The \textit{in vitro} transmission frequency of the $t^{12}$ mutation in the mouse

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\section*{SUMMARY}

Male mice which are heterozygous for a recessive lethal mutation ($+/t^L$) transmit the $t^L$ mutation in non-Mendelian ratios. In the present studies, spermatozoa obtained from $+/t^{12}$ males were used to fertilize ova from $+/t^{12}$ females \textit{in vitro}. The frequency of transmission of the $t^{12}$ mutation determined from these \textit{in vitro} studies was compared with the frequency of transmission of this mutation in normal and delayed matings. The data show that the transmission frequency of the $t^{12}$ mutation, \textit{in vitro}, is Mendelian and is the same as the transmission frequency of the $t^{12}$ mutation \textit{in vivo} when matings are delayed until the time of ovulation.

\section*{INTRODUCTION}

The 'T-region' in the house mouse ($Mus\ musculus$) is located on chromosome 17 and includes a dominant mutant allele $T$ (Brachyury), and a series of recessive mutations, $t^n$. Many of the recessive mutations result in embryonic death when homozygous. These lethal mutations (designated $t^L$) are generally transmitted in non-Mendelian frequencies through the male parent and have been found in both wild populations and laboratory strains of mice (for reviews see Bennett, 1975 and Sherman & Wudl, 1977). These $t^L$ mutations are transmitted in a Mendelian ratio by the female. The lethal mutations extracted from wild populations are transmitted by the male at an extremely high frequency, greater than 0·90, while those initially discovered in laboratory strains are transmitted at a lower frequency, less than 0·90 but generally greater than 0·50 (Dunn, 1960).

Previous investigators reported that the transmission frequency of some lethal mutations can be reduced by decreasing the time interval between insemination and fertilization. In general, those $t^L$ mutations which are transmitted in extremely high frequencies in normal matings (e.g. $t^{106}$ and $t^{1010}$, > 0·90) are not significantly affected when the time of mating is delayed (Yanagisawa, Dunn & Bennett, 1961). Conversely, certain of those $t^L$ mutations which are transmitted in a moderately high frequency in normal matings show a reduced transmission frequency in delayed matings (Braden, 1958, 1972; Yanagisawa \textit{et al.} 1961; Erickson, 1973). In normal matings approximately

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six hours elapse between the time of insemination and fertilization, whereas in delayed matings this time period is reduced to two hours or less (Braden & Austin, 1954).

In the present series of investigations we determined and compared the \textit{in vivo} transmission frequency of one of the lethal mutations (t\textsuperscript{12}) in normal and delayed matings. The t\textsuperscript{12} mutation is transmitted in a moderately high frequency \textit{in vivo} in normal matings (Smith, 1956; Mintz, 1964; Hillman, Hillman & Wileman, 1970). After determining the \textit{in vivo} transmission frequencies, we established the \textit{in vitro} frequency of transmission of this mutation. The \textit{in vivo} and \textit{in vitro} transmission frequencies of the t\textsuperscript{12} mutation are presented and compared in this report.

\textbf{MATERIALS AND METHODS}

Hybrid females were obtained by mating C57BL/6J females with T/t\textsuperscript{12} males. The progeny from this cross are phenotypically distinguishable: T/+ offspring are short-tailed while +/t\textsuperscript{12} animals have tails of normal length. The +/t\textsuperscript{12} females were used for the mating transmission frequency studies and served as the source of the ova for the \textit{in vitro} fertilization experimental series. T/+ females served as controls for both the mating and fertilization studies.

For the mating studies the F\textsubscript{1} females were injected intraperitoneally with pregnant mare serum gonadotrophin (PMSG, 5 i.u.) followed 48 h later with an injection of human chorionic gonadotrophin (HCG, 5 i.u.). In normal matings females were caged with +/t\textsuperscript{12} males immediately following the HCG injection. In delayed matings the females were caged with the males 12 h after the HCG injection. In both mating series, single females were caged with single males.

