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The transmission ratio distortion of the t^{h2}-haplotype *in vivo* and *in vitro*

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Summary

The t^{h2}-haplotype is transmitted at low frequencies ($< 0{\cdot}30$) *by* $+/t^{h2}$ males in normal matings. In the studies described here, the transmission frequency of the t^{h2}-haplotype from $Rb7/t^{h2}$ males was determined for normal and delayed matings and *in vitro* inseminations. The data show the transmission frequency from the two *in vivo* inseminations to be less than 0·30 and to be statistically equivalent. However, the *in vitro* transmission frequency (0·80) is significantly greater than either of the *in vivo* frequencies. The results show that the environment in which fertilization occurs affects the transmission frequency of this specific *t*-haplotype significantly.

1. Introduction

The distorted transmission frequency of some *t*-haplotypes (those which are complete haplotypes and those missing only the proximal sterility factor) can be altered by the genetic background of the male (Gummere *et al.* 1986), by the numbers of genetic modifier loci (Bennett *et al.* 1983), by the genetic background of the females to which the heterozygous males are mated (Braden & Weiler, 1964; McGrath & Hillman, 1980*a*) and by the amount of time elapsing between insemination and ovulation (Braden, 1958, 1972; Yanagisawa *et al.* 1961; McGrath & Hillman, 1980*a*, *b*). McGrath & Hillman (1980*a*, *b*) have also found that the transmission frequencies are modified when the spermatozoa from $+/t$ males are used for *in vitro* fertilization.

The present study was undertaken to determine the effects of varying the time period elapsing between insemination and ovulation *in vivo* (i.e. normal vs. delayed matings) and the effects of *in vitro* fertilization on the transmission frequency of the partial haplotype, t^{h2}. Embryos homozygous for this haplotype are viable *in vivo* and males which are heterozygous transmit the t^{h2}-bearing chromosome in normal matings at frequencies which are significantly lower than Mendelian (Lyon & Meredith, 1964; Sherman & Wudl, 1977).

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2. Materials and Methods

$Rb7/t^{h2}$ males were used to determine the transmission frequency of the t^{h2}-haplotype. The Robertsonian translocation *Rb(16·17)7Bnr* is used in the present studies as a marker. The $Rb7/t^{h2}$ males were mated with hormone-induced (C57BL/6J♀ × T/t^{h2}♂) F_1 females [5IU pregnant mare serum gonadotropin (PMS) followed 48 h later with an injection of 5IU human chorionic gonadotropin (HCG)]. The hybrid $+/t^{h2}$ females served as the experimental females and the $T/+$ as the control females. These females are phenotypically distinguishable from each other by tail length: $+/t^{h2}$ mice have tails of normal length while those with the genotype $T/+$ are short-tailed.

In the first series of studies, $Rb7/t^{h2}$ males were individually mated with $+/t^{h2}$ females to determine the transmission frequency of the t^{h2}-haplotype in normal and delayed matings. Ten hours after each type of mating, the zygotes were obtained from the excised oviducts and placed into culture medium (McGrath & Hillman, 1980*a*, *b*). The same males were used as the source of mature spermatozoa for the *in vitro* fertilizations. This protocol and the subsequent embryo culture methods for the zygotes from the three types of inseminations were previously described (McGrath & Hillman, 1980*a*, *b*). The zygotes were allowed to develop to the blastocyst stage and then karyotyped (Garside & Hillman, 1985). An embryo not carrying the *Rb7* chromosome was scored as being fertilized by a t^{h2}-bearing spermatozoon. The transmission frequency was determined from the ratio of the number of embryos lacking the *Rb7* marker to the

total number of embryos scored. Only euploid embryos were included in the results and at least two chromosome spreads from each blastocyst-staged embryo were counted to minimize error caused by non-disjunction resulting from the presence of the *Rb7* chromosome (Gropp & Winking, 1981).

The development of zygotes obtained from experimental ($+/t^{h2}♀ \times Rb7/t^{h2}♂$) and control ($T/+♀ \times Rb7/t^{h2}♂$) matings through the preimplantation stages was compared to ensure that the death of the experimental embryos was not above background. One of the genotypes of the zygotes obtained from experimental females mated to $Rb7/t^{h2}$ males, or through *in vitro* fertilization using these same males as sources of spermatozoa, is t^{h2}/t^{h2}. Although t^{h2}/t^{h2} embryos are viable *in vivo*, there have been no studies on their development *in vitro*. Differences in the preimplantation viability of t^{h2}/t^{h2} embryos *in vivo* and *in vitro* could result in an erroneous calculation of the transmission frequency. None of the zygotes from the control matings are homozygous for any preimplantation lethal mutations.

The transmission frequency percentages were arcsine transformed and Student's t test was used to determine the significance of differences among them. A χ^2 test was used to compare the transmission frequencies with Mendelian ratios. Significant differences in the preimplantation development of control and experimental embryos were determined by a contingency χ^2 test. Significance was set at $P < 0{\cdot}05$ for all statistical tests.

3. Results

The data from the first series of insemination studies (Table 1) show that the transmission frequency of the t^{h2}-haplotype is 0·26 and 0·23 in normal and delayed matings, respectively. These frequencies are not significantly different from each other ($t = 1{\cdot}67$, $0{\cdot}10 > P > 0{\cdot}05$); however, both are significantly lower than Mendelian (normal mating, $\chi^2 = 97$, $P < 0{\cdot}05$; delayed mating, $\chi^2 = 120$, $P < 0{\cdot}05$).

