MALE STERILITY IN MICE HOMOZYGOUS FOR THE t^{w2} ALLELE

By P. G. JOHNSTON*

[*Manuscript received February* 8, 1968]

Summary

The causes of sterility in males homozygous for the male sterile viable t^{w2} allele were investigated. The numbers, motility, and morphology of sperm from sterile and fertile males in the vas deferens, and in the uterus and fallopian tubes of females inseminated by these males were examined.

High morphological abnormality was found in sperm from sterile males, and also a significant reduction in numbers and motility was observed. No sperm from these males reached the fallopian tubes. Significantly lower testes weights of sterile males further suggested disturbed spermatogenesis. There are indications that the cause of sterility may vary, depending on the particular combinations of t alleles.

I. INTRODUCTION

The T locus in the house mouse, *Mus musculus*, has been a source of interest over the years, primarily because of the many varied properties shown by its alleles. One of these properties, male sterility, has received considerable attention. However, some of the findings have been contradictory — which suggests that the causes of sterility may vary, depending on the particular allele or combination of alleles.

Male sterility can be brought about by practically all combinations of the t alleles, whether they are lethal, viable, or wild viable male sterile. These combinations are as follows: (1) compounds of two different lethals, L/L (Bryson 1943; Braden and Gluecksohn-Waelsch 1958); (2) compounds of one lethal and one viable, L/V (Gluecksohn-Waelsch, Segal, and Fitch 1950; Rajasekarasetty 1954; Braden and Gluecksohn-Waelsch 1958); (3) compounds of two different or homozygous viables, V/V (Braden and Gluecksohn-Waelsch 1958); (4) compounds of one lethal and one viable male sterile, L/V^{MS} (Bennett and Dunn 1967); (5) compounds of different or homozygous wild viable male steriles, V^{MS}/V^{MS} (Bennett and Dunn 1967). Although this latter type has been observed and the three alleles t^{w2}, t^{w8}, and t^{w36} have been found to be completely male sterile when homozygous, no detailed study has been carried out.

In this study the numbers, motility, and morphology of sperm from mice homozygous for the t^{w2} allele were compared with the sperm from fertile genotypes. The sperm were obtained from the vas deferens and, after mating, from the uterus and fallopian tubes. Testes weights of the mice were also analysed.

* Division of Animal Genetics, CSIRO, P.O. Box 90, Epping, N.S.W. 2121.

II. Materials and Methods

(a) *Stock*

Tailless mice Tt^{w2} were obtained from Dunn in 1963 and have since been crossed with a number of lines—*Brachy* $T+$, random wild type, and feral mice from the Sydney area (tested to be free of the t alleles). The genotypes Tt^{w2}, $t^{w2}t^{w2}$, $+t^{w2}$, and $++$, which were maintained in the laboratory, were used in this experiment.

(b) *Sperm from the Vas Deferens*

Males homozygous for the t^{w2} and $+$ alleles were killed and the sperm from their vas deferens was placed on a slide, diluted with 0·9% NaCl, and examined under a phase-contrast microscope. Subjective classifications (0, 1, 2, 3, and 4) were made of the relative numbers of sperm and their motility. One hundred sperm were selected at random and estimates of morphological abnormality made on a similar basis to that of Bryson (1943) and Rajasekarasetty (1954).

(c) *Sperm from the Uterus*

Females were placed with males at 4 p.m. and examined for vaginal plugs on the following morning at 6 a.m. The contents of the uteri were placed on slides and estimates made of the number of sperm ejaculated and their motility. Four genotypes ($++$, $+t^{w2}$, Tt^{w2}, and $t^{w2}t^{w2}$) were examined.

(d) *Sperm from the Fallopian Tubes*

A similar procedure to the investigation *in utero* was followed. The fallopian tubes were separated from the uteri by severing the isthmus of the tube in order to prevent contamination with sperm from the uteri (Austin 1952). These were then separated from the ovary, 0·9% NaCl added on a slide, and the contents eased out and examined under a phase-contrast microscope. The number of sperm in each tube was counted.

III. Results

Table 1 shows the mean scores of relative numbers and motility and the percentage abnormality of the sperm in the vas deferens of mice of $t^{w2}t^{w2}$ and $++$ genotypes. The differences were analysed by the Student's t-test and highly significant differences were found for these characteristics.

Table 1

Mean relative numbers, motility, and percentage abnormalities of sperm from sterile and fertile genotypes

Source of Sperm	Genotype	No. of Mice	Relative No. of Sperm	Relative Motility	Percentage Abnormal
Vas deferens	$t^{w2}t^{w2}$	16	2·563	0·625	63·6
	$++$	9	3·778	3·333	14·6
Significance between genotypes			$P<0·001$	$P<0·001$	$P<0·01$
Uterus	$t^{w2}t^{w2}$	21	1·524	0·286	
	$+t^{w2}$	15	2·400	1·267	
	Tt^{w2}	11	2·455	1·273	
	$++$	15	2·730	0·933	
Significance between genotypes			$P<0·05$	n.s.	

Table 1 also shows the relative numbers of sperm recovered from the uterus and their motility. An analysis of variance showed significant difference in numbers

between genotypes. A test for comparing individual means based on Tukey's method (Snedecor 1956, p.251) showed a significant difference between $t^{w2}t^{w2}$ and $++$ genotypes but not between $t^{w2}t^{w2}$ and $+t^{w2}$ or Tt^{w2} genotypes, in which the differences just fell below the level of significance. No significant difference was found between the fertile genotypes. The analysis of variance of relative motility showed no significant differences between genotypes.

