Genet. Res., Camb (1993), **62**, *pp.* 183–193 *With 5 text-figures*

Inheritance of a meiotic abnormality that causes the ovulation of primary oocytes and the production of digynic triploid mice

JOHN D. WEST[1]*, SHEILA WEBB[2] AND MATTHEW H. KAUFMAN[2]

[1] *Department of Obstetrics and Gynaecology, University of Edinburgh, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW, UK*

[2] *Department of Anatomy, Medical School, University of Edinburgh, Teviot Place, Edinburgh EH8 9AG, UK*

(*Received 7 June 1993 and in revised form 26 July 1993*)

Summary

Previous studies have demonstrated that the LT/SvKau strain of mice ovulates a high proportion of oocytes as diploid primary oocytes rather than secondary oocytes. These ovulated primary oocytes are arrested at meiotic metaphase I but may be fertilized to produce digynic triploid embryos. In the present study, 40·4% of eggs analysed from LT/SvKau females were ovulated as primary oocytes, compared to 1·2% from control C57BL/Ws strain mothers. These two inbred strains were intercrossed to produce eight sets of F1, F2 and backcross females and the frequency of triploidy was investigated. The results are compatible with segregation of a co-dominant, autosomal gene that has a major influence on the incidence of triploidy. We suggest that the provisional gene symbol *Poo* (primary oocyte ovulation) be assigned to this gene, with alleles Poo^{l} (the 'mutant' allele present in the LT/SvKau strain) and Poo^{b} (the normal allele present in C57BL/Ws mice). *Poo* is incompletely penetrant and has variable expressivity because the proportion of oocytes ovulated as primary oocytes by LT/SvKau mice was variable and, in some cases, nil. In putative Poo^{l}/Poo^{b} heterozygotes the frequency of ovulated primary oocytes was increased only marginally (from 1·2% to 6·6%) by the presence of one copy of the Poo^{l} allele, but this increase was found consistently (in two reciprocal F1 crosses) and was statistically significant. No evidence was found for tight genetic linkage between *Poo* and two Mendelian loci (brown on chromosome 4 and glucose phosphate isomerase on chromosome 7), that were segregating in the crosses. The Poo^{l} mutant in the LT/SvKau strain of mice provides a valuable resource to study the cell and molecular biology of mammalian oocyte maturation and the control of meiosis.

1. Introduction

Prior to ovulation, the mammalian oocyte is arrested at the diploid, dictyate stage of the first meiotic division. After release from dictyate, it progresses through metaphase I and extrudes the first polar body, thereby becoming a haploid, secondary oocyte. The secondary oocyte continues through the second meiotic division until it is arrested again at metaphase II. It is ovulated in this arrested state and the second meiotic division is only completed after fertilization, when the second polar body is extruded and the secondary oocyte has become a fertilized egg.

The LT/SvKau strain of mice is unusual in that some oocytes are ovulated at the diploid primary oocyte stage (Kaufman & Howlett, 1986). Both primary and secondary oocytes ovulated by this strain are capable of being fertilized, and give rise to digynic triploid and normal diploid embryos, respectively (O'Neill & Kaufman, 1987; Speirs & Kaufman, 1990). There seems to be an effect of maternal age on the incidence of digynic triploidy in this strain. Without superovulation, about 40–50% of the oocytes ovulated by 6-week-old females are primary oocytes, whereas at 12 weeks of age only about 20% are of this type, and by 30 weeks of age only about 6% of the eggs ovulated are primary oocytes (Speirs & Kaufman, 1990). At 8–12 weeks, superovulation increased the frequency of primary oocytes ovulated from 24 to 34% (Speirs & Kaufman, 1988).

The mouse strains A/He (Takagi, 1970; Takagi & Oshimura, 1973; Takagi & Sasaki, 1976) and NMRI/Han (Hansmann & El-Nahass, 1979; Jenderny *et al.* 1980; Bartels, Jenderny & Hansmann, 1984) also ovulate some primary oocytes. The spontaneous frequency is about 4·5% for A/He and 1% for

* Corresponding author.

https://doi.org/10.1017/S001667230003189X Published online by Cambridge University Press

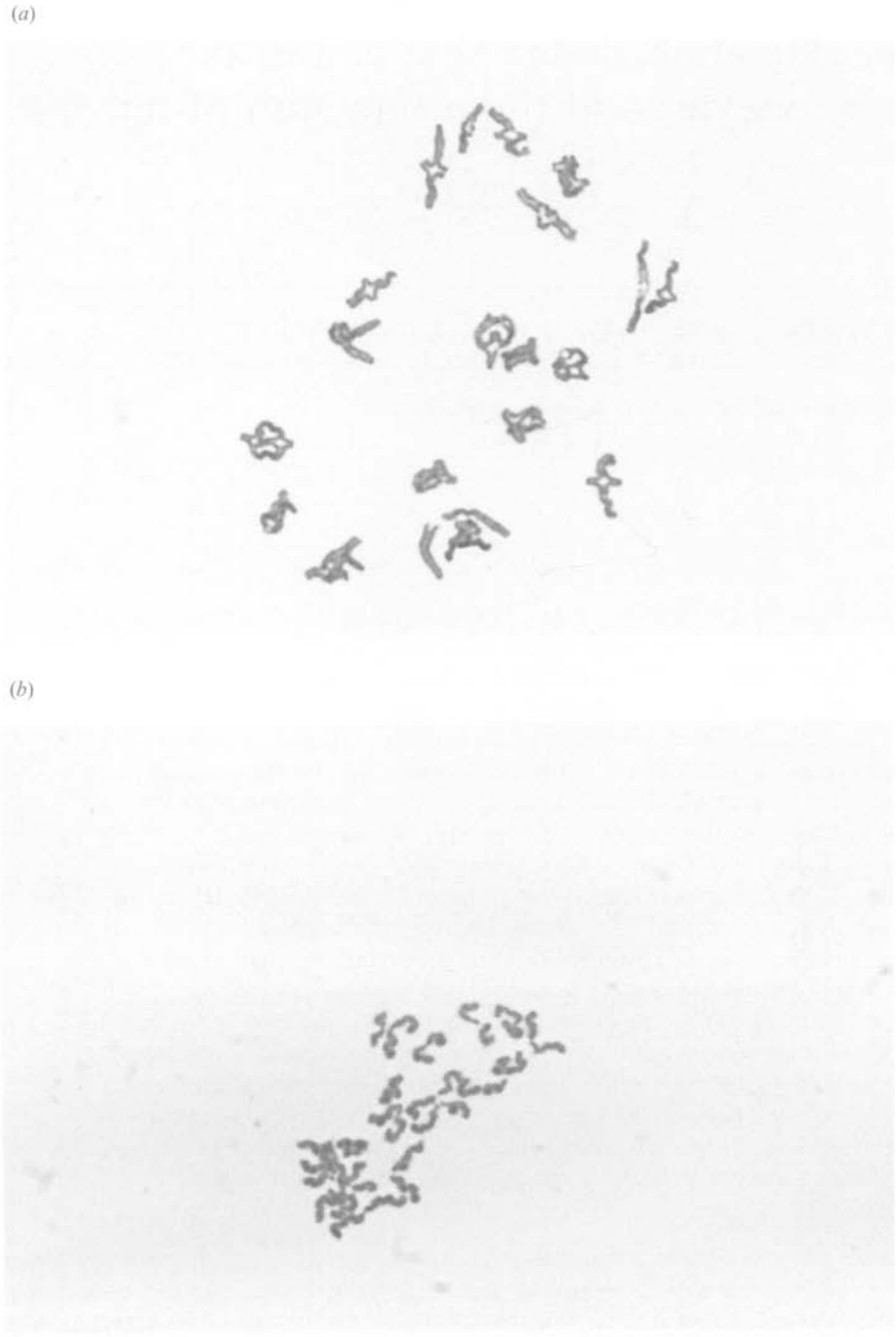

Fig. 1. (*a*) Characteristic 'tetrad' arrangement of the chromosomes in an unfertilized primary oocyte which has not extruded the first polar body. (*b*) Characteristic 'dyad' arrangement of the chromosomes in an unfertilized secondary oocyte which has extruded the first polar body.

