Genet. Res. Camb. (1995), **66**, *pp.* 189–193 *With text-figure*

The cellular basis for interaction of sterility factors in the mouse *t* haplotype

LINDA R. JOHNSON[1], STEPHEN H. PILDER AND PATRICIA OLDS-CLARKE[2]
Department of Anatomy and Cell Biology, Temple University School of Medicine, Philadelphia, PA 19140, U.S.A.

(*Received 20 May 1995 and in revised form 21 July 1995*)

Summary

The *t* haplotypes are variant forms of the proximal one-third of chromosome 17 in the mouse. They contain four inversions (relative to the wildtype DNA) extending over most of this region and house a number of male sterility factors. Males carrying two complete *t* haplotypes (*t*/*t*) are sterile, as are males homozygous for *S2*, the sterility factor located in the most distal (relative to the centromere) inversion. Males homozygous for the sterility factor *S1*, located in the most proximal inversion, are not sterile; however, if such a male also is heterozygous for other sterility factors, then sterility results. It has been suggested therefore that homozygosity for *S1* enhances the detrimental action of other sterility factors. Sperm from *t*/*t* males have severe motility defects and are unable to penetrate investment-free eggs, while sperm from fertile *t*/+ mice have less serious motility defects and exhibit a delay in penetration of investment-free eggs. To determine whether homozygosity for *S1* enhances the cellular defects exhibited by sperm from mice heterozygous for other sterility factors, we compared the motility and egg-penetrating ability of sperm from fertile mice homozygous for *S1* to that of sperm from mice carrying one complete *t* haplotype and one proximal or distal partial *t* haplotype. The data suggest that sperm from males carrying a proximal partial *t* haplotype and a complete *t* haplotype have serious defects in motility and penetration of the investment-free egg, and support the hypothesis that *S1* enhances the detrimental effects of other sterility factors within the *t* haplotype.

1. Introduction

The *t* haplotypes are structural variants of the proximal one-third of mouse chromosome 17 containing a group of linked genes which affect male fertility (Lyon, 1991). Males carrying two complete *t* haplotypes (t^x/t^y) are sterile, while males carrying one *t* haplotype are usually fertile (Lyon, 1987). It has been difficult to identify the genes within the *t* haplotypes which cause sterility, due to the presence of four inversions (*In1-In4*; see Fig. 1) which extend over most of the *t* haplotype region (Forejt *et al.* 1994). However, use of partial *t* haplotypes, each of which contains a subset of these inversions, has determined that *t* haplotypes contain at least three sterility factors: *S2*, located in *In4*; *S3*, located in *In3*; and *S1*, located in *In1* (Lyon, 1986). *S2* is the strongest sterility factor, since males homozygous for *In4* alone are sterile (Lyon, 1986; Pilder *et al.* 1993). It has been suggested that *S2* could consist of several separate loci that each contribute to sterility (Silver & Buck, 1993).

Studies of the cellular basis for sterility of t^x/t^y males support the theory that there are several separate sterility factors within *t* haplotypes. Sperm are produced by t^x/t^y mice in nearly normal numbers and most sperm have normal morphology at the light and electron microscope levels (Nadijcka & Hillman, 1980). However, they have very little forward motility, either *in vitro* (McGrath & Hillman, 1980; Olds-Clarke & Johnson, 1993) or when recovered from the female genital tract (Bennett & Dunn, 1967; Olds-Clarke, 1986). Moreover, these sperm have a chronic negative bend in the flagellum, referred to as a 'curlicue' (Olds-Clarke & Johnson, 1993). The locus responsible for the curlicued flagellum has been mapped to *In4*, suggesting that this defect is a result of

[1] Present address: Division of Reproductive Biology, Department of Obstetrics & Gynecology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.
[2] To whom correspondence and reprint requests should be addressed: Department of Anatomy and Cell Biology, Temple University School of Medicine, 3400 North Broad St., Philadelphia, PA 19140, U.S.A., Telephone: 215-707-3562, Fax: 215-707-2966.