Twelve to thirteen hours following fertilization, the females were sacrificed by cervical dislocation and their oviducts placed into drops of modified Whitten’s medium (Abramczuk, Solter & Koprowski, 1977). The one-cell embryos were flushed from the oviducts, washed through three drops of the modified medium and placed into a fourth drop of this medium under silicone oil (Dow Corning 200 Fluid, 50 cs viscosity) contained in a 10 x 35 mm tissue culture dish (Falcon Plastics). The medium and oil were equilibrated with 5 % O\textsubscript{2}, 5 % CO\textsubscript{2} and 90 % N\textsubscript{2}.

For the \textit{in vitro} fertilization studies, ova were obtained from gonadotrophin injected (PMSG, 5 i.u.; HCG, 5 i.u.) females (+/t\textsuperscript{12} and T/+ ) 12–13 h after the HCG injection. The females were sacrificed, and their oviducts were excised and placed into modified Tyrode’s medium (Fraser & Drury, 1975) under silicone oil. The sodium chloride concentration in the modified Tyrode’s medium was reduced to 85.2 mM so that the fertilization and culture media were of identical osmolarities. The cumulus cell-surrounded ova were removed from the oviducts, washed through a second drop of medium and placed into a third
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0.2 ml drop under silicone oil in a 10 x 35 mm tissue culture dish. The spermatozoa were obtained from the excised vasa deferentia and cauda epididymides of \(+/t^{12}\) males. These excised ducts were placed into a 1 ml drop of modified Tyrode's medium, minced, and the spermatozoa allowed to disperse for 20 min. After dispersal, a 100 \( \mu l \) aliquot of the spermatozoan suspension was diluted approximately 1:4, v/v, with modified Tyrode's medium. Ten \( \mu l \) of the diluted suspension were then added to the drop of medium containing the cumulus cell-surrounded ova. The final spermatozoan concentrations in the insemination dishes ranged from 1.5 to 4.0 \( \times 10^5 \) spermatozoa/ml.

The insemination dishes were placed into an anaerobic jar (Torbal) inside a 37 °C incubator. The jar was gassed with 5 % \( O_2 \), 5 % \( CO_2 \) and 90 % \( N_2 \) for 20 min and then sealed. The gametes were allowed to co-incubate for six hours. At the completion of this incubation period, the ova were removed and washed through four drops of modified Whitten's medium.

One-cell embryos, obtained from both the in vivo and in vitro fertilizations, were maintained in modified Whitten's medium in a sealed anaerobic jar which had been gassed for 20 min with 5 % \( O_2 \), 5 % \( CO_2 \) and 90 % \( N_2 \). Ova that subsequently underwent regular cleavage to the two-cell stage were regarded as fertilized. At the four-cell stage, the culture dishes were transferred to an incubator with a continuous gas flow of 5 % \( CO_2 \) in air. The embryos were allowed to develop to the blastocyst stage.

It was possible to determine the transmission frequency of the \( t^{12} \) mutation, since homozygous mutant embryos from \(+/t^{12} \) inter se matings are developmentally arrested at the morula stage (Smith, 1956). This criterion was used to distinguish the \( t^{12} \) homozygotes from their phenotypically wild-type littermates in the genetically mixed embryo populations.

Background mortality for both the mating frequency studies and the experimental in vitro fertilization series was calculated from the number of embryos developing to the blastocyst stage from control ova from \( T/ + \) females fertilized in vivo and in vitro by spermatozoa from \(+/t^{12}\) males. These fertilizations do not produce any genetically lethal embryos. The percentage viability of embryos in the control matings, which reflects the non-genetic lethality, can be expressed as \( a \). The lethality of embryos obtained from \(+/t^{12}\) inter se matings is affected by both the non-genetic lethality, \( a \), and the lethality due to \( t^{12} \) homozygosity, \( 0.5b \), where 0.5 is the transmission frequency of the \( t^{12} \) allele by the female, and \( b \) is the transmission of this mutation by the male. The percentage of viable experimental embryos, expressed as \( c \), is therefore

\[
c = a(1.0 - 0.5b).
\]