NMRI/Han, and after superovulation this increases to 20% and 2–4% respectively. A preliminary genetic analysis indicated that this trait found in NMRI/Han mice was heritable (Bartels *et al.* 1984; Beermann *et al.* 1987). The mode of inheritance was not defined precisely, but autosomal recessive inheritance and X-chromosome linkage were excluded (Beermann *et al.* 1987) and the provisional gene symbol *DipII* (diploid oocytes, metaphase I arrested) was assigned. Also, the frequency of ovulation of primary oocytes was thought to be modified by a maternal (possibly mitochondrial) factor. The LT/SvKau strain mice produce a substantially higher proportion of primary oocytes than NMRI/Han mice (40–50% versus 2–4%), and this makes a genetic investigation more feasible.

An understanding of the genetic basis of the meiotic abnormality that causes oocytes to be ovulated before completing their first meiotic division would be an important step towards the understanding of the mechanisms that control meiosis. In order to examine the inheritance of this trait, we have determined the frequency of triploid fertilized eggs (ovulated primary

https://doi.org/10.1017/S001667230003189X Published online by Cambridge University Press

oocytes) in individual females of LT/SvKau strain mice, control C57BL/Ws strain mice and females from eight series of crosses (F1, F2 and backcrosses) made between these two strains.

2. Materials and methods

LT/SvKau and C57BL/Ws inbred mice and various intercross progeny were reared under conventional conditions at the Centre for Reproductive Biology. LT/SvKau mice were homozygous for the light allele (B^{lt}) of the brown locus (chromosome 4) and the *Gpi-1s*^{a} allele of glucose phosphate isomerase (chromosome 7); C57BL/Ws mice were homozygous for the wildtype black allele (B) of the brown locus and the *Gpi-1s*^{b} allele. Females aged between 5 and 7 weeks were induced to ovulate by injecting 5 IU pregnant mares' serum gonadotrophin (PMSG) between 12.00 and 16.00 h, followed 48 h later by 5 IU human chorionic gonadotrophin (hCG). They were then caged overnight with (C57BL/Ws × CBA/Ca) F_1 males, and mating was verified the following morning by the presence of a vaginal plug. Prior to superovulation, each female was assigned an individual code number, ear-tagged and the coat colour was classified as black (homozygous B/B), light (homozygous B^{lt}/B^{lt}) or intermediate (heterozygous B/B^{lt}).

Mice were analysed in batches at approximately weekly intervals and batches often contained individuals from more than one cross. The cytogeneticist responsible for the analysis of ovulated oocytes was unaware of the identity of the females at the time of the analysis. At 20–24 h after the hCG injection the female mice were killed by cervical dislocation, a blood sample was removed and stored at −20 °C for electrophoresis of glucose phosphate isomerase and the oviducts were isolated into Dulbecco's phosphate-buffered saline, which contained 4 mg/ml of bovine serum albumin. The cumulus mass containing the oocytes was released from the oviduct and then transferred into a microdrop of M16 culture medium (Whittingham, 1971), which contained 1 mg/ml of hyaluronidase to facilitate removal of the cumulus cells. After a few minutes, the oocytes were washed through three washes of M16 and cultured overnight in M16 with added colcemid solution (1 μg/ml), and the following morning air-dried chromosome preparations were made using the Tarkowski (1966) method. Ovulated, unfertilized oocytes were also collected from females that failed to mate and were treated in the same way as fertilized oocytes.

Cytogenetic analysis of the fertilized oocytes arrested at the first cleavage mitosis revealed that the fertilized primary oocytes had a triploid chromosome constitution (i.e. 60 chromosomes) and the fertilized secondary oocytes had a normal diploid constitution (i.e. 40 chromosomes). The cytogenetic analysis of the unfertilized oocytes showed both primary and secondary oocytes arrested at metaphase of the first meiotic and second meiotic divisions, respectively. The primary oocytes exhibited the characteristic 'tetrad' chromosome configuration (see Fig. 1*a*) whereas the secondary oocytes exhibited the 'dyad' chromosome configuration (see Fig. 1*b*) usually observed in recently ovulated oocytes. Details of all the analysed oocytes were recorded separately for each individual female.

Blood samples were lysed by three cycles of freeze/thawing. Cellulose acetate electrophoresis and staining for glucose phosphate isomerase (GPI-1) activity were carried out as previously described (West & Fisher, 1984) in order to type the female mice as homozygous *Gpi-1s*^{a}/*Gpi-1s*^{a}, homozygous *Gpi-1s*^{b}/*Gpi-1s*^{b} or heterozygous *Gpi-1s*^{a}/*Gpi-1s*^{b}.

Statistical comparisons (χ^2 tests) were performed on an Apple Macintosh computer either using the 'MultiStat' statistical package (Biosoft, Cambridge) or a routine established on a spreadsheet ('Microsoft Excel').

3. Results

A total of 391 female mice were superovulated and 2525 eggs and oocytes were successfully analysed from 299 (76%) of them. These results clearly show that the trait causing ovulation of primary oocytes in LT/SvKau mice is heritable. The frequencies of ovulated primary oocytes produced by ten groups of female mice are shown in Table 1, together with the percentages predicted by different modes of single-gene inheritance. A parental source effect (genomic imprinting) seems unlikely because the observed ratios of triploids to diploids in reciprocal crosses 3 and 4 were not significantly different (12:210 versus 21:249; $\chi^2 = 1{\cdot}10$, $P = 0{\cdot}295$). Similarly, there was no significant difference between reciprocal crosses 5 and 6, 7 and 8 or 9 and 10 (see below). If the trait is inherited as a single gene, several modes of inheritance can be excluded.