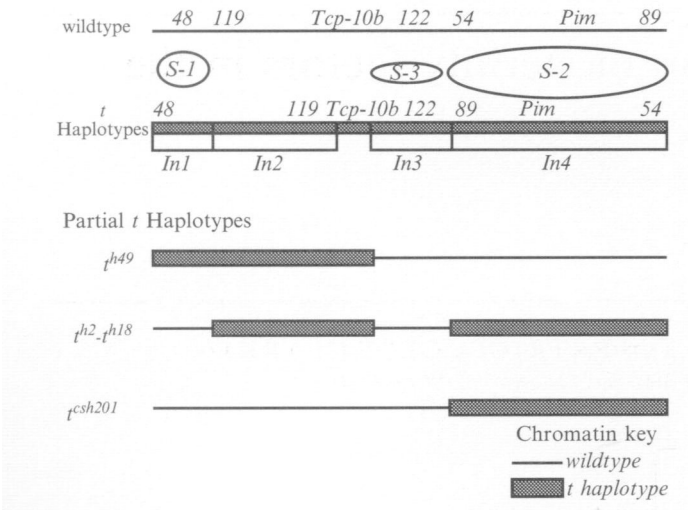

Fig. 1. The extent of *t* haplotype DNA in the partial *t* haplotypes used in this study. At the top of the diagram are the wildtype and *t* haplotype forms of the *t* complex, with the position of probes used to determine the extent of *t* haplotype DNA listed just above each chromosome. The position and extent of each of the sterility factors is shown, with their relative strengths indicated by their sizes. The position of each inversion is indicated by the open rectangles just below the complete *t* haplotype chromosome. Underneath these chromosomes are shown the extent of the proximal and distal partial *t* haplotype chromosomes used in this study.

the *S2* sterility factor (Pilder *et al.* 1993). Sperm from t^x/t^y males are also unable to penetrate oocytes *in vitro*, even when all oocyte investments have been removed (McGrath & Hillman, 1980; Johnson *et al.* 1995). Since motility is not necessary for penetration of investment-free oocytes (Wolf *et al.* 1993; Terriou *et al.* 1993), it appears that the sperm penetration defect is separate from the sperm motility defect. However, the location of the locus or loci affecting sperm penetration is not known.

In contrast to the other sterility factors of the *t* haplotype, *S1* does not cause male sterility by itself. However, males homozygous for *S1* and heterozygous for *S2* are sterile, suggesting that the *S1* sterility factor might somehow enhance the deleterious action of other sterility factors (Lyon, 1986, 1991). To examine this hypothesis at the cellular level, we have compared the motility and egg-penetrating ability of sperm from sterile mice carrying a complete *t* haplotype and different partial *t* haplotypes on the homologous chromosome.

2. Materials and methods

All *t* haplotypes were from the colonies of S. Pilder or P. Olds-Clarke. Complete *t* haplotypes (carrying *S1*, *S2* and *S3*) used were t^{w32} and t^{w5} (Olds-Clarke & Johnson, 1993). Partial *t* haplotypes used were: t^{h49} (Lyon, 1986); t^{h18} and t^{CSH201} (Mains, 1986); and t^{h2}-t^{h18}, a recombinant of two partial *t* haplotypes (t^{h2} and t^{h18}) identified in Dr Pilder's colony. See Fig. 1 for a diagram of the extent of each *t* haplotype, inversion and sterility factor.

Genotyping of mice was done by Southern blotting of DNA from tail tips, as described in Pilder *et al.* (1991). Probes used to determine the extent of *t* haplotype DNA (Fig. 1) included: *D17Leh48* (Fox *et al.* 1985), *D17Leh119* (Hermann *et al.* 1986), *Tcp-10b* (Schimenti *et al.* 1988), *D17Leh122* (Fox *et al.* 1985), *D17Leh54* and *D17Leh89* (Bucan *et al.* 1987). *Pim-1* was used to distinguish t^{w32} from t^{w5} (Nadeau & Phillips, 1987). All males were tested for fertility as described previously (Pilder *et al.* 1991).