This equation includes both non-genetic and genetic lethality. Solving for the transmission frequency of the \( t^{12} \) mutation by the male,

\[
b = \frac{a-c}{0.5a}.
\]
The incidence of polyspermy and of parthenogenesis among ova fertilized *in vitro* and the effect of parthenogenesis on cleavage-stage embryo development were also quantitated. The former was determined by a light microscopic examination of ova from +/*t*/* and *T/+ females following their incubation with spermatozoa. These ova were fixed immediately after fertilization (6 h after the initiation of gamete co-incubation) in 2.5% glutaraldehyde, placed on a microscope slide with four drops of vaseline–paraffin and compressed with a coverslip. The slide was placed in neutral buffered formalin overnight at 4 °C (Toyoda & Chang, 1974). The ova were stained with 0.25% aceto-lacmoid and each was scored for the numbers of pronuclei, the numbers of spermatozoan tails within the ooplasm, and for the presence of a second polar body. This scoring method established the frequency of both polyspermy and parthenogenic activation of the *in vitro* fertilized ova.

To determine the effect of parthenogenesis on the ability of embryos to develop *in vitro*, ova from +/*t*/* and *T/+ females were parthenogenetically activated according to the protocol described by Surani, Azim & Kaufman (1977). Following this protocol, cumulus cell-surrounded ova were removed from the ampullary region of the oviducts 18–20 h post-HCG injection. The ova were incubated in modified Whitten’s medium for 5–6 h and then freed of their cumulus cells by a 5 min incubation in medium containing hyaluronidase (300 i.u. hyaluronidase/ml medium). The ova were then examined and those that had extruded their second polar bodies (i.e. were activated) were placed into modified Whitten’s medium and incubated for 125–140 h until the activated ova should have reached the late blastocyst stage of development. The culture techniques were the same as those described above.

Significant differences (*P < 0.05*) between the mean transmission frequencies, derived by angular transformation (Biggers & Brinster, 1965), were determined by the Student *t*-test. The *in vivo* and *in vitro* transmission frequencies were compared with Mendelian transmission by a $\chi^2$ test.

**RESULTS**

In the first series of studies, the normal and delayed average transmission frequencies of the +/*t*/* males were determined. Ova fertilized *in vivo* at both mating times were removed from control (*T/+*) and experimental (+/*t*/* females and allowed to develop *in vitro*. Table 1 shows that 93% of the control embryos from the normal matings developed to the blastocyst stage in culture, whereas only 57% of the experimental embryos reached this developmental stage. After correcting for the background lethality (7%), the transmission frequency of the *t*/* mutation was calculated to be 0.78. In the delayed matings, 93% of the fertilized control ova developed to the blastocyst stage *in vitro*, whereas only 69% of the experimental embryos reached this developmental stage. An allowance for the background lethality (7%) established the delayed
transmission frequency as 0.51. This value is significantly less than the transmission frequency at normal mating times but not significantly different from the Mendelian segregation ratio.

In the second series of experiments, specific males were tested for their individual transmission frequencies in either normal or delayed matings before they were used for the in vitro fertilization studies. Table 2 shows that the transmission frequencies of the \( t^{12} \) mutation from the different males in normal matings was highly variable, ranging from 0.58 to 0.98. The average frequency, 0.74, is, however, not significantly different from the average transmission frequency calculated for the normal matings in the first series of experiments. The transmission frequencies of the males used for the delayed matings were also variable; however, the average frequency, 0.51, was equivalent to the averaged delayed frequency reported above.