X-chromosome linkage can be ruled out because cross 8 produced a significantly lower proportion of triploids than cross 1 (21·6% *vs* 40·4%; $\chi^2 = 18{\cdot}37$, $P < 0{\cdot}0001$) although, in each cross, both X chromosomes would be from the LT strain. Also, although cross 6 produced a lower proportion of triploids than cross 5, this was not statistically significant ($\chi^2 = 1{\cdot}30$, $P = 0{\cdot}255$). As noted above, the proportion of triploids produced by females from crosses 7 and 8 was not significantly different ($\chi^2 = 0{\cdot}09$, $P = 0{\cdot}764$). However, the possibility of X-linked dominant inheritance is discounted by the similarity between the results for crosses 9 and 10. Although more triploids were produced in cross 9 than cross 10 this was not statistically significant ($\chi^2 = 3{\cdot}24$, $P = 0{\cdot}072$), and much of the excess was attributable to a single anomalous female (female M391 – the last female used in the study!) which produced 6 triploids from a total

https://doi.org/10.1017/S001667230003189X Published online by Cambridge University Press

Table 1. *Ovulation of primary oocytes by females from ten crosses*

Cross				No. of female progeny tested	Observed number of primary oocytes (3*n* embryos)		Expected percentage of primary oocytes*							
							Autosomal				X-linked			
									Semi-Dom				Semi-Dom	
	Female	×	Male		Proportion	%	Dom	Rec	*a*†	*b*†	Dom	Rec	*a*†	*b*†
1	LT		LT	33	84/208	40·4								
2	C57		C57	31	3/256	1·2								
3	LT		C57	19	12/222	5·4	40·4	1·2	20·8	*6·6*‡§	40·4	1·2	20·8	*6·6*‡§
4	C57		LT	31	21/270	7·7	40·4	1·2	20·8	*6·6*‡§	40·4	1·2	20·8	*6·6*‡§
5	(LT × C57) F1		(LT × C57) F1	28	27/229	11·8	30·6	*11·0*‡	20·8	*13·7*‡	40·4	20·8	30·6	23·5
6	(C57 × LT) F1		(C57 × LT) F1	35	29/328	8·8	30·6	*11·0*‡	20·8	13·7	20·8	1·2	*11·0*‡	3·9
7	(LT × C57) F1		LT	34	60/293	20·5	40·4	*20·8*‡	30·6	*23·5*‡	40·4	*20·8*‡	30·6	*23·5*‡
8	LT		(LT × C57) F1	25	50/232	21·6	40·4	*20·8*‡	30·6	*23·5*‡	40·4	40·4	40·4	40·4
9	(LT × C57) F1		C57	41	22/272‖	8·1	20·8	1·2	*11·0*‡‖	3·9‖	20·8	1·2	*11·0*‡‖	3·9‖
10	C57		(LT × C57) F1	22	8/215	3·7	20·8	1·2	11·0	*3·9*‡	40·4	1·2	20·8	*6·6*‡

Abbreviations: Dom, dominant; Rec, recessive; Semi-dom, semi-dominant; C57, C57BL/Ws; LT, LT/SvKau.

* Calculations assume percentage triploidy is 1·2% for C57BL and 40·4% for LT.

† Expected results for semi-dominant inheritance if frequency of triploids from heterozygous females is: (*a*) mean of LT and C57; (*b*) mean of the two F1 crosses.

§ Assumed from observed data – see (*b*) above.

‡ Observed frequencies are not significantly different from the expected percentages shown in italics.

‖ Excluding one female, who ovulated 6/13 primary oocytes, the proportion of primary oocytes in females from cross 9 was 16/259 (6·2%). This was significantly different from the 11·0% expectation for version (*a*) considered for semi-dominant inheritance ($\chi^2 = 6{\cdot}15$; $P < 0{\cdot}05$) but not significantly different from the 3·9% expectation for version (*b*) ($\chi^2 = 3{\cdot}55$; $P > 0{\cdot}05$).

Table 2. *Tests for autosomal, semi-dominant inheritance (version* b *from Table 1)*

		Expected frequency of genotypes*			Expected proportions†	Observed proportions	Statistical significance	
Cross: female × male		+/+	m/+	m/m	Triploid:Diploid	Triploid:Diploid	χ^2	P
5	(LT × C57) × (LT × C57)	0·25	0·5	0·25	0·137:0·863	27:202	0·71	> 0·05
6	(C57 × LT) × (C57 × LT)	0·25	0·5	0·25	0·137:0·863	29:299	6·55	*< 0·02*‡
7	(LT × C57) × LT	0	0·5	0·5	0·235:0·765	60:233	1·49	> 0·05
8	LT × (LT × C57)	0	0·5	0·5	0·235:0·765	50:182	0·49	> 0·05
9	(LT × C57) × C57	0·5	0·5	0	0·039:0·961	22:250	12·73	*< 0·001*‡
						16:243§	3·58	> 0·05
10	C57 × (LT × C57)	0·5	0·5	0	0·039:0·961	8:207	0·02	> 0·05

* Semi-dominant inheritance of a single autosomal gene: + is the wildtype, C57BL-strain allele; *m* is the mutant, LT-stain allele. Presence of the *m* allele is assumed to increase the probability of ovulation of a primary oocyte.
† Expected proportion of triploidy for the three hypothetical genotypes is calculated from observed frequencies in crosses 1–4 as: 0·012 for +/+, 0·066 for *m*/+ and 0·404 for *m*/*m*. The overall expected proportion of triploidy for each cross is calculated as the sum of the expected frequency of each genotype multiplied by the relevant expected proportion of triploidy.
‡ Probability values less than 0·05 are shown in *italics* and indicate a significant departure from expectations.
§ Excluding one female, who ovulated 6/13 primary oocytes.

of 13 eggs that were analysable. If this female is ignored, the similarity between crosses 9 and 10 is greater (16/259 versus 8/215; $\chi^2 = 1{\cdot}01$, $P = 0{\cdot}315$).

Autosomal dominant inheritance can be ruled out because the proportion of triploids produced by F1 hybrid females (crosses 3 and 4) was much lower than by LT females (cross 1). This leaves either autosomal semi-dominant or autosomal recessive as possible modes of inheritance. The frequency of triploids produced in crosses 3 and 4 (12/222 and 21/270, respectively) was much less than the mean of the frequencies produced by LT and C57BL mice (20·8%), and these differences were highly significant ($\chi^2 = 30{\cdot}82$, $P < 0{\cdot}001$ for cross 3 and $\chi^2 = 22{\cdot}23$, $P < 0{\cdot}001$ for cross 4). This rules out semi-dominant inheritance with a linear relationship between gene dosage and proportion of triploids (version *a* in Table 1), but does not exclude other types of semi-dominant inheritance.

For the second type of semi-dominance (version *b* in Table 1), it was assumed that the proportion of triploids expected from heterozygous females was the mean of that observed in the two F1 crosses (i.e. 6·6%). Comparison of observed and expected frequencies for crosses 5–10 shows that four of these six crosses (crosses 5, 7, 8 and 10) produced triploids at frequencies compatible with this type of semi-dominant inheritance (Tables 1 and 2), but the results are also compatible with recessive inheritance for four of these crosses (crosses 5, 6, 7 and 8).

Two crosses produced results that differed from those predicted by autosomal semi-dominant inheritance (version *b*) in Table 1. Cross 6 failed to match expectations and produced fewer triploids (8·8%) than the expected 13·7% (see Table 2 for statistics). The data for this cross are more compatible with the 11% predicted by autosomal recessive inheritance ($\chi^2 = 1{\cdot}56$, $P > 0{\cdot}05$). Cross 9 also departed from expectations but, as shown in Table 2, exclusion of the data from one female would adequately resolve this discrepancy.

Overall, the discrepancies with the predictions for autosomal recessive inheritance seem more significant (Table 1). Most importantly, the two groups of F1 females (crosses 3 and 4) both produced significantly more triploids than the control C57BL mice in cross 2 ($\chi^2 = 5{\cdot}69$, $P = 0{\cdot}017$ for cross 3 and $\chi^2 = 11{\cdot}68$, $P = 0{\cdot}0006$ for cross 4). This makes it unlikely that the trait is inherited as an autosomal recessive gene.