Sperm were isolated from the cauda epididymides in medium that will support fertilization *in vitro* (IVF medium; Johnson *et al.* 1995). Sperm concentration, percent of sperm that were motile, net velocity, and percent of motile sperm with curlicued flagella were determined within 5–10 min after release from the epididymides, as described in Pilder *et al.* (1993).

Sperm were incubated at 37 °C in 5% CO_2 in air to allow capacitation; sperm were then diluted to the appropriate concentration and 10 μl added to five to ten investment-free oocytes in 2 μl of IVF medium. Oocytes were obtained from CF1 females (Charles River Laboratory) and their investments removed by sequential treatment with hyaluronidase and collagenase (Johnson *et al.* 1995). Gametes were co-incubated for 2 h, then washed six times in IVF medium, fixed, stained with Hoechst 33342 and viewed under epifluorescence optics (Johnson *et al.* 1995). Sperm were classified as intact (bound to the oocyte) or as decondensed (within the ooplasm). Only experiments in which the mean of intact sperm/oocyte was $> 1{\cdot}0$ were included in the results. The sperm:oocyte ratio was calculated from the concentration in the diluted sperm sample, divided by the number of oocytes inseminated. Significant differences between groups were determined by the Newman–Keuls test after analysis of variance. Eggs groups inseminated at the two sperm:egg ratios were analysed separately.

3. Results

(i) *Fertility*

The t^{h49}/t^{h49} males were, as expected, fertile. All t^{h49}/t^{c} (where t^{c} = complete *t* haplotypes t^{w5} or t^{w32}), t^{h2}-t^{h18}/t^{w5}, and t^{CSH201}/t^{w32} males used in these studies were sterile as evidenced by vaginal plugs but no progeny from matings with a pair of females for a month or more (Pilder *et al.* 1991).

(ii) *Motility*

The percentage of sperm that were motile was determined by calculating the percent of sperm with a moving flagellum, whether or not the sperm head changed position. This parameter was not different among genotypes of mice examined in this study

https://doi.org/10.1017/S0016672300034637 Published online by Cambridge University Press

Table 1. *Motility Characteristics of Sperm from Males Carrying Partial and Complete* t *Haplotypes*

Male genotype (no. males)	Sterility factors	Mean ± S.E.M. % motile (range)	Mean ± S.E.M. net velocity in μ/sec* (range)	Mean ± S.E.M. % curlicue flagella (range)
+/+ (6)†	+ + + / + + +	52 ± 2[a]‡ (45–56)	156 ± 3[a] (147–168)	0 ± 0[a]
t^{h49}/t^{h49} (4)	*S1* + + / *S1* + +	53 ± 5[a] (42–62)	106 ± 6[b] (93–122)	1 ± 1[a] (0–4)
$+/t^{c}$ (10)†	+ + + / *S1 S3 S2*	56 ± 2[a] (46–63)	97 ± 7[b] (62–133)	1 ± 1[a] (0–4)
t^{h49}/t^{c} (11)	*S1* + + / *S1 S3 S2*	54 ± 2[a] (43–67)	48 ± 3[c] (37–63)	13 ± 5[b] (0–54)
t^{h2}-t^{h18}/t^{w5} and t^{CSH201}/t^{w32} (3)	+ + *S2* / *S1 S3 S2*	45 ± 3[a] (40–51)	40 ± 2[c] (37–44)	65 ± 2[c] (61–68)
t^{w32}/t^{w5} (7)†	*S1 S3 S2* / *S1 S3 S2*	23 ± 2[b] (17–30)	43 ± 3[c] (29–49)	91 ± 2[d] (85–98)

* A measure of forward progression.
† Data taken from Olds-Clarke & Johnson, 1993.
‡ Means in the same column with different superscript letters are significantly different (Newman–Keuls test, $P < 0.05$).