After the individual males were tested for their in vivo transmission frequencies, the spermatozoa from each male were used for the in vitro inseminations of both control and experimental ova. The spermatozoa from two additional untested males were also used. The data from these studies (Table 2) show that 84% of the control ova developed to the blastocyst stage in vitro. Conversely, only 61% of the experimental ova reached this developmental stage. Therefore, the transmission frequency of the \( t^{12} \) mutation in vitro is 0.55. This value is significantly lower than the normal mating transmission frequency, but not significantly different from the delayed mating frequencies of the \( t^{12} \) mutation established in either the first or second series of experiments.

Both polyspermic fertilization and spontaneous activation of the ova could affect the calculated transmission frequencies. Determining the incidence of polyspermic fertilization was particularly important since previous investigators have shown a significantly higher incidence of polyspermy among ova fertilized in vitro than among ova fertilized in vivo (Fraser, Zanelloti, Paton & Drury, 1976; Maudlin & Fraser, 1977; Fraser & Maudlin, 1978). To establish the incidence of polyspermy, 310 ova from \(+/t^{12}\) and \(T/\) females fertilized in vitro by spermatozoa from \(+/t^{12}\) males were scored for the number of pronuclei and for the presence of spermatozoan tails. Two hundred and eighty-seven of the ova were scored as monospermic, 22 as dispermic, and one as trispermic. These data show that 7% of the embryos developing from ova.
Table 2. In vitro transmission frequency – t^{12} mutation

<table>
<thead>
<tr>
<th>Male no.</th>
<th>Normal mating</th>
<th>Delayed mating</th>
<th>% Fertilization</th>
<th>In vivo*</th>
<th>% Fertilization</th>
<th>Control cross $T/\varphi \times +/t^{12}\delta$</th>
<th>% Fertilization</th>
<th>Experimental cross $+/t^{12}\varphi \times +/t^{12}\delta$</th>
<th>Transmission frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T0·71 (55)</td>
<td>—</td>
<td>91</td>
<td>93/97 (96%)</td>
<td>98</td>
<td>62/97 (64%)</td>
<td>0·67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0·62 (65)</td>
<td>—</td>
<td>89</td>
<td>79/95 (83%)</td>
<td>93</td>
<td>63/97 (65%)</td>
<td>0·44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0·88 (56)</td>
<td>—</td>
<td>99</td>
<td>111/139 (80%)</td>
<td>100</td>
<td>43/61 (70%)</td>
<td>0·23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0·98 (72)</td>
<td>—</td>
<td>98</td>
<td>71/89 (80%)</td>
<td>100</td>
<td>30/65 (46%)</td>
<td>0·84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0·77 (85)</td>
<td>—</td>
<td>72</td>
<td>97/111 (87%)</td>
<td>74</td>
<td>82/150 (55%)</td>
<td>0·75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0·58 (58)</td>
<td>—</td>
<td>82</td>
<td>74/85 (87%)</td>
<td>90</td>
<td>80/131 (61%)</td>
<td>0·60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0·78 (78)</td>
<td>—</td>
<td>87</td>
<td>76/98 (78%)</td>
<td>92</td>
<td>56/90 (62%)</td>
<td>0·40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>—</td>
<td>—</td>
<td>88</td>
<td>58/74 (78%)</td>
<td>91</td>
<td>66/13 (58%)</td>
<td>0·51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>—</td>
<td>—</td>
<td>95</td>
<td>83/101 (82%)</td>
<td>97</td>
<td>60/115 (52%)</td>
<td>0·73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0·58 (55)</td>
<td>—</td>
<td>66</td>
<td>72/83 (87%)</td>
<td>73</td>
<td>37/66 (56%)</td>
<td>0·71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>—</td>
<td>0·64 (78)</td>
<td>92</td>
<td>75/86 (87%)</td>
<td>90</td>
<td>109/162 (67%)</td>
<td>0·46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>—</td>
<td>0·40 (57)</td>
<td>93</td>
<td>87/92 (95%)</td>
<td>90</td>
<td>92/126 (73%)</td>
<td>0·46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>—</td>
<td>0·48 (93)</td>
<td>84</td>
<td>51/68 (75%)</td>
<td>98</td>
<td>82/120 (68%)</td>
<td>0·18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>—</td>
<td>0·50 (98)</td>
<td>78</td>
<td>64/76 (84%)</td>
<td>88</td>
<td>78/140 (56%)</td>
<td>0·68</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Average 0·74          0·51 Total 1091/1294 (84%) 940/1533 (61%) 0·55