These data also show that the *in vitro* transmission frequency of the t^{h2}-haplotype is 0·80. The difference between the transmission frequency of the t^{h2}-haplotype following either the normal or the delayed matings and the *in vitro* transmission frequency of this mutation are significant ($t = 9{\cdot}67$, $P < 0{\cdot}001$ and $t = 13{\cdot}14$, $P < 0{\cdot}001$, respectively). Also, the *in vitro* transmission frequency is significantly higher than Mendelian ($\chi^2 = 169{\cdot}44$, $P < 0{\cdot}001$).

There are no significant differences between the percentages of blastocyst embryos which develop from experimental and control zygotes obtained from females following normal matings, delayed matings and *in vitro* fertilization (Table 2). Thus, the development of the homozygous t^{h2} embryos through the preimplantation stages is the same as that of their littermates ($+/+$; $+/t^{h2}$) and of the control embryos.

Table 1. *The transmission frequency of the t^{h2}-haplotype*

	In vivo		
	Normal mating	Delayed mating	*In vitro*
Number of males used	34	30	22
Number of blastocysts scored	431	403	476
Number without *Rb7* marker	113	91	380
Mean transmission frequency	0·26	0·23	0·80

4. Discussion

The transmission frequency of the t^{h2}-haplotype is not significantly modified by altering the length of time between insemination and fertilization *in vivo* (normal vs. delayed matings). This finding is in agreement with earlier findings that other partial t-haplotypes are transmitted at equivalent frequencies in the two types of matings (Braden, 1972). Also, these frequencies are not significantly different from those reported for the t^{h2}-haplotype in normal matings (Lyon & Meredith, 1964; Sherman & Wudl, 1977).

The data so show, however, that the transmission frequency of the t^{h2}-haplotype can be significantly

Table 2. *Survival of embryos from the zygote stage to the blastocyst stage of development*

	Normal matings		Delayed matings		*In vitro* fertilization	
	Control (12)*	Experimental (15)	Control (9)	Experimental (13)	Control (12)	Experimental (11)
Zygotes	181	293	229	321	160	376
Blastocysts	173	276	206	294	131	316
Percentage	95·6	94·2	90·0	91·6	81·9	84·0
χ^2	0.426		0·438		0·370	
P	> 0·50		> 0·50		> 0·20	

* Numbers of males used.

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increased above its *in vivo* frequency when fertilization occurs *in vitro*. These effects differ from the effects of delayed matings and of *in vitro* fertilization on the transmission frequencies of the t^{12}-, t^{6}- and t^{w5}-haplotypes (McGrath & Hillman, 1980*a*, *b*; Garside & Hillman, 1989).

The t^{12}-haplotype (a complete *t*-lethal haplotype; Silver *et al.* 1980) is transmitted at a moderate frequency (0·70) in normal matings and at lower and equivalent frequencies in both delayed matings and *in vitro* fertilizations. The t^{6}-haplotype (a 'complete' lethal haplotype except that the proximal sterility factor, *tcs-1*, and the proximal distortion factor, *Tcd-1*, are missing; Lyon & Mason, 1977; Silver *et al.* 1983; Lyon, 1984; Fox *et al.* 1985) is transmitted at nearly normal frequencies during normal matings and at significantly reduced and equivalent frequencies in delayed matings and when insemination occurs *in vitro*. Thus, in both cases, the transmission frequencies drop significantly below their normal mating frequencies in delayed matings and in *in vitro* fertilization. Conversely, the transmission frequency of the t^{w5}-haplotype, which, like t^{12}, is a complete haplotype, is not affected by the type of insemination. The t^{w5}-bearing spermatazoa from $+/t^{w5}$ males fertilize eggs with equivalent frequencies ($> 90\%$) in normal and delayed matings and *in vitro* Garside & Hillman, 1989.

The t^{h2}-haplotype, a partial haplotype, is missing both the proximal and distal regions of the *t*-complex DNA. The distal region contains: the lethal factor, *tcl*; two sterility factors, *tcs-2* and *tcs-3*; and at least two (*Tcd-2* and *Tcd-3*) or possibly four (*Tcd-2*, *Tcd-3*, *Tcd-5* and *Tcd-6*) transmission distortion factors (Silver *et al.* 1983; Silver, 1985; Lyon, 1984, 1986). Since the t^{h2}-haplotype was derived from the t^{6}-haplotype, it is also missing those proximal factors which are deleted from the t^{6} chromosome. The portion of the *t*-complex DNA retained by the t^{h2}-haplotype contains only the tail interaction factor (*tct*), the responder (*Tcr*) and *Tcd-4* (Silver *et al.* 1983; Lyon, 1984, 1986; Silver & Remis, 1987). It is probable that the deleted portions of the *t*-complex DNA are necessary for aberrantly high *in vivo* transmission frequencies since a loss of these regions results in aberrantly low transmission frequencies *in vivo* (Lyon, 1984; this study).

The combined results of our earlier and present studies of the transmission frequencies following *in vivo* and *in vitro* fertilization suggest, however, that the presence of this region does not affect the transmission frequencies when insemination occurs *in vitro*. The obvious difference between the *in vivo* and the *in vitro* fertilizations is the environment in which the inseminations take place. Spermatozoa bearing different but complete *t*-haplotypes and those bearing partial *t*-haplotypes respond differently to oviducal fluid and to capacitation medium. In this study the response of the t^{h2}-bearing spermatozoa to the capacitation medium resulted in an enhancement of their ability to fertilize eggs.

The present observations show that the *in vitro* transmission frequencies of mutations in eukaryotes do not necessarily reflect their *in vivo* transmission frequencies. This point must be considered seriously in light of the increased usage of *in vitro* fertilization techniques to circumvent human infertility.

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