

Fertility of sperm from $t/+$ mice: evidence that $+ -$ bearing sperm are dysfunctional

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Summary

Male mice carrying one complete t haplotype transmit it to virtually all offspring. This implies that either the sperm carrying the t haplotype have an advantage in fertilization, or that the sperm not carrying a t haplotype are dysfunctional. To distinguish between these possibilities, epididymal sperm from $t^{w32}/+$ and congenic $+/+$ males were artificially inseminated into the uterus of $T/+$ females, and the transmission ratio determined from the phenotype of the offspring. After artificial insemination (AI) of sperm from $t^{w32}/+$ males, the mean ratio was 0.95 (3 experiments, 5 litters), demonstrating that the transmission ratio remains high after AI into the uterus. After AI of a mixture of equal numbers of motile sperm from $t^{w32}/+$ and congenic $+/+$ males, the mean ratio was 0.22 (6 experiments, 8 litters). These results suggest that sperm carrying a t haplotype from $t/+$ males are not superior to sperm from $+/+$ males, and therefore imply that sperm not carrying a t haplotype from $t/+$ males are dysfunctional.

1. Introduction

Male mice heterozygous for a complete t haplotype transmit that t haplotype to virtually all of their offspring, a phenomenon called transmission distortion (for a review of t haplotypes, see Silver, 1986). Since these males produce equal proportions of germ cells carrying a t haplotype (t -sperm) and germ cells not carrying a t haplotype ($+ -$ sperm) (Hammerberg & Klein, 1975), either t -sperm have an advantage in fertilization or $+ -$ sperm are disadvantaged. To determine the fertility of these sperm relative to normal sperm, we have mixed epididymal sperm from $t/+$ and $+/+$ males and artificially inseminated the mixture into the uterus of $T/+$ females, and observed the phenotype of the offspring to determine the transmission of the t haplotype in the presence of sperm from $+/+$ males. Because it has long been known that sperm of different strains often do not have equal chances of fertilization when mixtures of equal quantities are used for artificial insemination (Edwards, 1955), $t^{w32}/+$ males and congenic $+/+$ males were used for these experiments, so that sperm from the two types of males differed genetically only in the region of the t complex. Our results support the hypothesis that $+ -$ sperm from $t/+$ mice are dysfunctional.

2. Materials and Methods

Mice

Males used were hybrids of two inbred strains, C57BL/6 (B6) and C3H, where either parent carried the t^{w32} haplotype (for a complete description of this strain, see Tessler, Carey & Olds-Clarke, 1981). Females used for natural and artificial insemination were either of the same hybrid strain, hereafter called F_1 , or were outbred from crosses of the F_1 , B6 or C3H strains. All females carried *Brachyury*, T .

Each male used for artificial insemination was first tested to determine his transmission ratio after natural mating. Each $F_1-t^{w32}/+$ male was bred with $T/+$ females until 7–11 litters (containing a minimum of 25 short-tail and tailless offspring) were collected. Because the genetic background can have a significant influence on the phenotype of $T/+$ (Mickova & Ivanyi, 1974), all $F_1-+/+$ males were also tested with $T/+$ females and 5–7 litters (containing a minimum of 16 short-tail and tailless offspring) were collected.

Artificial Insemination

Males were killed by cervical dislocation, the cauda epididymides removed and cut open in several places

to release sperm from the tubules, then incubated in 300–400 μl of medium under silicon oil at 37 °C in 5% CO_2 in air. The medium used was a modified Krebs–Ringer-bicarbonate solution (Wolf & Inoue, 1976) containing 0.3% bovine serum albumin. After 15 min the epididymal tissue was removed from the medium, and two 10- μl samples of the sperm suspension were diluted and assayed in a hemocytometer for sperm density and frequency of motility. Sperm from one male of each genotype were used for each insemination.