The conclusion is supported by the two backcrosses to C57BL, which also produced more triploids than expected for autosomal recessive inheritance, i.e. significantly higher than the C57BL females. For cross 9 this difference was highly significant ($\chi^2 = 12{\cdot}49$, $P = 0{\cdot}0004$ or $\chi^2 = 7{\cdot}73$. $P = 0{\cdot}005$ if female M391 is excluded – see above). For cross 10 this difference is only of borderline significance. [Cross 10 produced 3·7% triploids (8/215) which was significantly greater than the expected 1·2% when 8/215 was compared with the expected frequency of 2·58/215 (the calculation, used in Table 1: with Yates correction, $\chi^2 = 3{\cdot}99$, $P < 0{\cdot}05$). However, when compared to the observed frequency of 3/256 (from cross 2) the χ^2 value of 2·30 is not significant in either a two-tailed, $P = 0{\cdot}129$ or one-tailed test, $P = 0{\cdot}065$].

Thus, although some minor inconsistencies remain, autosomal (or pseudoautosomal) semi-dominant inheritance seems most likely from the results shown in Table 1. Distributions of the proportion of triploids per female from the ten crosses should also help to decide the mode of inheritance, but estimation of these proportions is likely to be inaccurate if the number of eggs analysed is low. For this reason, data were plotted only for those females that produced at least 10 analysable oocytes. Many females produced less than 10 analysable oocytes (Fig. 2), so results were

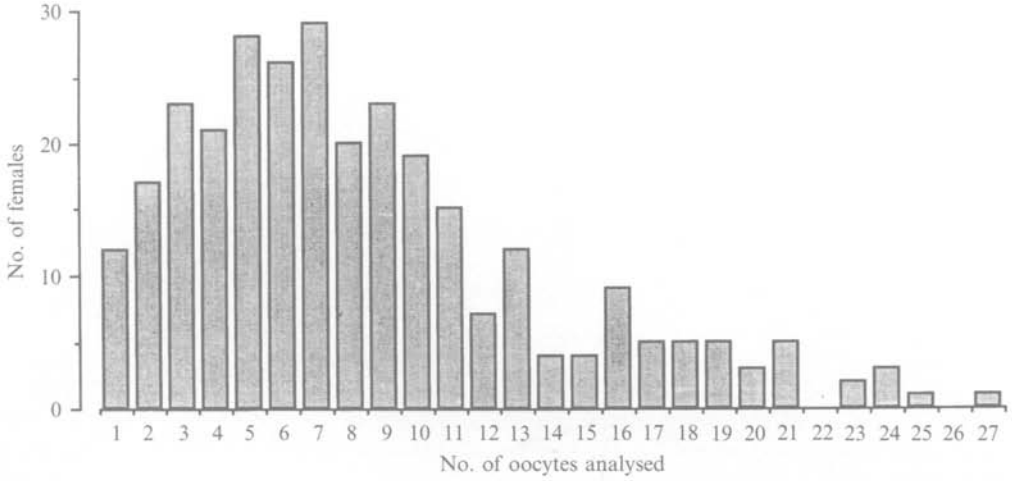

Fig. 2. Distribution of the number of oocytes analysed from each of the 299 females used in the study.

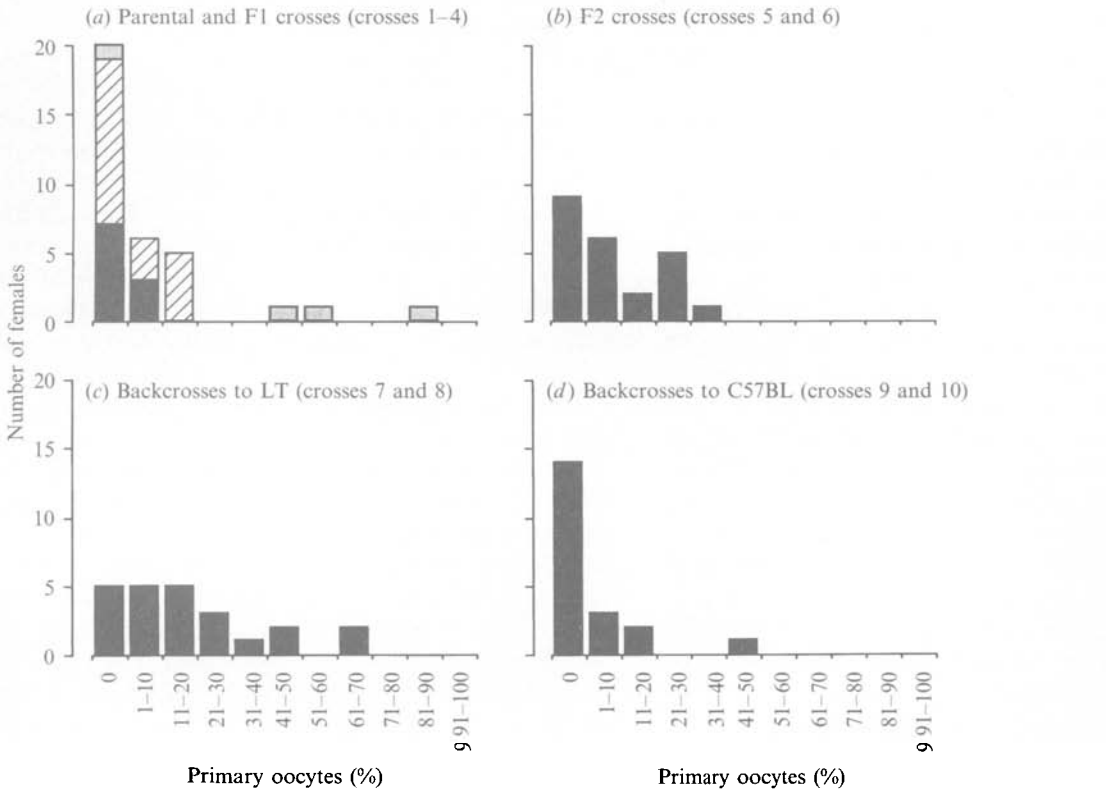

Fig. 3. Distributions of the percentage of oocytes that were ovulated as primary oocytes by females from the ten genetic crosses, excluding females who produced less than ten analysable oocytes. Data from reciprocal crosses have been pooled. The distributions for parental LT/SvKau (▨), parental C57BL/Ws (■) and F1 (▨) females are shown in (*a*). Distributions for F2 and backcrosses are shown in (*b–d*), as indicated.

pooled for reciprocal crosses, since they were not significantly different, and the results were plotted (Fig. 3). This severely reduced the number of females in the two parental crosses. Only 4 of the 33 LT females (cross 1) produced at least 10 analysable oocytes, but these four females illustrated the spread of the data. Of 31 C57BL females (cross 2), 10 produced at least 10 analysable oocytes, and these included the three females which each ovulated a single primary oocyte.

