The transmission ratio distortion of the $t^h_2$-haplotype in vivo and in vitro

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Summary
The $t^h_2$-haplotype is transmitted at low frequencies (< 0.30) by +$/t^h_2$ males in normal matings. In the studies described here, the transmission frequency of the $t^h_2$-haplotype from $Rb_7/t^h_2$ 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, 1980a) and by the amount of time elapsing between insemination and ovulation (Braden, 1958, 1972; Yanagisawa et al. 1961; McGrath & Hillman, 1980a, b). McGrath & Hillman (1980a, 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, $t^h_2$. Embryos homozygous for this haplotype are viable in vivo and males which are heterozygous transmit the $t^h_2$-bearing chromosome in normal matings at frequencies which are significantly lower than Mendelian (Lyon & Meredith, 1964; Sherman & Wudl, 1977).

2. Materials and Methods
$Rb_7/t^h_2$ males were used to determine the transmission frequency of the $t^h_2$-haplotype. The Robertsonian translocation $Rb(16-17)7Bnr$ is used in the present studies as a marker. The $Rb_7/t^h_2$ males were mated with hormone-induced (C57BL/6J x T/th2)$\times T/+$ females [5IU pregnant mare serum gonadotropin (PMS) followed 48 h later with an injection of 5IU human chorionic gonadotropin (HCG)]. The hybrid +$/t^h_2$ females served as the experimental females and the $T/+$ as the control females. These females are phenotypically distinguishable from each other by tail length: +$/t^h_2$ mice have tails of normal length while those with the genotype $T/+$ are short-tailed.

In the first series of studies, $Rb_7/t^h_2$ males were individually mated with +$/t^h_2$ females to determine the transmission frequency of the $t^h_2$-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, 1980a, b). The zygotes were allowed to develop to the blastocyst stage and then karyotyped (Garside & Hillman, 1985). An embryo not carrying the $Rb_7$ chromosome was scored as being fertilized by a $t^h_2$-bearing spermatozoon. The transmission frequency was determined from the ratio of the number of embryos lacking the $Rb_7$ 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 (+/t2 x Rb7/t2) and control (T/ + x Rb7/t2) matings 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/t2 males, or through in vitro fertilization using these same males as sources of spermatozoa, is t2/t2. Although t2/t2 embryos are viable in vivo, there have been no studies on their development in vitro. Differences in the preimplantation viability of t2/t2 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 pre-implantation 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 χ² 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 χ² test. Significance was set at P < 0.05 for all statistical tests.

3. Results

The data from the first series of insemination studies (Table 1) show that the transmission frequency of the t2-haplotype is 0.26 and 0.23 in normal and delayed matings, respectively. These frequencies are not significantly different from each other (t = 1.67, 0.10 > P > 0.05); however, both are significantly lower than Mendelian (normal mating, χ² = 97, P < 0.05; delayed mating, χ² = 120, P < 0.05).

These data also show that the in vitro transmission frequency of the t2-haplotype is 0.80. The difference between the transmission frequency of the t2-haplotype following either the normal or the delayed matings and the in vitro transmission frequency of this mutation are significant (t = 9.67, P < 0.001 and t = 13.14, P < 0.001, respectively). Also, the in vitro transmission frequency is significantly higher than Mendelian (χ² = 169.44, P < 0.001).

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 t2 embryos through the preimplantation stages is the same as that of their littermates (+/+; +/t2) and of the control embryos.

4. Discussion

The transmission frequency of the t2-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 t2-haplotype in normal matings (Lyon & Meredith, 1964; Sherman & Wudl, 1977).

The data so show, however, that the transmission frequency of the t2-haplotype can be significantly

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Table 1. The transmission frequency of the t2-haplotype

|                      | In vivo |              |              |
|----------------------|---------|--------------|--------------|
|                       | Normal matings | Delayed matings | 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         |

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            |
| χ²                   | 0.426          | 0.438           | 0.370      |               |              |                 |
| P                    | > 0.50         | > 0.50          | > 0.20     |               |              |                 |

* Numbers of males used.
Transmission frequencies of the $t^{ab}$-haplotype

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 $t^{ab}$-, $t^a$- and $t^{ab}$-haplotypes (McGrath & Hillman, 1980a,b; Garside & Hillman, 1989).

The $t^{ab}$-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 $t^a$-haplotype (a 'complete' lethal haplotype except that the proximal sterility factor, $tcs-I$, and the proximal distortion factor, $Tcd-I$, 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 $t^{ab}$-haplotype, which, like $t^{ab}$, is a complete haplotype, is not affected by the type of insemination. The $t^{ab}$-bearing spermatozoa from $+//t^{ab}$ males fertilize eggs with equivalent frequencies (> 90%) in normal and delayed matings and in vitro (Garside & Hillman, 1989).

The $t^{ab}$-haplotype, a partial haplotype, is missing both the proximal and distal regions of the $t$-complex DNA. The distal region contains: the lethal factor, $tcI$; 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 $t^{ab}$-haplotype was derived from the $t^a$-haplotype, it is also missing those proximal factors which are deleted from the $t^a$ chromosome. The portion of the $t$-complex DNA retained by the $t^{ab}$-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 $t^{ab}$-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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