For sperm populations mixed prior to artificial insemination, the percent motile sperm varied from 34 to 71, and density varied from 2.0 to 6.5×10^7 sperm/ml. When necessary, volumes of the two types of sperm were adjusted so that the sperm suspension used for insemination contained approximately equal numbers of motile sperm from each type of male. In two inseminations a larger volume of sperm suspension from $F_1-t^{w32}/+$ males was used, in one insemination a larger volume of sperm suspension from the $F_1-+/+$ male was used, and in three inseminations equal volumes of sperm suspension from the two types of males were used. Sperm were mixed and inseminated as soon as the motile sperm density had been determined.

Females were induced to ovulate with intraperitoneal injections of 5-IU pregnant mare's serum gonadotrophin (Sigma) followed 48 h later by 5-IU human chorionic gonadotrophin (hCG) (Sigma). The hCG injection was timed so that ovulation would occur 1–2 h after artificial insemination.

Artificial insemination was done by the method of West *et al.* 1977, except that the vagina was not stimulated artificially by insertion of a vibrating brass rod or by a cotton wool ball soaked in isotonic saline. Instead, immediately after the insemination, females were placed with vasectomized males for 2–3 h. Females were then examined for a vaginal plug and isolated. Only one of the females delivered normally; the other pregnant females were killed by cervical dislocation 20–21 days after artificial insemination. The proportion of artificially-inseminated females that became pregnant was similar to that of West *et al.* (1977). The tails of all fetuses and pups were examined and classified as normal (*nt*), short (*st*), or tailless (*ot*). Fetuses and pups from all females inseminated with the same type of sperm were pooled for calculation of the mean transmission ratio.

3. Results

Seven of the eight $F_1-+/+$ males used for artificial insemination were first tested for fertility and transmission ratio by natural matings with $T/+$ females. A total of 152 *st* and 1 *ot* pups resulted from these matings, demonstrating that the appearance of 'false' *ot*, i.e. $T/+$ pups with no apparent tail, was rare in this study. The nine $F_1-t^{w32}/+$ males used for

artificial insemination were tested more extensively; a total of 12 *st* and 289 *ot* pups resulted, indicating that the mean transmission ratio for these males was 0.96 (Table 1). The transmission ratio for individual males varied between 0.92 and 1.00.

Of the 11 fetuses resulting from artificial insemination of sperm from $F_1-+/+$ males, 5 were *st* and none were *ot*. Of the 42 fetuses resulting from artificial insemination of sperm from $F_1-t^{w32}/+$ males, 1 was *st* and 19 were *ot*; the transmission ratio in individual litters varied from 0.89 to 1.00. These results demonstrate that the transmission ratio of t^{w32} after artificial insemination of sperm into the uterus is similar to the transmission ratio after normal mating. Of the 53 fetuses and pups resulting from artificial insemination of a mixture of sperm from $F_1-+/+$ and $F_1-t^{w32}/+$ males, 18 were *st* and 5 *ot*; the transmission ratio in individual litters varied from 0.00 to 0.50. The mean transmission ratio after artificial insemination of a mixture of sperm from $F_1-+/+$ and $F_1-t^{w32}/+$ males, 0.22, is significantly different from the ratio after artificial insemination of sperm from $F_1-t^{w32}/+$ males (2×2 contingency table, $P < 0.005$). These results indicate that when sperm from a $+/+$ male are present in the female genital tract with sperm from a congenic $t^{w32}/+$ male, the transmission ratio of t^{w32} is substantially lower than when only sperm from a $t^{w32}/+$ male are present.

