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The *in vivo* and *in vitro* transmission frequencies of the t^{w5}-haplotype in mice

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Summary

The recessive t^{w5}-haplotype, a complete haplotype, is transmitted by heterozygous male mice at very high frequencies (> 0·90) in normal matings. The present studies were undertaken to determine the effects of delayed matings and *in vitro* fertilizations on this phenotypic expression. Males carrying the t^{w5}-haplotype ($+/t^{w5}$) were first tested for their frequencies of transmission of the mutant 17th chromosome in both normal and delayed matings. Spermatozoa obtained from these same males were then used to fertilize eggs *in vitro*. The *in vivo* and *in vitro* transmission frequencies were found to be statistically equivalent in all types of inseminations. An *in vitro* fertilization time course study showed that the same percentages of eggs are fertilized by t^{w5}-bearing spermatozoa when the gametes are coincubated for either 2 or 6 h. The data lead to the conclusion that the transmission frequency of the t^{w5}-haplotype is not affected either by the length of time elapsing between insemination and fertilization or by the environment in which fertilization occurs.

1. Introduction

In wild populations, 17th chromosomes bearing *t*-haplotypes are transmitted from heterozygous males ($+/t$) at very high frequencies (i.e. > 0·90; Dunn & Suckling, 1956; Dunn, 1957). When these mice are maintained in the laboratory, the frequency of transmission of the haplotype may remain high, be reduced to a moderately high frequency (0·70–0·80) or to a frequency near Mendelian. This change is observed in normal matings in which ovulation and fertilization occur approximately 6–8 h after insemination (Braden & Austin, 1954; Braden, 1972). For example, in our colony males bearing the t^6-haplotype originally transmitted the mutation to 94% of their offspring (Nadijcka & Hillman, 1975). Following maintenance in the colony for five years, males transmitted the t^6-haplotype to only 52% of their offspring (McGrath & Hillman, 1980*a*). The amount of the decrease in the transmission frequency following colony maintenance is dependent upon the specific *t*-haplotype and upon other factors such as the genetic background of the male and the number of modifiers located in the residual genotype (Bennett *et al.* 1983; Gummere *et al.* 1986; Lyon, 1984, 1986).

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It has been reported that the transmission frequencies of those haplotypes which are transmitted at either moderately high or near normal percentages in normal matings are decreased in delayed matings in which fertilization occurs within 2 h after insemination (Braden & Austin, 1954; Braden, 1958, 1972; Yanagisawa *et al.* 1961; McGrath & Hillman, 1980*a*, *b*). The studies by McGrath and Hillman show that in normal matings the mean transmission frequency of the t^{12}-haplotype is 78% and, as stated above, of the t^6-haplotype, 52%. The percentages of eggs fertilized by spermatozoa carrying either of these haplotypes are significantly reduced in delayed matings. Also, when spermatozoa from these same males are used for *in vitro* inseminations, the frequency of transmission of each haplotype is reduced and equivalent to its respective frequency in delayed matings (McGrath & Hillman, 1980*a*, *b*).

Unlike the *t*-haplotypes transmitted at either moderately high or near normal frequencies, the percentage of eggs fertilized by spermatozoa bearing *t*-haplotypes which are transmitted at high frequencies in normal matings are not significantly reduced in delayed matings (Yanagisawa *et al.* 1961). The present studies were undertaken to determine the effects of delayed matings and of *in vitro* fertilizations on the transmission frequency of the t^{w5}-haplotype, which is transmitted by heterozygous males ($+/t^{w5}$) to greater than 90% of their offspring in normal matings.

2. Materials and Methods

(i) *Transmission frequency*

The average *in vivo* transmission frequency of the t^{w5}-haplotype in our colony was determined by mating $Rb7/t^{w5}$ males to T/t^{w5} females. Embryos which are homozygous for the t^{w5}-haplotype die *in utero* between gestation days 7 and 10 (Bennett & Dunn, 1958). The remaining embryos are viable and are phenotypically distinguishable at birth by tail length; T/t^{w5} mice are tailless, $+/t^{w5}$ are normal tailed and $T/+$ mice, short tailed. Using the phenotypes of 3361 offspring, the mean transmission frequency of the t^{w5}-haplotype was calculated to be 0·95.

(ii) *Normal and delayed matings*

(C57BL/6J♀ × T/t^{w5}♂) F_1 females were injected intraperitoneally with pregnant mare serum (PMS; 5IU) and 48 h later with human chorionic gonadotropin (HCG; 5IU). The $T/+$ control and $+/t^{w5}$ experimental females were separately caged with an $Rb7/t^{w5}$ ($Rb7/Rb7$♀ × T/t^{w5}♂) F_1 male immediately following the HCG injection (normal matings) or 12 h after this injection (delayed matings). (*Rb7* is an abbreviation for the Robertsonian translocation *Rb(16·17)7Bnr* which was used as a marker in these studies.) On the following day the females were sacrificed by cervical dislocation. The control and experimental zygotes were flushed from the extirpated oviducts and placed into modified Whitten's medium (Abramczuk *et al.* 1977) following the protocol of McGrath & Hillman (1980*a*, *b*).