If the trait is inherited as a single autosomal, semi-dominant gene with normal (+) and mutant (*m*) alleles (from strains C57BL and LT respectively), F1 females (from crosses 3 and 4) should all be genetically equivalent (all *m*/+ heterozygotes), but in crosses 5–10 some will be heterozygous and others will be homozygous. The distributions shown in Fig. 3 are more compatible with autosomal semi-dominant inheritance than autosomal recessive inheritance. Although LT females (cross 1) are all genetically identical, the percentage of oocytes that were ovulated as primary oocytes varied from 0/11 to 9/11 (0–81·8%). Nevertheless, the distribution differed from that of the control C57BL mice and of the two

https://doi.org/10.1017/S001667230003189X Published online by Cambridge University Press

F1 crosses, also shown in Fig. 3*a*. None of the control C57BL females ovulated more than 10% of their oocytes as primary oocytes, and none of the F1 females ovulated over 20% primary oocytes. Apart from the control C57BL × C57BL cross, each cross (including F1 crosses 3 and 4 and the backcrosses to C57BL – crosses 9 and 10) produced some females which ovulated more than 10% primary oocytes (Figs 3*a–d*). This is consistent with the conclusion that the trait is not inherited as an autosomal recessive gene. Apart from one female in cross 9 (already mentioned above), females which ovulated more than 20% of their oocytes as primary oocytes were only found in those crosses which would have been expected to produce homozygous *m/m* females (crosses 1 and 5–8). Again, this is consistent with the conclusions drawn from the overall proportions of oocytes ovulated (Table 1).

The data are not sufficiently precise to define absolute limits for each of the three putative genotypes. (For example, all crosses produced some females which ovulated no primary oocytes; also a single primary oocyte would represent 10% of those ovulated if only 10 were analysed.) It is, however, possible to define trends. Thus, +/+ females tend to ovulate only 0–10% of their oocytes as primary oocytes (usually none), *m*/+ tend to produce no more than 20% but *m/m* females frequently produce well over 20% primary oocytes. Although the results do not rule out the possibility of polygenic inheritance or minor modifier genes, the simplest explanation that fits the data is that the increased chance of ovulating primary oocytes in LT mice is caused by a single, autosomal semi-dominant gene. This gene has variable expressivity, because the proportion of primary oocytes ovulated by LT/SvKau mice is variable (Fig. 3) and age-dependent (Speirs & Kaufman, 1990). Some LT/SvKau mice ovulated only secondary oocytes (Fig. 3), so the gene should also be considered to show incomplete penetrance.

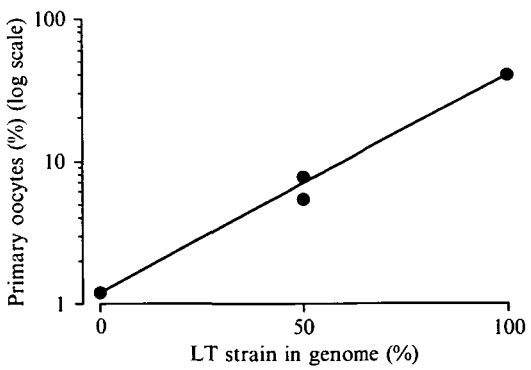

Fig. 4. Semi-logarithmic plot showing the relationship between the mean percentage of ovulated primary oocytes and the percentage of LT/SvKau strain present in the genome of the genetically homogeneous females from crosses 1–4.

The relationship between the gene dose and proportion of oocytes ovulated as primary oocytes was nonlinear. Fig. 4 shows that, for the genetically homogeneous females from crosses 1–4, this relationship was closer to a logarithmic one. Oocytes in putative *m*/+ heterozygotes are approximately 6 times as likely to be ovulated before completing the first meiotic division as those in homozygous wildtype (+/+) females of the C57BL strain. In turn, primary

Table 3. *Tests for segregation of triploidy production with* Gpi-1s *alleles (chromosome 7) in females produced by crosses 5–10*

Cross (female × male)		No. of females analysed			Proportion (%) of primary oocytes			Statistical significance	
	Gpi-1s genotype of females...	*a/a*	*a/b*	*b/b*	*a/a*	*a/b*	*b/b*	χ^2	*P*
5	(LT × C57) × (LT × C57)	4	15	9	5/34 (14·7)	20/122 (16·4)	2/73 (2·7)	8·51	*0·014**†
6	(C57 × LT) × (C57 × LT)	10	20	5	11/87 (12·6)	14/195 (7·2)	4/46 (8·7)	2·23	0·328
7	(LT × C57) × LT	18	16	—	27/153 (17·7)	33/140 (23·6)	—	1·58	0·209
8	LT × (LT × C57)	16	9	—	34/150 (22·7)	16/82 (19·5)	—	0·31	0·576
9	(LT × C57) × C57	—	15	26	—	6/108 (5·6)	16/164§ (9·8)	1·03	0·310
10	C57 × (LT × C57)	—	11	11	—	3/133 (2·3)	5/82 (6·1)	2·09	> 0·10

Alleles of the glucose phosphate isomerase locus are abbreviated as *a* (*Gpi-1s*^{a}) and *b* (*Gpi-1s*^{b}).

* Probability values less than 0·05 are shown in *italics* (2 degrees of freedom for crosses 5 and 6; 1 degree of freedom for crosses 7–10) and indicate a significantly unequal distribution of primary oocytes among the different genotypes.

† Homozygous, *b/b* females ovulated a significantly lower proportion of primary oocytes than either *a/b* mice ($\chi^2 = 7{\cdot}20$, $P = 0{\cdot}007$) or *a/a* animals ($\chi^2 = 3{\cdot}65$, $P < 0{\cdot}05$ for a one-tailed test). However, there was no significant difference between *a/a* and *a/b* females ($\chi^2 = 0{\cdot}0007$, $P = 0{\cdot}978$).

§ Excluding one female who ovulated 6/13 primary oocytes, the proportion is 10/151 (6·6%); $\chi^2 = 0{\cdot}008$, $P = 0{\cdot}928$.

https://doi.org/10.1017/S001667230003189X Published online by Cambridge University Press

Table 4. *Tests for segregation of triploidy production with brown alleles (chromosome 4) in females produced by crosses 5–10*

Cross (female × male)		No. of females analysed			Proportion (%) of primary oocytes			Statistical significance	
	Genotype of females...	*lt/lt*	*+/lt*	*+/+*	*lt/lt*	*+/lt*	*+/+*	χ^2	*P*
5	(LT × C57) × (LT × C57)	11	7	10	14/96 (14·6)	6/56 (10·7)	7/77 (9·1)	1·32	0·516
6	(C57 × LT) × (C57 × LT)	6	21	8	0/65 (0)	25/174 (14·4)	4/89 (4·5)	14·98*	*0·0006*†
7	(LT × C57) × LT	18	16	—	27/157 (17·2)	33/136 (24·3)	—	2·24	0·135
8	LT × (LT × C57)	11	14	—	29/118 (24·6)	21/114 (18·4)	—	1·30	0·254
9	(LT × C57) × C57	—	14	27	—	13/92‡ (14·1)	9/180 (5·0)	5·65	*0·017*†
10	C57 × (LT × C57)	—	12	10	—	7/125 (5·6)	1/90 (1·1)	1·82	> 0·10

Alleles of the brown locus are abbreviated as + (wildtype or black, *B* allele) and *lt* (light, B^{lt} allele).
* Heterozygous, *+/lt* females ovulated a significantly higher proportion of primary oocytes than *+/+* animals: $\chi^2 = 4{\cdot}89$, $P = 0{\cdot}027$. However, none of the 65 oocytes ovulated by six homozygous *lt/lt* females were primary oocytes.
† Probability values less than 0·05 are shown in *italics* (2 degrees of freedom for crosses 5 and 6; 1 degree of freedom for crosses 7–10) and indicate a significantly unequal distribution of primary oocytes among the different genotypes.
‡ Excluding one female who ovulated 6/13 primary oocytes, the proportion is 7/79 (8·9%); $\chi^2 = 1{\cdot}41$, $P > 0{\cdot}05$ (not significant).

oocytes in putative homozygous *m/m* females of the LT strain are approximately 6 times more likely to be ovulated than those in the *m/+* heterozygotes.