4. Discussion

Because transmission of *t* is so high after normal mating with a $t/+$ male, sperm carrying a *t* haplotype (*t*-sperm) have an advantage in fertilization over sperm in the same population not carrying a *t* haplotype ($+ -$ sperm). Many people have interpreted this to mean that *t*-sperm are superior to normal sperm (Bennett, 1975; Hammerberg & Klein, 1975; Olds-Clarke & Becker, 1978; Nadijcka & Hillman, 1980). Others have suggested that the $+ -$ sperm from a $t/+$ male may be inferior to normal sperm (Braden & Gluecksohn-Waelsch, 1958; Silver, 1986), as has been shown for a similar gene complex in *Drosophila*, *Segregation Distorter* (Tokuyasu, Peacock & Hardy, 1977). To compare the fertility of *t*-sperm to sperm from $+/+$ males, Seitz & Bennett (1985) constructed three male chimaeras from $t^{w73}/+$ and unrelated $+/+$ embryos carrying markers which could distinguish *t*-sperm from the $t^{w32}/+$ genotype, $+ -$ sperm from the $t^{w73}/+$ genotype and $+ -$ sperm from the $+/+$ genotype. While the pooled ratio of fertilizing sperm from the $t^{w73}/+$ genotype to fertilizing sperm from the $+/+$ genotype was 0.55, the ratios for individual males were 0.05, 0.67 and 0.82. This inconsistency in ratios between males could have been due to unequal numbers of germ cells of each genotype present, the different genetic background of the cells of each genotype, or both factors. Furthermore, variation in the time of mating (relative to ovulation) can also affect the relative proportions of the two types of

Table 1. Transmission ratios of congenic mice after natural and artificial insemination

Type of insemination	Male genotype	No. of males or insemination expts.	No. of litters	<i>nt</i>	<i>st</i>	<i>ot</i>	Transmission ratio $\left(\frac{ot}{st+ot}\right)$
Natural	Fl- +/+	7	41	191	152	1	0.01
	Fl- <i>t</i> ^{w32} /+	9	82	336	12	289	0.96
Artificial	Fl- +/+	2	3	6	5	0	0.00
	Fl- <i>t</i> ^{w32} /+	3	5	22	1	19	0.95
	Fl- +/+ and	6	8	30	18	5	0.22*
	Fl- <i>t</i> ^{w32} /+						

* Significantly different from the transmission ratio of Fl- *t*^{w32}/+ males after artificial insemination, using a χ -Square value for a 2 x 2 contingency table ($P < 0.005$).

progeny in litters of chimeric males containing different genotypes (Buehr & McLaren, 1984).

In the present study the genetic background of the sperm was similar except in the region of the *t*-complex, motile sperm of each genotype were mixed in equal proportions, and the time of insemination relative to ovulation was the same for all experiments. Though a relatively small number of informative offspring were recovered, the results were consistent between inseminations, the transmission ratio for mixes of *t*^{w32}/+ and congenic +/+ sperm never being more than 0.50. Since almost all of the + - sperm from the *t*^{w32}/+ sperm population were not fertile, among the sperm in the mix there should have been twice as many potentially fertile + - sperm as *t*-sperm, so that the expected transmission ratio, assuming neither *t*-sperm nor + - sperm from +/+ populations had an advantage, was 0.33. The mean transmission ratio for all mixed inseminations, 0.22, is not significantly different from this theoretical ratio. Thus the results of this study do not support the idea that *t*-sperm are superior to normal sperm.

If *t*-sperm are not superior to normal sperm, then the + - sperm produced by *t*/+ males must be dysfunctional. Whatever this dysfunction is, it does not affect the sperm until after they have been deposited in the female genital tract, since fertilization *in vitro* results in a normal transmission ratio (McGrath & Hillman, 1980) and delayed mating (relative to ovulation) can sometimes result in a normal transmission ratio (Braden, 1972). Even 90 min after coitus + - sperm are still present in the uterus in equal proportion to *t*-sperm (Silver & Olds-Clarke, 1984). Male accessory gland secretions are unlikely to play a role in the dysfunction since sperm from the cauda epididymis of *t*^{w32}/+ males, never exposed to seminal fluid, when used for artificial insemination into the uterus still yielded a high transmission of *t*^{w32} (Table 1).

The available evidence suggests that the transmission ratio distortion involves some process endemic to the female genital tract, such as sperm transport to the site of fertilization, or a feature of fertilization *in vivo* which does not occur *in vitro*. Regardless of which

sperm functions the distortion affects, the present study suggests that the result of the distortion is an impairment of the ability of + - sperm from a *t*/+ male to fertilize oocytes *in vivo*.

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