* Individual males were tested for their transmission frequencies in vivo prior to their being used for the in vitro fertilization experiments.
† Transmission frequency (number of embryos on which the transmission frequency value is based).
‡ Number of blastocysts/total number of fertilized embryos (%).
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fertilized \textit{in vitro} were polyploid, and almost all of these were triploid. In the experimental \textit{in vitro} fertilizations, triploidy, caused by dispermic fertilization, would result in +/+ +, +/ +/t\textsuperscript{12}, +/t\textsuperscript{12}/t\textsuperscript{12}, and t\textsuperscript{12}/t\textsuperscript{12}/t\textsuperscript{12} embryos. Fraser (1977) has reported that +/+ + ova develop normally to the blastocyst stage \textit{in vitro}. In a separate series of investigations (data to be reported elsewhere), we have found that t\textsuperscript{12}/t\textsuperscript{12}/t\textsuperscript{12} embryos are inviable whereas +/ +/t\textsuperscript{12} and +/t\textsuperscript{12}/t\textsuperscript{12} are viable. Therefore, only viable triploid +/t\textsuperscript{12}/t\textsuperscript{12} embryos would contribute to error in our calculations. The penetration by a + -bearing spermatozoon of a t\textsuperscript{12}-bearing ovum previously fertilized by a t\textsuperscript{12}-bearing spermatozoon would result in an underestimation of the transmission frequency by 1.7%. This underestimation, however, does not alter the significance of the data obtained from the \textit{in vitro} experiments.

To determine the frequency of spontaneous activation occurring during \textit{in vitro} fertilization, 120 ova were examined following their incubation with spermatozoa from +/t\textsuperscript{12} males. Of the ova scored, 100 were fertilized, one was activated but unfertilized, and 18 were neither fertilized nor activated. The frequency of activation in the \textit{in vitro} fertilization system was therefore 1%. Moreover, in a separate study, we found that only a small number of parthenogenetically activated ova develop to the blastocyst stage \textit{in vitro}. Of 167 spontaneously activated ova from T/+ females, only ten (6%) developed to the blastocyst stage, and of 127 activated ova from +/t\textsuperscript{12} females, 11 (9%) became blastocysts. Since only a low percentage of the ova become spontaneously activated (1%), and since parthenogenotes develop poorly \textit{in vitro}, regardless of their genotype, the error introduced by spontaneous activation in the determination of the transmission frequency of the t\textsuperscript{12} mutation is insignificant.

\textbf{DISCUSSION}

The transmission frequency (0.78) of the t\textsuperscript{12} mutation for the normal matings reported here is not significantly different from the frequencies reported for this mutation in those studies in which the embryos from +/t\textsuperscript{12} \textit{inter se} matings were allowed to continue development \textit{in vivo} (0.76, Smith, 1956) or were removed from the oviducts at the 2-cell stage and allowed to develop \textit{in vitro} (0.77, Mintz, 1964; 0.75, Hillman \textit{et al.} 1970). The equivalent transmission frequencies of the t\textsuperscript{12} mutation from these four independent investigations indicate that the methods used in the present studies are sufficient for determining the transmission frequencies of those \textit{t} mutations which are lethal during the early stages of embryogenesis. Consequently, the significant differences between the \textit{in vivo} normal and delayed mating frequencies are attributable solely to the decreased frequency of transmission of the t\textsuperscript{12} mutation in the latter matings. In addition, over 80% of the control ova fertilized \textit{in vitro} develop to the blastocyst stage. Therefore, the \textit{in vitro} fertilization and subsequent embryo culture protocols are adequate methods for determining the
frequency of transmission of cleavage-stage lethal \(t\) mutations. The data also show that the incidences of polyspermy and of spontaneous ovum activation which occur \textit{in vitro} do not introduce significant errors in determining the averaged frequency of transmission of the lethal mutations.