The females were also typed for two genetic markers that were known to be segregating in the crosses between C57BL and LT. The LT/SvKau strain carried the semi-dominant B^{lt} allele (abbreviated to *lt*) of the brown coat colour gene and the semi-dominant *Gpi-1s*^{a} allele of glucose phosphate isomerase, whereas C57BL/Ws mice carried alternative alleles *B* and *Gpi-1s*^{b}. Although individual females could not always be classified as *m/m*, *m/+* or *+/+*, it was still possible to test for genetic linkage. If either brown or *Gpi-1s* was closely linked to the gene responsible for triploidy, mice with alleles that were derived from the LT strain (B^{lt} and *Gpi-1s*^{a}) would be expected to ovulate significantly more primary oocytes than other females from the same cross. The results (Tables 3 and 4) showed that this was not a consistent trend.

Only cross 5 showed any significant association between the triploidy trait and *Gpi-1s*^{a} (Table 3). Homozygous, *b/b* females ovulated a significantly lower proportion of primary oocytes than either *a/b* or *a/a* animals, but there was no significant difference between *a/a* and *a/b* females. In Table 4, two crosses (6 and 9) showed a significant association between the trait and B^{lt} (*lt*). Heterozygous *+/lt* females from cross 6 ovulated a significantly higher proportion of primary oocytes than *+/+* animals, but since none of the 65 oocytes ovulated by six homozygous *lt/lt* females was a primary oocyte, the biological significance of this difference remains unclear. Heterozygous, *+/lt* females from cross 9 also ovulated a significantly higher proportion of primary oocytes than *+/+* animals (Table 4). In order to test for differences between *lt/lt* and *+/lt* females, data were pooled from crosses 7 and 8. Overall, the proportion of triploids produced by *lt/lt* females (56/275; 20·4%) was no different from that produced by *+/lt* females (54/250; 21·6%: $\chi^2 = 0{\cdot}12$, $P = 0{\cdot}728$). There is no evidence, from these results, that the triploidy trait is tightly linked to either brown on chromosome 4 or glucose phosphate isomerase on chromosome 7.

4. Discussion

The trait causing ovulation of primary oocytes in LT/SvKau mice is clearly heritable. The genetic trait that results in the ovulation of primary oocytes in NMRI/Han mice (Bartels *et al.* 1984; Beermann *et al.* 1987) was assigned the provisional gene symbol *Dipll* (diploid oocytes, metaphase I arrested). The inhibition of the first meiotic division in NMRI/Han mice was thought to be a consequence of impaired communication between the oocyte and the surrounding somatic cells of the follicle (Bartels *et al.* 1984; Hansmann *et al.* 1985) rather than a defect within the oocyte itself, but this is not known for the trait found in LT/SvKau strain mice. As pointed out in the Introduction, LT/SvKau strain mice ovulate a much higher proportion of primary oocytes than NMRI/Han mice either with or without superovulation. At 8–12 weeks, superovulation increased the frequency of primary oocytes ovulated from 24 to 34% (Speirs & Kaufman, 1988), but the frequency recovered, after superovulation of 5 to 7-week-old mice, in the present

study (40·4%) was no higher than that reported for spontaneous ovulations in similar-aged mice (Speirs & Kaufman, 1990).

Many genetic lesions could result in a block at metaphase I, and it is not known whether the semi-dominant gene in the LT/SvKau strain is allelic with *DipII*. For this reason we propose to assign a different symbol to the gene present in the LT/SvKau, for use until it is shown to be allelic or identical with another known gene. We propose to name this semi-dominant gene *Poo* (primary oocyte ovulation) with allele designations based on the type strains used in the present study. The allele Poo^{t} is the 'mutant' allele present in the LT/SvKau strain and the allele Poo^{b} is the normal allele present in C57BL/Ws.

Another unusual feature of ovaries of LT/Sv strain mice is that they produce teratomas. These ovarian teratomas arise within granulosa cell-deficient follicles (Eppig, 1978). Twenty-one of 23 teratomas, arising in $Gpi\text{-}1s^{a}/Gpi\text{-}1s^{b}$ heterozygous mice, were homozygous at *Gpi-1s* and were, therefore, derived from oocytes that had completed the first meiotic division (Eppig *et al.* 1977). The remaining two teratomas were heterozygous and could have arisen either at the same stage, with genetic recombination between the centromere and the *Gpi-1s* locus, or from heterozygous primary oocytes. The incidence of ovarian teratomas in the LT/SvKau sub-strain of LT/Sv is low (Speirs & Kaufman, 1990) and it is not clear whether the ovulation of primary oocytes from apparently morphologically normal follicles and the production of teratomas within depleted follicles are both caused by a common genetic lesion.

The possibility also exists that a similar genetic mutation in human females may be responsible for the differentiation of benign ovarian teratomas. These are known to result from the spontaneous parthenogenetic activation of ovarian oocytes that have previously completed the first meiotic division and continued to develop *in situ* (Linder & Power, 1970; Linder, McCaw & Hecht, 1975). It is unclear whether these develop within granulosa cell-deficient follicles, equivalent to those observed in LT/Sv mice (Eppig, 1978).

The timing of ovulation is dependent on changes that are initiated in the ovarian follicle by the surge of luteinizing hormone (LH). This LH surge also triggers the production of maturation promoting factor (MPF) and the release of the oocyte from the dictyate stage of meiotic prophase I. Normally the timing is such that the oocyte progresses through meiosis and has arrested at metaphase II by the time it is ovulated. If the timing of the two events is uncoupled, the oocyte may fail to reach metaphase II by the time of ovulation. This could occur if progress through the normal meiotic divisions was unusually slow or if the process was blocked, as in the case of the oocytes from LT/SvKau mice which block at metaphase of the first meiotic division (Kaufman & Howlett, 1986; O'Neill & Kaufman, 1987). Progress from prophase I to metaphase II is dependent on the successful completion of a sequence of events, including germinal vesicle breakdown, chromosome condensation and the extrusion of the first polar body. These events are dependent on communication between the oocyte and the surrounding follicle cells and an interplay of molecules within the oocyte (Eppig & Downs, 1984). Some of these molecules have now been identified.

