanent cohabitation with males. Very weak IgA antisperm antibody was observed in control rats only after the vaginal deposition of immobile sperm and this weak activity persisted through the 180-d mating period. Comparison of test groups vs. control rat antisperm antibody titers before mating

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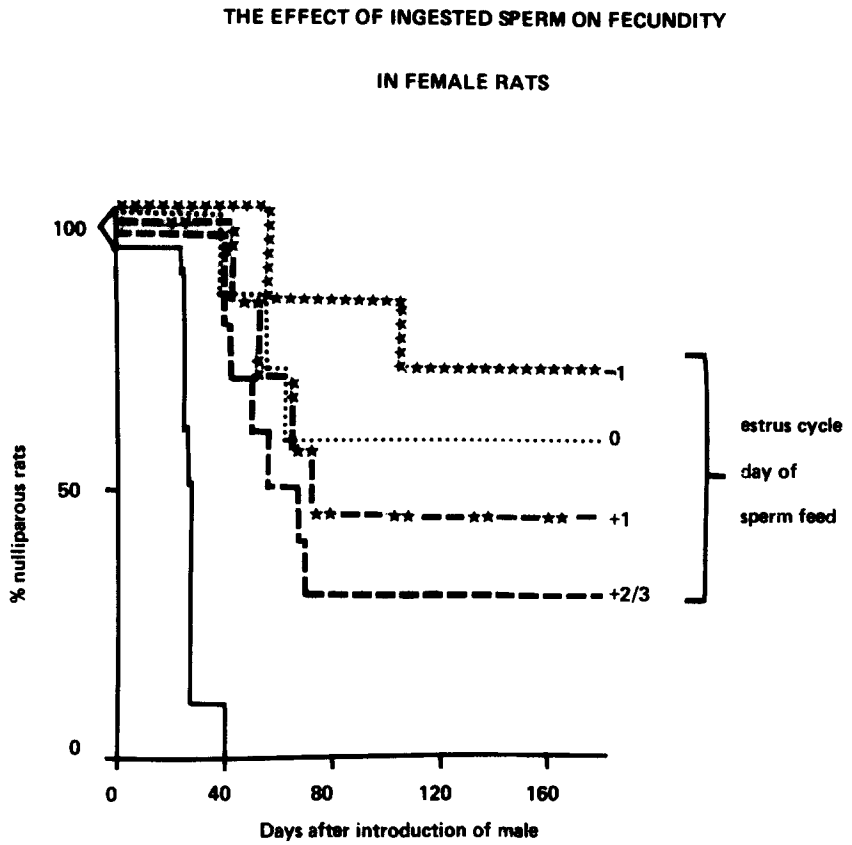

FIGURE 1. The effect of ingested sperm on fecundity in female rats. The breeding performance (decrease in percentage of nulliparous rats) of test groups of inbred female rats following two intragastric doses of syngeneic epididymal sperm on the same estrus cycle day of two consecutive cycles is compared with that of matched control females that received no sperm. Test rats dosed with sperm on days 0 (·····), estrus; +1 (--★★--), metestrus; +⅔ (– – –), diestrus and −1 (★★★★★), proestrus produced fewer litters much later than controls (——). A comparison of test groups indicates the relationship between the stage of estrus cycle sperm administration and the degree of impaired fecundity.

indicated significant differences in each instance ($P < 0.001$, Student's t test). Early IgA antibodies were generally strongest in day −1, proestrus, sperm-fed rats while progressively weaker positive antisperm reactions were noted in day 0, +1, and + ⅔ groups, respectively ($P > 0.02$, <0.05). In addition, the strongest IgA antisperm reactions within the four test groups were observed before mating in those females that failed to bear young ($P < 0.001$) (Table I).