The mechanism which results in the non-Mendelian transmission frequencies of the \(t^L\) mutations from \(T/t^L\) and \(+/t^L\) males is unknown. An early study by Bryson (1944) showed that the increased transmission frequency of the \(t^L\)-bearing gamete was not caused by extra post-meiotic mitoses of the \(t^L\)-bearing spermatogenic cells. Data which corroborates Bryson's observation that equivalent numbers of \(t^L\)-bearing and \(+\) or \(T\)-bearing gametes (from \(+/t^L\) or \(T/t^L\) males) are produced are found in two recent reports. First, immunological studies (using anti-\(t^L\), anti-\(+\), and anti-\(T\) sera) have shown that there are equal numbers of \(t^L\)- and \(+\) (or \(T\))-bearing spermatozoa obtained from heterozygous males (Yanagisawa, \textit{et al.} 1974). Second, unequal chromosome distribution has been ruled out by recent cytogenetic studies (Hammerberg & Klein, 1975).

One hypothesis for the increased transmission frequency was advanced by Yanagisawa (1965a). He suggested that a distorted transmission ratio would be seen if most of the non-\(t^L\)-bearing spermatozoa from heterozygous males were ultrastructurally defective while the \(t^L\)-bearing gametes were morphologically normal. His hypothesis was based on a comparative ultrastructural study of vasa deferentia spermatozoa from \(T/t^L\), \(T/+\) and \(+/+\) mice. Yanagisawa observed that the gametes had tail defects only and that these defects were limited to the \(T/t^L\) mice. Based on these observations, Yanagisawa proposed that the presence of the \(t^L\) mutation caused tail abnormalities in \(T\)-bearing gametes. These defects would render the \(T\)-bearing spermatozoa less motile than \(t^L\)-bearing gametes. As a consequence, the morphologically normal \(t^L\)-bearing spermatozoa would be more likely to reach the site of fertilization and would be at a selective advantage in fertilizing ova. This advantage would produce the aberrant transmission frequency of the \(t^L\) mutation.

To establish the validity of this hypothesis, Hillman & Nadiejka (1978\textit{a}, \textit{b}) undertook two studies. In the first they compared spermiogenesis in mice heterozygous for the \(t^{12}, t^{1432}\), or \(t^6\) mutation with that in inbred and outbred strains of mice which did not carry a \(t^L\) mutation. In the second, they examined the cauda epididymal spermatozoa from these same males at both the light and electron microscopic levels. They reported that aberrant spermiogenesis occurs in males of all strains and genotypes and that the same specific types of abnormal spermatids are found in all of the males examined. No unique morphological defect which could be correlated with the increased transmission frequency of the \(t^L\)-bearing gametes was found in males heterozygous for the \(t^L\) mutation. They also noted that all of the males contain abnormal spermatozoa and that all contain the same types of defective gametes. Again, no unique defect was found which could be correlated with the increased transmission frequency of the \(t^L\) mutation.
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Yanagisawa (1965b) also suggested that spermatozoa from heterozygous males (\( T/t^L, +/t^L \)) were composed of two populations which differed in their viability and that this differential longevity could result in the aberrant transmission frequencies of the mutation. For these studies, Yanagisawa separately incubated spermatozoa from \( +/-, T/+ \) and \( T/t^L \) males in Tyrode's solution for up to 6 h, examined aliquots of the spermatozoa at hourly intervals, and scored the percentage viable by counting the numbers of motile (viable) spermatozoa. He found that the numbers of dead spermatozoa from \( +/- \) and \( T/+ \) males gradually increased from zero-time to 6 h in vitro. Conversely, an abrupt increase in death occurred between 3 h and 4 h in samples of spermatozoa obtained from \( T/t^L \) males. From these observations he postulated that a large proportion of \( + - \) and \( T \)-bearing spermatozoa live less than 4 h in vitro while most \( t^L \)-bearing spermatozoa live longer. He suggested that this longer viability might also occur in normal matings, in which approximately 6 h elapse between copulation and fertilization. If this selective death occurred in utero, more \( t^L \)-than \( + - \) or \( T \)-bearing spermatozoa would be present at the site of fertilization, and more ova would be fertilized by \( t^L \)-bearing than by non-\( t^L \)-bearing gametes.