Table 2. *Oocyte Penetrating Ability of Sperm from Males Carrying Partial and Complete* t *Haplotypes*

Male genotype (no. males)	Mean ± S.E.M. sperm:oocyte ratio (range)	Mean ± S.E.M. intact sperm per oocyte (range)	Mean ± S.E.M. % penetrated oocytes (range)	Mean ± S.E.M. no. swollen sperm per oocyte (range)
(A) Sperm:oocyte ratio near 100:1				
t^{h49}/t^{h49} (3)	110 ± 10 (90–120)	13 ± 1·6[a]* (9·9–16)	93 ± 7[a] (80–100)	1·7 ± 0·4[a] (1·0–2·2)
t^{h49}/t^{c} (4)	185 ± 60 (100–360)	6·4 ± 1·6[b] (2·8–10)	25 ± 16[b] (0–67)	0·3 ± 0·2[b] (0–0·8)
t^{h2}-t^{h18}/t^{w5} and t^{CSH201}/t^{w32} (4)	128 ± 12 (100–160)	4·0 ± 1·6[b] (1·4–8·2)	0 ± 0[b]	0 ± 0[b]
(B) Sperm:oocyte ratio near 1000:1				
t^{h49}/t^{h49} (3)	807 ± 97 (700–1000)	21 ± 9·0[c] (11–39)	100 ± 0[c]	3·2 ± 0·8[c] (1·9–4·7)
t^{h49}/t^{c} (4)	982 ± 124 (700–1300)	17 ± 4·4[c] (11–30)	56 ± 19[d] (22–100)	1·0 ± 0·6[d] (0·2–2·6)
t^{h2}-t^{h18}/t^{w5} and t^{CSH201}/t^{w32} (4)	1150 ± 233 (700–1600)	19 ± 9·4[c] (2·5–42)	0 ± 0[e]	0 ± 0[d]

* Means in the same column with different superscript letters are significantly different (Newman–Keuls test, $P < 0.05$).

(Table 1). For comparison purposes, data from motility tests of sperm from +/+, $+/t^{c}$ (where t^{c} = complete *t* haplotypes t^{w5} or t^{w2}), and congenic t^{w32}/t^{w5} mice (Olds-Clarke & Johnson, 1993) are also shown in Table 1. Sperm from t^{w32}/t^{w5} mice had significantly fewer motile sperm than all of the other genotypes (Table 1).

The net velocity of a sperm is a measure of its forward progression. The mean net velocity of sperm from all sterile genotypes (t^{h49}/t^{c}, t^{h2}-t^{h18}/t^{w5}, t^{CSH201}/t^{w32} and t^{w32}/t^{w5} mice) was only half that of the mean net velocity of the sperm from fertile t^{h49}/t^{h49} and $+/t^{c}$ mice, a significant difference (Table 1). In addition, the mean net velocity of wildtype sperm was significantly higher than that of sperm from fertile t^{h49}/t^{h49} and $+/t^{c}$ males.

The abnormal 'curlicue' flagellar conformation was typical of virtually all motile sperm from t^{w32}/t^{w5} mice, and the majority of sperm from t^{h2}-t^{h18}/t^{w5} and t^{CSH201}/t^{w32} mice had curlicued flagella (Table 1). While the mean percentage of sperm from t^{h49}/t^{c} mice with curlicued flagella was small, there was a large S.E.M. This variability was due largely to one t^{h49}/t^{w5} male with 54% curlicue flagella. Without this male, the mean ± S.E.M. would have been 9 ± 2, and the range 0·26. Since the genetic backgrounds of these mice were diverse, it is possible that different alleles at loci outside the *t* haplotype influence the expression of

https://doi.org/10.1017/S0016672300034637 Published online by Cambridge University Press

curlicue flagella. Nevertheless, with or without the outlying male, the mean percentage of curlicue flagella for t^{h49}/t^{c} mice was significantly different from all other genotypes (Table 1).