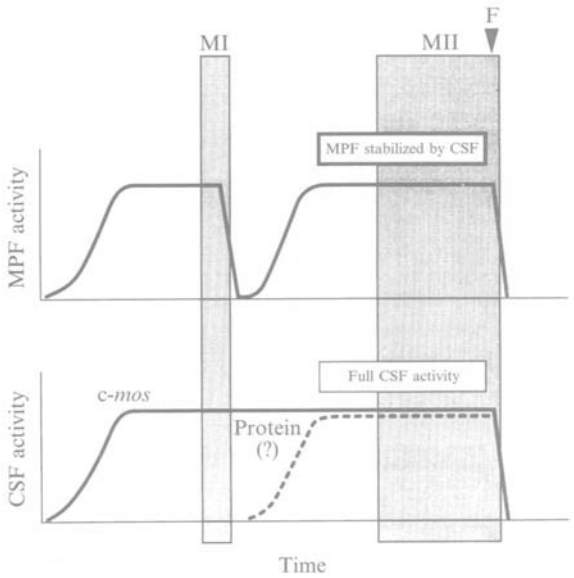

Fig. 5. Schematic representation of proposed MPF and CSF levels during maturation of *Xenopus* oocytes, based on diagram in Sagata *et al.* (1989) but revised to take account of subsequent results (Yew *et al.* 1992). The MPF level falls transiently at the end of MI but remains high in MII (stabilized by full CSF activity: c-*mos* protein plus a second protein) and the oocyte remains arrested until CSF is degraded by the activation of calpain at fertilization. See the text for further details. MI, meiotic metaphase I; MII, meiotic metaphase II; F, fertilization.

The identification of the various molecules involved has been largely the result of work on oocytes of non-mammalian species, such as *Xenopus* (e.g. Murray & Kirschner, 1989; Murray, Solomon & Kirschner, 1989; Sagata *et al.* 1989; Watanabe *et al.* 1989; Kanki & Donoghue, 1991; Yew, Mellini & Vande Woude, 1992; Yew, Strobel & Vande Woude, 1993). However, the molecules involved occur in other species, and it is likely that much of the *Xenopus* model (Fig. 5) is also valid for mice. MPF has two subunits: cyclin and $p34^{cdc2}$ kinase, homologous to the *cdc2* (cell division cycle 2) gene of *Schizosaccharomyces pombe*, which is thought to phosphorylate molecules involved in cell division. MPF activity rises and falls at both meiotic and mitotic divisions. At the end of each interphase, MPF activity increases and initiates the next cell division; MPF levels fall again as the cell exits from metaphase and progresses to the next interphase. Another factor, the cytostatic factor (CSF) is produced during meiosis but not during mitosis. CSF stabilizes MPF levels after meiotic metaphase I, and this causes the secondary oocyte to arrest in meiotic metaphase II. Fertilization results in an influx of calcium and the

https://doi.org/10.1017/S001667230003189X Published online by Cambridge University Press

activation of a calcium-dependent protease (calpain) which degrades CSF. As a result of the loss of the stabilizing CSF activity, MPF levels fall and the oocyte completes the second meiotic metaphase division to extrude the second polar body.

Sagata *et al.* (1989) showed that the protein product of the c-*mos* proto-oncogene (pp39^{mos}) is necessary to block secondary oocytes in metaphase II, and Kanki & Donoghue (1991) showed that *Xenopus* oocytes needed c-*mos* protein to progress from the first to second meiotic divisions. Recent experiments, reported by Yew *et al.* (1992) showed that another protein is needed for CSF function. This implies that CSF includes c-*mos* protein and another, as yet unidentified, protein. It is thought that synthesis of this protein does not begin until after meiotic metaphase I, so the oocyte can exit from the first metaphase before full CSF activity is available to stabilize MPF (Fig. 5). At the next meiotic division full CSF activity is present and the oocyte arrests in metaphase II.

A genetic defect that made CSF less efficient at stabilizing MPF would be unlikely to block the oocyte in metaphase I. Although failure to stabilize MPF would block progress to meiosis II, the oocyte would be expected to be arrested between divisions, because low levels of MPF are thought to cause cells to exit from metaphase. This type of arrest has been demonstrated for mouse oocytes by O'Keefe *et al.* (1989). Mouse oocytes depleted in c-*mos*, by the injection of antisense c-*mos* oligonucleotides, completed the first meiotic division but failed to initiate the second division. After meiosis I, the chromosomes decondensed to form a nucleus and the oocyte cleaved to produce two cells.

According to the scheme shown in Fig. 5, oocytes could arrest in meiotic metaphase I if MPF levels remained high. Cyclin degradation is required to inactivate MPF and exit from meiotic metaphase I. Murray, Solomon & Kirschner (1989) cited evidence that protease inhibitors arrest starfish oocytes in meiosis I and stabilize cyclin. These authors also demonstrated that injection of protease-resistant cyclin into *Xenopus* oocytes or eggs caused them to arrest at the next cell division, whether meiotic or mitotic. If the *Poo*^{l} gene, present in LT mice, caused cyclin to be more resistant to degradation, oocytes might block in meiotic metaphase I, but subsequent mitotic divisions would also probably be affected. An effect specific to meiotic divisions is more likely to involve the components of CSF or the capacity of MPF to be stabilized by CSF. For example, oocytes could arrest at metaphase I if MPF was stabilized prematurely (for example, by early synthesis of the second protein component of CSF, before the oocyte had progressed beyond metaphase I or by a mutant form of c-*mos* protein that didn't require the second protein to stabilize MPF).

The act of fertilization must be capable of releasing both metaphase I and metaphase II blocks because both primary and secondary oocytes can be fertilized to produce viable embryos. Evidence from *Xenopus* (Sagata *et al.* 1989; Watanabe *et al.* 1989) suggests that this release involves the degradation of the protein product of the c-*mos* proto-oncogene by an endogenous calcium-dependent protease (calpain). This is consistent with an involvement of the c-*mos* protein in the metaphase-I block.

Whatever is responsible for the metaphase I block in LT/SvKau oocytes, it is worth noting that even control, putatively +/+, C57BL females ovulate some oocytes as primary oocytes. This suggests that there is scope for the control systems to fail and that the *Poo*^{l} allele increases the probability of this failure. A detailed mapping study should reveal whether *Poo* is allelic with any known genes that encode components of CSF or MPF. In the mouse, the c-*mos* protein is encoded by *Mos* (Moloney sarcoma oncogene), which maps to mouse chromosome 4, approximately 25 cM proximal to brown (Dandoy *et al.* 1989). Although we found no evidence for tight linkage between *Poo* and *b*, the results do not rule out weaker linkage of the magnitude expected between *Mos* and *b*. Since MPF is composed of two subunits, a cyclin and a *cdc2* protein, some of these genes may be relevant to the mapping of *Poo*. It will be of interest to test for allelism between *Poo* and various mouse cyclin genes and related sequences, including *Ccna* (chromosome 3, formerly *Cyca*, encoding Cyclin A; Sakaguchi *et al.* 1991), *Ccnb1-rs13* (chromosome 13, formerly *Cycb-1*, encoding a sequence which may be the functional Cyclin B1 gene) and *Ccnb2* (formerly *Cycb-2*, encoding Cyclin B2). Other cyclin B1-related sequences have been mapped to chromosomes 1, 4, 5, 7, 8, 13, 14, 15, 17 and the X (for a recent listing see Peters & Cocking, 1993). It will also be worth testing for allelism with the three known cell division cycle proteins, *Cdc2a* (chromosome 10), *Cdc2b* (chromosome 17) and *Cdc25* (Justice *et al.* 1990; Sakaguchi *et al.* 1991).