Discussion

Several important points are suggested by these results. First, oral or intragastric administration of intact sperm may initiate an immunological process leading to reduced fecundity in the rat. This is consistent with a recent report using mice (11). However, the two studies differ in two respects. The failure of Curtis and

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TABLE I
Genital Tract IgA Antisperm Antibodies

Group (estrus cycle day of sperm feed)	*n*	IgA antisperm activity (titer range):					
		20 d after sperm feeds		7 d after sperm douche		50 d after ♂ introduction*	
		Aggl.‡	IF§	Aggl.	IF	Aggl.	IF
0	7	1:16–64	1:16–128	1:8–16	1:8–32	1:2–4	1:4–8
+1	7	1:16–64	1:16–64	1:4–8	1:4–16	1:2	1:4–8
+2 3	10	1:8–32	1:16–64	1:8	1:8–16	1:4–8	1:4–16
−1	7	1:64–128	1:64–256	1:8–32	1:16–64	1:4–8	1:4–16
Control	10	0	0	1:2–4	1:2–8	1:2–4	1:2–8
Sperm-fed test females							
Without pups	15	1:32–128	1:64–256	1:8–32	1:16–64	1:2–8	1:4–16
With pups	16	1:8–64	1:16–128	1:4–16	1:4–32	1:2–8	1:4–16

* IgA antisperm antibody levels were representative of the period following female/male cohabitation (assessed to day 180).
‡ Group range of positive sperm agglutination endpoint titers.
§ Group range of positive sperm immunofluorescent endpoint titers.

Ryan to link antisperm antibodies with infertility may reflect the fact that sera, not genital tract secretory fluids, were assayed and that this was done later rather than early in the experiment. Also, in the present study treated female rats continuously cohabited with breeding males for six months, as opposed to their removal after one or two mating opportunities. This approach allowed for the long-term analysis of the effects of intragastric sperm administration and indicated the possible effect of continued intermittent mucosal antigenic challenge on fecundity. Whether the ingestion of sperm may be similarly related to reduced fertility in human females must remain a speculation. Secondly, because of that possibility, this study may provide the framework for a contraceptive strategy excluding exogenous hormonal therapy, mechanical and surgical intervention. However, since the effects of ingested sperm on other than virgin females were not tested, the possible applications of such a strategy must await tests conducted on groups of previously mated and breeding females. Thirdly, recognition of the trend suggesting the possible importance of hormonal influences on the outcome of local immune responses at mucosal sites remote from the gut after per oral immunization may lead to a reconsideration of the conditions under which clinically successful oral vaccination against pathogenic microorganisms might be achieved. Supportive evidence indicates that estradiol has a marked stimulatory effect on IgA and IgG levels in the rat uterus (12, 13) and intragastric immunization may lead, through estrogen influence, to the accumulation of gut IgA antibodies in uterine secretion (14), while increasing the numbers of IgA positive lymphoid cells that migrate into the rat uterus (15) and cervix (6).

The present evidence, including prolonged reduction of fertility in sperm-fed rats and the detection of IgA antisperm antibodies in test genital tract secretions, suggests that immunological mechanisms may reduce fecundity in this rat model. Early work using sperm-injected rats, as well as many more recent investigations (see reviews 1, 2), supports this interpretation. Whether the hormonally influenced transfer of functional antisperm immune reactivity from the gut to the genital tract is dependent upon the transport of gut-synthesized immunoglobulins

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via the blood (5, 14, 16, 17), the migration of sensitized lymphoid cells (6, 15, 18), or both, remains to be demonstrated.

Finally, since some sperm antigens cross-react with those of the blastocyst (19–22), Menge's observation that uterine secretory IgA from sperm-sensitized rabbits can express embryocytotoxic activity (21) suggests a mechanism active in reducing fecundity in this model additional to the more direct effects of genital tract antibodies on sperm. It therefore remains to be established in humans whether some of the early abortions reported in infertile couples (22) are associated with the generation of an immune response to ingested sperm.

Summary

The intragastric administration of homologous strain epididymal sperm to adult virgin NZBW (Rt 1^1) female rats was shown to induce short- to long-term infertility. Infertility was associated with an early rise of genital secretory fluid IgA antisperm antibody preceding mating.

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