(ii) *Egg penetration*

Sperm were tested for their ability to penetrate investment-free oocytes at two sperm:egg ratios, approximately 100:1 (Table 2*A*) and 1000:1 (Table 2*B*). Under these conditions, sperm from both +/+ and congenic $+/t^{c}$ mice were able to penetrate virtually all the oocytes (Johnson *et al.* 1995). At both ratios, sperm from t^{h49}/t^{h49} males (homozygous for *S1*) were able to penetrate virtually all the oocytes, and most oocytes contained more than one decondensed sperm head. At the lower sperm:egg ratio, sperm from t^{h49}/t^{c} males (homozygous for *S1* and heterozygous for *S2*) were able to penetrate significantly fewer oocytes and had a significantly lower mean number of penetrated sperm/oocyte at the comparable sperm:egg ratio than sperm from t^{h49}/t^{h49} mice (Table 2*A*). At the higher sperm:egg ratio, the mean percentage of eggs penetrated by sperm from t^{h49}/t^{c} males was significantly different from that of t^{h49}/t^{h49} males and from that of males carrying a complete *t* haplotype and *In4* of the *t* haplotype (and thus homozygous for *S2*; Table 2*B*). Sperm from males homozygous for *S2* were unable to penetrate any oocytes at either sperm:egg ratio (Table 2).

4. Discussion

The *S1* sterility factor in the *t* haplotypes is unusual in that it does not cause sterility when homozygous. However, while mice heterozygous for all sterility factors are fertile, addition of another copy of *S1* to the genome induces sterility (Lyon, 1986). This suggests that *S1* somehow enhances the detrimental action of other sterility factors in the *t* haplotypes (Lyon, 1986). The data presented here support this theory. Sperm from t^{h49}/t^{c} mice (homozygous for *S1* and heterozygous for other sterility factors) have an initial net velocity and a mean percent of curlicue flagella intermediate between that of fertile t^{h49}/t^{h49} and $+/t^{c}$ mice (homozygous for *S1* or heterozygous for all sterility factors) and that of sterile t^{w32}/t^{w5} mice (homozygous for all sterility factors) (Table 1). Sperm from t^{h49}/t^{c} mice also have investment-free egg penetrating ability which is significantly impaired relative to those same genotypes, i.e. t^{49}/t^{h49} (Table 2) or $+/t^{c}$ mice (Johnson *et al.* 1995). Yet sperm from t^{h49}/t^{c} mice have some degree of penetrating ability at high sperm:egg ratios (Table 2), while sperm from t^{x}/t^{y} mice do not (McGrath & Hillman, 1980; Johnson *et al.* 1995). This suggests that homozygosity for *S1* enhances the defects in motility and in egg-penetrating ability of sperm from $+/t^{c}$ mice (Johnson *et al.* 1995) nearly to the level seen in sperm from mice homozygous for all sterility factors. The interaction of *S1* with other sterility factors could involve *t*-specific proteins produced by genes located in the most proximal region of the *t* haplotype (e.g. *Tcp-4*, *Tcp-5*, Silver *et al.* 1987; *Tctex-1*, O'Neill & Artzt, 1995; *Iph-1*, Olds-Clarke *et al.* 1995).