The *Poo*^{l} mutant in the LT/SvKau strain of mice provides a valuable resource to study the cell and molecular biology of mammalian oocyte maturation and the control of meiosis.

We thank Drs Verne M. Chapman, Ian Jackson and Roger Gosden for helpful comments, Mrs Jean Flockhart for performing the GPI electrophoresis and Mr Denis Doogan and Mrs Maureen Ross for expert mouse husbandry. We are grateful to the Wellcome Trust (grant 032409 to M.H.K. and J.D.W.) for financial support.

References

Bartels, I., Jenderny, J. & Hansmann, I. (1984). Control of meiosis by somatic cells in mice: Inheritance of the meiosis I error 'diploidy' and nondisjunction in sensitive NMRI/Han oocytes ovulated from chimaeras. *Cell Differentiation* **15**, 189–194.

Beermann, F., Jenderny, J., Hummler, E. & Hansmann, I.

(1987). Inheritance of a meiosis I error expressed in mouse oocytes and modulated by a maternal factor. *Genetical Research* **49**, 239–243.

Dandoy, F., De Maeyer-Guignard, J. & De Maeyer, E. (1989). Linkage analysis of the murine *mos* proto-oncogene on chromosome 4. *Genomics* **4**, 546–551.

Eppig, J. J. (1978). Granulosa cell deficient follicles. Occurrence, structure and relationship to ovarian teratocarcinogenesis in strain LT/Sv mice. *Differentiation* **12**, 111–120.

Eppig, J. J. & Downs, S. M. (1984). Chemical signals that regulate mammalian oocyte maturation. *Biology of Reproduction* **30**, 1–11.

Eppig, J. J., Kozak, L. P., Eicher, E. M. & Stevens, L. C. (1977). Ovarian teratomas in mice are derived from oocytes that have completed the first meiotic division. *Nature* **269**, 517–518.

Hansmann, I. & El-Nahass, E. (1979). Incidence of nondisjunction in mouse oocytes. *Cytogenetics and Cell Genetics* **24**, 115–121.

Hansmann, I., Bartels, I., Beermann, F., Caspari, D., Hummler, E. & Theuring, F. (1985). Mechanisms of nondisjunction: facts and perspectives. In *Aneuploidy: Aetiology and Mechanisms* (ed. V. L. Dellarco, P. E. Votek, A. Hollaender, B. R. Brinkley, E. B. Hook and M. J. Moses), pp. 417–432. New York: Plenum.

Jenderny, J., Düls, C., Probeck, H. D., Richter, P., Schulz, H. & Hansmann, I. (1980). Genetically determined diploidy in mouse oocytes. *European Journal of Cell Biology* **22**, 23.

Justice, M. J., Siracusa, L. D., Gilbert, D. J., Heisterkamp, N., Groffen, J., Chada, K., Silan, C. M., Copeland, N. G. & Jenkins, N. A. (1990). A genetic linkage map of mouse chromosome 10: localization of eighteen molecular markers using a single interspecific backcross. *Genetics* **125**, 855–866.

Kanki, J. P. & Donoghue, D. J. (1991). Progression from meiosis I to meiosis II in *Xenopus* oocytes requires de novo translation of the *mos*^{xe} protooncogene. *Proceedings of the National Academy of Sciences, USA* **88**, 5794–5798.

Kaufman, M. H. & Howlett, S. K. (1986). The ovulation and activation of primary and secondary oocytes in LT/Sv strain mice. *Gamete Research* **14**, 255–264.

Linder, D., McCaw, B. K. & Hecht, F. (1975). Parthenogenetic origin of benign ovarian teratomas. *New England Journal of Medicine* **292**, 63–66.

Linder, D. & Power, J. (1970). Further evidence for postmeiotic origin of teratomas in the human female. *Annals of Human Genetics* **34**, 21–30.

Murray, A. W. & Kirschner, M. W. (1989). Cyclin synthesis drives the early embryonic cell cycle. *Nature* **339**, 275–280.

Murray, A. W., Solomon, M. J. & Kirschner, M. W. (1989). The role of cyclin synthesis and degradation in the control of maturation promoting factor activity. *Nature* **339**, 280–286.

O'Keefe, S. J., Wolfes, H., Kiessling, A. A. & Cooper, G. M. (1989). Microinjection of antisense c-*mos* oligonucleotides prevents meiosis II in the maturing mouse egg. *Proceedings of the National Academy of Sciences, USA* **86**, 7038–7042.

O'Neill, G. T. & Kaufman, M. H. (1987). Ovulation and fertilization of primary and secondary oocytes in LT/Sv strain mice. *Gamete Research* **18**, 27–36.

Peters, J. & Cocking, Y. (1993). Mouse gene list. *Mouse Genome* **91**, 156–249.

Sagata, N., Watanabe, N., Vande Woude, G. F. & Ikawa, Y. (1989). The c-*mos* proto-oncogene product is a cytostatic factor responsible for meiotic arrest in vertebrate eggs. *Nature* **342**, 512–518.

Sakaguchi, A. Y., Martinez, L., Lalley, P. A., Sylvia, V. L., Hans, E. S., Killary, A. M. & Ghosh Choudhury, G. (1991). Chromosome mapping in mouse of genes that regulate the eukaryotic cell cycle. *Cytogenetics and Cell Genetics* **58** (Human Gene Mapping 11th London Conference, p. 2138, abstract 27428).

Speirs, S. & Kaufman, M. H. (1988). Effect of exogenous hormones on the ovulation of primary and secondary oocytes in LT/Sv strain mice. *Gamete Research* **21**, 179–184.

Speirs, S. & Kaufman, M. H. (1990). Effect of maternal age on the incidence of digynic triploidy in LT/Sv strain mice: implications for the ovulation of primary and secondary oocytes in this strain. *Journal of Experimental Zoology* **253**, 83–87.

Takagi, N. (1970). High incidence of triploid embryos associated with artificial polyovulation in mice. Chromosome Information Service no. 11, p. 32.

Takagi, N. & Oshimura, M. (1973). Developmental fate of triploid mouse embryos induced by superovulation. Chromosome Information Service no. 14, p. 36.

Takagi, N. & Sasaki, M. (1976). Digynic triploidy after superovulation in mice. *Nature* **264**, 278–281.

Tarkowski, A. K. (1966). An air-drying method for chromosome preparations from mouse eggs. *Cytogenetics* **5**, 394–400.

Watanabe, N., Vande Woude, G. F., Ikawa, Y. & Sagata, N. (1989). Specific proteolysis of the c-*mos* proto-oncogene product by calpain on fertilization of *Xenopus* eggs. *Nature* **342**, 505–511.

West, J. D. & Fisher, G. (1984). A new allele of the *Gpi-1t* temporal gene that regulates the expression of glucose phosphate isomerase in mouse oocytes. *Genetical Research* **44**, 169–181.

Whittingham, D. G. (1971). Culture of mouse ova. *Journal of Reproduction and Fertility* (Supplement 14), 7–21.

Yew, N., Mellini, M. L. & Vande Woude, G. V. (1992). Meiotic initiation by the *mos* protein in *Xenopus*. *Nature* **355**, 649–652.

Yew, N., Strobel, M. & Vande Woude, G. V. (1993). Mos and the cell cycle: the molecular bases of the transformed phenotype. *Current Opinions in Genetics and Development* **3**, 19–25.