Results from a more recent study, however, do not support the hypothesis that \( t^L \)-bearing spermatozoa live longer than \( + - \) bearing spermatozoa in vitro (McGrath & Hillman, 1980). In this study, spermatozoa from \( +/t^6 \) males were incubated in vitro for different time periods (0 to 6 h) prior to their being used for the in vitro inseminations of ova from \( +/t^6 \) females. (The \( t^6 \) mutation in a homozygous condition is lethal to the embryo between gestation days 5.5–6.5; Nadijcka & Hillman, 1975.) In this investigation there was no significant difference between the transmission frequency of the lethal allele by spermatozoa used for in vitro insemination at zero time and by those incubated for 6 h prior to use. Spermatozoa from the same male were used for both inseminations. Had selective death occurred, one would expect a higher transmission frequency of the \( t^6 \) allele by those populations of spermatozoa incubated for the longer time period.

Recently, Olds-Clarke & Carey (1978) reported data which suggested that the increased transmission frequency of the lethal \( t^{uc32} \) mutation might be expressed in vitro. In these studies, the investigators compared both the incidence and the rapidity of fertilization of ova from \( +/- \) females by spermatozoa from experimental \( +/-t^{uc32} \) and control \( +/- \) congenic males. By examining ova at hourly intervals up to 5 h following insemination, they found that spermatozoa from the experimental males fertilized more ova and did so in a shorter time period than did control spermatozoa. Since the spermatozoa were obtained from males which were congenic except at the \( T \)-locus, the investigators proposed that the enhanced fertilization by the spermatozoa from the \( +/-t^{uc32} \) males was the result of the presence of the \( t^{uc32} \) mutation and possibly that more \( t^{uc32} \)-bearing spermatozoa than \( + - \) bearing spermatozoa were fertilizing ova.
In the current studies we used embryo lethality to establish the transmission frequency and found that the \( t^{12} \) mutation is not transmitted with an aberrant frequency \textit{in vitro}. Both the \( t^{u32} \) and the \( t^{12} \) mutations belong to the same complementation group (Bennett, 1975), both are cleavage-stage lethals (Bennett & Dunn, 1964), and both are transmitted with similar frequencies \textit{in vivo} (Hillman & Hillman, 1975). Since the two tested \( t^L \) mutations (\( t^{12}; t^6 \), McGrath & Hillman, 1979) are both transmitted \textit{in vitro} in a frequency which is significantly lower than their normal mating frequencies, we can presume that the \( t^{u32} \textit{in vitro} \) transmission frequency would also be lowered. We suggest that all of the moderately high transmission frequency \( t^L \) mutations are transmitted \textit{in vitro} in frequencies which are lower than their \textit{in vivo} normal frequencies and which do not differ significantly from their delayed mating frequencies.

In 1958, Braden proposed that the distorted transmission frequency of the lethal mutation was the result of a physiological superiority of \( t^L \)-bearing spermatozoa and that this advantage occurred between the time of ejaculation and fertilization. Since this physiological superiority is expressed in normal matings but not in delayed matings, Braden also proposed that the expression of this advantage was time dependent. If this hypothesis is correct, the superiority could result from factors intrinsic to the \( t^L \)-bearing gametes and/or might be affected by the response of the spermatozoa to extrinsic factors present in the uterine and oviducal environments.

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REFERENCES


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