It is not clear whether *S1* by itself causes sperm defects. Although mice homozygous for *S1* are fertile, this does not eliminate the possibility that their sperm are dysfunctional. For example, $+t^{c}$ mice are fertile, yet their sperm have abnormal motility and exhibit a delay in penetrating the investment-free oocyte (Olds-Clarke & Johnson, 1993; Johnson *et al.* 1995). The net velocity of t^{h49}/t^{h49} sperm was significantly lower than that of wildtype sperm studied by Olds-Clarke & Johnson (1993; Table 1), consistent with the idea that these sperm have subtle defects. However, since the wildtype sperm were of a different genetic background, it is not clear whether the difference is due to action of genes within or outside the *t* haplotype. Moreover, the oocyte penetration ability of sperm from t^{h49}/t^{h49} mice was not tested under the stringent conditions which were able to detect a significant decrease in the penetrating ability of sperm from $+/t^{c}$ mice, relative to congenic wildtype sperm (Johnson *et al.* 1995). Thus additional experiments with congenic controls will be necessary to determine whether subtle defects in sperm function are characteristic of sperm from mice homozygous for *S1*.

Data from this study also suggest that a genetic factor located in the distal inversion, *In4*, affects penetration of the oolemma. Sperm from mice homozygous for *In4* (t^{h2}-t^{h18}/t^{w5}, t^{CSH201}/t^{w32}) were unable to penetrate any zona pellucida-free oocytes at either of the two sperm:egg ratios tested here (Table 2). However, since sperm from mice homozygous only for *S2* were not tested, it is not clear whether the factor in *In4* which affects egg penetration acts independently, or depends on interaction with more proximal factors. In either case, these data, together with the evidence for genes in *In4* affecting sperm motility (Pilder *et al.* 1993), support the suggestion that the *S2* sterility factor consists of several loci (Silver & Buck, 1993), each causing dysfunction in a different sperm organelle.

Sperm from t^{h49}/t^{c} mice are defective in both motility and ability to penetrate the investment-free mouse oocyte, yet their net velocity is significantly better than that sperm from t^{w32}/t^{w5} mice (Table 1), and they are able to penetrate at least some eggs (Table 2). Why then are these males completely sterile? Since good forward progression is necessary for sperm transport to the site of fertilization *in vivo* (Olds-Clarke & Wivell, 1992; Olds-Clarke & Sego, 1992) as well as for penetration of the egg investments (Johnson *et al.* 1995), it appears that the 'contraceptive' action of each of these sperm defects could be additive. Thus the motililty defects might allow few sperm to reach the site of fertilization *in vivo*, and

fewer still to penetrate the egg investments; because the chances of such sperm being able to penetrate the oolemma are also very low, the chances of successful fertilization are so small as to be virtually undetectable.

The authors would like to thank Mr. Larry Dugan for his expert technical assistance. This research was supported by NIH grant HD15045 to P.O-C. and HD31164 to S.H.P.

References

Bennett, D. & Dunn, L. (1967). Studies of effects of *t*-alleles in the house mouse on spermatozoa. I. Male sterility effects. *Journal of Reproduction & Fertility* **13**, 421–428.

Bucan, M., Herrmann, B., Frischauf, A., Bautch, V., Bode, V., Silver, L., Martin, G. & Lehrach, H. (1987). Deletion and duplication of DNA sequences is associated with the embryonic lethal phenotype of the t9 complementation group of the mouse t complex. *Genes & Development* **1**, 376–385.

Forejt, J., Arzt, K., Barlow, D., Hamvas, R., Lindahl, K., Lyon, M., Klein, J. & Silver, L. (1994). Mouse chromosome 17. *Mammalian Genome* **5**, S238–S258.

Fox, H., Martin, G., Lyon, M., Herrmann, B., Frischauf, A., Leharach, H. & Silver, L. (1985). Molecular probes define different regions of the mouse *t* complex. *Cell* **40**, 63–69.

Herrmann, B., Bucan, M., Mains, P., Frischauf, A., Silver, L. & Lehrach, H. (1986). Genetic analysis of the proximal portion of the mouse *t* complex: evidence for a second inversion within *t* haplotypes. *Cell* **44**, 469–476.