No sperm of the sterile genotype $t^{w2}t^{w2}$ were found in the fallopian tubes. On the other hand, the mean number of sperm at this site from the fertile genotype $++$ was 223 (range 40–912).

TABLE 2

COMPARISON OF MEAN BODY AND TESTES WEIGHTS OF FERTILE AND STERILE MALES

Genotype	No. of Mice Tested	Mean Body Weight (g) ± S.E.	Mean Testes Weight (g) ± S.E.	Correlation Coefficient
$++$ (fertile)	49	$28{\cdot}1184 \pm 3{\cdot}5285$	$0{\cdot}1858 \pm 0{\cdot}0306$	$0{\cdot}3714$**
$t^{w2}t^{w2}$ (sterile)	25	$24{\cdot}4240 \pm 2{\cdot}9017$	$0{\cdot}1175 \pm 0{\cdot}0035$	$0{\cdot}2343$

** $P < 0{\cdot}01$.

Table 2 shows the mean body and testes weight of the two genotypes. As the correlation coefficient between body and testes weight for the fertile genotype was significant, it was necessary to use a covariance analysis to test the difference between the testes weight of the two genotypes. This analysis is shown in Table 3 where it becomes apparent that there was a highly significant difference between the body and testes weights of the two genotypes.

TABLE 3

SIGNIFICANCE OF DIFFERENCE BETWEEN TESTES WEIGHTS IN FERTILE AND STERILE MALES WHEN CORRECTED BY COVARIANCE FOR BODY WEIGHT

x = body weight, y = mean testes weight, B = constant

Source of Variation	D.F.	x^2	xy	y^2	D.F.	$(y-Bx)^2$	Mean Square	F
Between	1	—	—	—	1	0·04561	0·04561	45·65**
Within	72	820·9956	2·0098	0·07585	71	0·07093	0·000999	
Total	73	1046·9346	6·1879	0·15311	72	0·11654		

** $P < 0{\cdot}01$.

IV. DISCUSSION

Braden and Gluecksohn-Waelsch (1958) and Bennett and Dunn (1967) found the number of sperm ejaculated by sterile genotypes V/V, V/L, L/L, and V^{MS}/L did not differ appreciably from that of fertile males. However, the present results suggest that the sperm production of the sterile genotype $t^{w2}t^{w2}$ is significantly reduced as judged by the numbers present in the vas deferens and in the uterus after mating.

Bryson (1943) found a high proportion of morphologically abnormal sperm in the vas deferens of sterile (L/L) males but felt this was insufficient to account for the sterility. Braden and Gluecksohn-Waelsch (1958) support these findings for they showed that some of the more fertile genotypes (V/V) produced a higher proportion of morphologically abnormal sperm than did the less fertile or sterile genotypes (V/L, L/L). Rajasekarasetty (1954), on the other hand, concluded that morphological abnormality was an important factor and could result in the number of normal spermatozoa falling below a threshold value in V/L genotypes, thus reducing or preventing fertilization. Head abnormalities, together with a reduction in size and an increase in headless sperm, were frequently found in our $t^{w2}t^{w2}$ males, and the incidence would appear to be too high for fertilization to take place.

Bryson (1943) and Braden and Gluecksohn-Waelsch (1958) found that the sperm from sterile genotypes (V/V, V/L, and L/L) had reduced motility, but they did not attach much importance to it.

Bennett and Dunn (1967) found that the sterility of V^{MS}/L genotypes was correlated with reduced motility and low numbers of sperm at the site of fertilization and not with reduced numbers inseminated. The motility of $t^{w2}t^{w2}$ sperm in this experiment was significantly reduced and differed in action to that of the fertile genotypes. The motion of the tails, instead of showing a regular flowing movement, was irregular and disjointed in action.

Braden and Gluecksohn-Waelsch (1958) found virtually no sperm of L/L or V/L genotypes in the fallopian tubes after mating and suggested this might be due to inability of the sperm to traverse the utero–tubule junction. There was, however, some penetration of eggs by sperm from V/L males. Bennett and Dunn (1967), working with V^{MS}/L males, also found reduced numbers of sperm in the fallopian tubes but these were totally incapable of fertilization. In our study, no sperm was found in the fallopian tubes, and any one of the sperm's characteristics, namely amount, motility, or morphological abnormality, could have prevented it from reaching the site of fertilization.

Bryson (1943) noted a significant reduction in the testes size of L/L mice which was correlated with the diameter of the seminiferous tubules and suggested that the combination of two lethal alleles had a deleterious effect on spermatogenesis. A similar reduction in testes weight has been found in this study which further supports the fact that spermatogenesis is highly abnormal in these mice.

Clearly the degree of sterility can vary and the number of sperm ejaculated as well as their morphology, motility, and perhaps fertilizing capacity can differ considerably depending on the combination of t alleles. This appears to be the reason why the results of previous workers have been contradictory, for they have considered causes of sterility to be the same for all combinations. There is evidence that spermatogenesis is not as severly affected in the V/V and V/L genotypes as in the L/L and V^{MS}/V^{MS} combinations. The causes of sterility can all be directly related to abnormal spermatogenesis, but the ways in which fertilization is prevented may differ from one combination of t alleles to another.

V. Acknowledgments

The author wishes to thank Miss Marilyn Daley for her valuable technical assistance and Dr. J. M. Rendel for reading the manuscript.

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