(iii) In Vitro *fertilization*

For the *in vitro* transmission frequency determinations, ova were obtained from F_1 control and experimental hormone-stimulated females and inseminated with spermatozoa obtained from the same males used for the normal and delayed transmission frequency determinations. Spermatozoa from both the caudae epididymides and vasa deferentia were used for these inseminations (McGrath & Hillman, 1980*a*). After 6 h of coincubation, the eggs were washed and placed into modified Whitten's medium.

(iv) *Time course studies*

Eggs from hormone-stimulated F_1 hybrid experimental females were inseminated *in vitro* with spermatozoa from $Rb7/t^{w5}$ males and allowed to coincubate for either 2 or 6 h. These time periods were chosen to simulate delayed and normal matings. Following each of these periods of coincubation, the eggs were washed and placed into modified Whitten's medium.

(v) *Embryo culture*

The protocol of McGrath and Hillman (1980*a*) was used for culturing the zygotes until they reached the blastocyst stage of development.

(vi) *Karyotyping*

The embryos were karyotyped at the blastocyst stage and scored for the presence or absence of the metacentric *Rb7* chromosome (Garside & Hillman, 1985). It has been reported that the *Rb7* chromosome causes approximately 4% non-disjunction in heterozygous males (Gropp & Winking, 1981). Since this percentage could introduce error into the calculation of the transmission frequency, at least two chromosome spreads from each blastocyst embryo were counted to minimize the possibility of error. Only euploid embryos were included in the results.

(vii) *Significance determinations*

A contingency χ^2 test was used to determine significant differences in the preimplantation development of control and experimental embryos and in the numbers of eggs fertilized by t^{w5}-bearing spermatozoa in the time course studies. Significant differences among the transmission frequency percentages were determined by Student's *t* test using arcsine transformed data. The transmission frequencies were compared with Mendelian ratios using the χ^2 test. Significance was set at $P < 0{\cdot}05$.

3. Results

Before the transmission frequencies for the three types of inseminations were compared, it was necessary to determine if the different methods of insemination and the subsequent methods of culture uniquely affected the embryonic viability of the zygotes obtained from the experimental matings during preimplantation development. Therefore, experimental and control zygotes were obtained from hybrid females following normal matings, delayed matings, and from *in vitro* inseminations and compared for their development to the blastocyst stage. The same ten $Rb7/t^{w5}$ males were used for all of the studies. In the first study, 74% (191/257) of the control zygotes and 69% (557/807) of the experimental zygotes developed to the blastocyst stage; in the second, 70% of the control (139/199) and 75% of the experimental (734/980) developed to the blastocyst stage; and, in the third, 69% (230/355) of the control and 67% (608/907) of the experimental reached the blastocyst stage of development. There are no significant differences between the numbers of control and experimental zygotes developing to the blastocyst stage from the different types of inseminations (normal mating, $P > 0{\cdot}10$; delayed mating, $P > 0{\cdot}10$; *in vitro* fertilization,

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Table 1. *The* in vivo *and* in vitro *transmission frequency of the* t^{w5}*-haplotype*

	In vivo		
	Normal mating	Delayed mating	*In vitro*
Number of males used	17	17	17
Number of blastocysts scored	399	451	549
Number without *Rb7* marker	368	400	507
Mean transmission frequency	0·92	0·89	0·92

Table 2. *Time course study of the* in vitro *transmission frequency of the* t^{w5}*-haplotype*

	Time period of coincubation	
	2 h	6 h*
Number of males	17	36
Number of eggs inseminated	3667	1784
Number developing to the 2-cell stage	778 (21 %)	1427 (80 %)
Number developing from 2-cell to blastocyst	345 (44 %)	1011 (71 %)
Mean transmission frequency	0·90	0·92

* Includes the embryos from the *in vitro* column in Table 1.

$P > 0{\cdot}05$). Thus, the three methods of insemination and the subsequent handling of the experimental zygotes do not adversely or uniquely affect their subsequent development.

The results obtained from the karyotypic analyses (Table 1) show the normal mating transmission frequency of the t^{w5}-haplotype to be 0·92 and that of the delayed matings, 0·89. These frequencies are not significantly different from each other ($P > 0{\cdot}05$), nor are they significantly different from that expected, 0·95 (normal mating, $0{\cdot}10 > P > 0{\cdot}05$; delayed mating, $0{\cdot}50 > P > 0{\cdot}20$). However, both frequencies are significantly different from Mendelian (normal mating, $P < 0{\cdot}001$ delayed mating, $P < 0{\cdot}001$).

In vitro, following 6 h of coincubation of the gametes, the transmission frequency of the t^{w5}-haplotype is 0·92 (Table 1). This frequency is equivalent to the normal and delayed mating frequencies (normal mating, $0{\cdot}50 > P > 0{\cdot}20$; delayed mating, $0{\cdot}20 > P > 0{\cdot}10$) and to the expected frequency of 0·95 ($0{\cdot}20 > P > 0{\cdot}10$). As in the *in vivo* fertilizations, there is a significant difference between the *in vitro* and Mendelian frequencies of transmission ($P < 0{\cdot}001$).