Johnson, L., Pilder, S., Bailey, J. & Olds-Clarke, P. (1995). Sperm from mice carrying one or two *t* haplotypes are deficient in investment and oocyte penetration. *Developmental Biology* **168**, 138–149.

Lyon, M. (1986). Male sterility of the mouse *t* complex is due to homozygosity of the distorter genes. *Cell* **44**, 357–363.

Lyon, M. (1987). Distorter genes of the mouse *t*-complex impair male fertility when homozygous. *Genetical Research* **49**, 57–60.

Lyon, M. (1991). The genetic basis of transmission-ratio distortion and male sterility due to the *t* complex. *American Naturalist* **137**, 349–358.

Mains, P. (1986). The cis-trans test shows no evidence for a functional relationship between two mouse t complex lethal mutations: Implications for the evolution of t haplotypes. *Genetics* **114**, 1225–1237.

McGrath, J. & Hillman, N. (1980). Sterility in mutant (t^{Lx}/t^{Ly}) male mice. III. *In vitro* fertilization. *Journal of Embryology & experimental Morphology* **59**, 49–58.

Nadeau, J. & Phillips, S. (1987). The putative oncogene *Pim-1* in the mouse: its linkage and variation among *t* haplotypes. *Genetics* **117**, 533–541.

Nadijcka, M. & Hillman, N. (1980). Sterility in mutant (t^{Lx}/t^{Ly}) male mice. II. A morphological study of spermatozoa. *Journal of Embryology and experimental Morphology* **59**, 39–47.

O'Neill, M. & Artzt, K. (1995). Identification of a germ-cell-specific transcriptional repressor in the promoter of *Tctex-1*. *Development* **121**, 561–568.

Olds-Clarke, P. (1986). Motility characteristics of sperm from the uterus and oviducts of female mice after mating to congenic males differing in sperm transport and fertility. *Biology of Reproduction* **34**, 453–467.

Olds-Clarke, P. & Sego, R. (1992). Calcium alters capacitation and progressive motility of uterine spermatozoa from +/+ and congenic $t^{w32}/+$ mice. *Biology of Reproduction* **47**, 629–635.

Olds-Clarke, P. & Wivell, W. (1992). Impaired transport and fertilization *in vivo* of calcium-treated spermatozoa from +/+ or congenic $t^{w32}/+$ mice. *Biology of Reproduction* **47**, 621–628.

Olds-Clarke, P. & Johnson, L. (1993). *t* haplotypes in the mouse compromise sperm flagellar function. *Developmental Biology* **155**, 14–25.

Olds-Clarke, P., Pilder, S., Visconti, P., Moss, S., Orth, J. & Kopf, G. 1995. Sperm from mice carrying two *t* haplotypes do not possess a tyrosine phosphorylated form of hexokinase. *Molecular Reproduction and Development*, in press.

Pilder, S., Hammer, M. & Silver, L. (1991). A novel mouse chromosome 17 hybrid sterility locus: implications for the origin of *t* haplotypes. *Genetics* **129**, 237–246.

Pilder, S., Olds-Clarke, P., Phillips, D. & Silver, L. (1993). *Hybrid sterility-6*: A mouse *t* complex locus controlling sperm flagellar assembly and movement. *Developmental Biology* **159**, 631–642.

Schimenti, J., Cebra-Thomas, J., Islam, S., Pilder, S. & Silver, L. (1988). A candidate gene family for the mouse *t Complex Responder* (*Tcr*) locus responsible for haploid effects on sperm function. *Cell* **55**, 71–78.

Silver, L., Kleene, K., Distel, R. & Hecht, N. (1987). Synthesis of mouse *t* complex proteins during haploid stages of spermatogenesis. *Developmental Biology* **119**, 605–608.

Silver, L. & Buck, C. (1993). The mouse *t* complex distorter-3 (*Tcd*-3) locus and transmission ratio distortion. *Genetical Research* **62**, 133–137.