The data from the comparative *in vitro* time-course studies are shown in Table 2. The percentages of t^{w5}-bearing embryos obtained from these two time periods of coincubation are not significantly different ($0{\cdot}50 > P > 0{\cdot}25$). Also, the percentages of eggs fertilized by t^{w5}-bearing spermatozoa following either the shorter or longer coincubation period are not significantly different from those expected based upon those previously determined for normal and delayed mating studies and for the *in vitro* inseminations.

4. Discussion

In earlier studies, it was noted that the expression of the moderate and near normal transmission frequency phenotypes require that the *t*-bearing spermatozoa reside in the female reproductive tract for 6 h or longer prior to fertilization. The frequency of transmission of these haplotypes is significantly reduced when insemination occurs in delayed matings or *in vitro* (McGrath & Hillman, 1980*a*, *b*). In addition, the study of Braden & Weiler (1964) and that of McGrath & Hillman (1980*a*) show that the genotype of the female affects the frequency of transmission of these haplotypes. Based on these observations, McGrath & Hillman (1980*a*) suggested that the expression of the aberrant transmission frequency is 'dependent on an interaction between the female reproductive tract and that this interaction is time dependent'. Since the moderate and near normal transmission frequencies of the *t*-haplotypes are significantly affected by the genotype of the females, it was also suggested that the enhancing factor is intrinsic to the uterine and oviducal environments and is variable among the females of different genotypes.

Although these conclusions can be drawn from the previous studies, the current studies show that the expression of the high transmission frequency does not require spermatozoan–uterine interaction and is not time-dependent except for the length of time required to facilitate capacitation. These conclusions are based on the results obtained from the delayed matings and from the *in vitro* time-course studies. [Capacitation and fertilization can occur *in vitro* within 2 h of insemination; however, higher percentages of eggs are fertilized with longer periods of coincubation (Fraser & Drury, 1976).] Furthermore, the transmission frequency of the t^{w5}-haplotype has remained high during nine years of colony maintenance and is not affected by the genotypes of the females to which the t^{w5}-bearing males are mated. The mean transmission frequencies of this haplotype in matings between $Rb7/t^{w5}$ males and C57BL/6J, BALB/c or (C57BL/6J♀ × BALB/c♂) F_1 females are all greater than 90 % (unpublished observation). These data show therefore, that the requirements for the expression of the high transmitting phenotype differ from those required for the expressions of the moderate and normal transmitting phenotypes.

Recently, Olds-Clarke and Peitz (1986) found that the t^{w32}-haplotype from $+/t^{w32}$ males is transmitted in

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normal matings to greater than 95% of the progeny. When the gametes from these same males are used for artificial insemination, 95% of the embryos or progeny carry the t^{w32}-haplotype. In these studies, artificial insemination occurred one to two hours following hormone (HCG) induced ovulation. This period of time is equivalent to that elapsing between ovulation and insemination in delayed matings. In both cases, the gametes reside in the female reproductive tract for no longer than 2 h prior to ovulation. Therefore, spermatozoa bearing the t^{w32}-haplotype, like those bearing the t^{w5}-haplotype, do not require an extended length of time (i.e. longer than 2 h) in the female reproductive tract to be able to fertilize greater than 90% of the eggs.

Olds-Clarke and Peitz (1986) suggest that the *t*-bearing gametes from a heterozygous (+/*t*) male do not differ from wild-type gametes; rather, the +-bearing spermatozoa from these males are dysfunctional. They hypothesize that this dysfunction involves a 'process endemic to the female reproductive tract'. However, the results of the current study show that the high transmission frequency of the t^{w5}-haplotype is expressed *in vitro*. Therefore, if the dysfunction hypothesis is correct, then the inability of the +-bearing gametes to fertilize eggs is not dependent upon spermatozoan–uterine interaction. Because of the similarities between the t^{w32}- and t^{w5}-haplotypes in the maintenance of their high transmission frequencies in normal matings, delayed matings and in artificial inseminations which simulate delayed matings, the high transmission phenotype of the t^{w32}-haplotype should also be expressed in *in vitro* inseminations.

Since the different types of inseminations have different effects on the transmission frequencies of three complete haplotypes (t^{12}, t^{w5}, t^{w32}) an overall control mechanism for the transmission distortion phenotype can not yet be proposed. Furthermore, the differences between the maintenance of or changes in the transmission frequencies of the different *t*-haplotypes under the various methods of insemination are not readily explainable on the basis of the current understanding of the genetic control of the aberrant transmission frequency phenomenon (Lyon, 1984, 1986, 1987). The cause or causes of this phenomenon and the effect of *in vivo* and *in vitro* fertilization on this phenotypic expression will be elucidated only when the products of, and the interaction of the products of, the factors which are known to be associated with the transmission distortion phenotypes are defined.

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