Genet. Res., Camb. (2000), **75**, *pp.* 199–208. *With 2 figures.* Printed in the United Kingdom © 2000 Cambridge University Press

Correlated responses to selection for large body size in oMt1a-oGH transgenic mice: reproductive traits

K. R. PARKS[1], E. J. EISEN[1*], I. J. PARKER[1], L. G. HESTER[1] AND J. D. MURRAY[2]
[1] *Department of Animal Science, Box 7621, North Carolina State University, Raleigh, NC 27695 USA*
[2] *Department of Animal Science and Department of Population Health and Reproduction, University of California, Davis, CA 95616, USA*

(*Received 30 April 1999 and in revised form 9 August 1999*)

Summary

Correlated responses in female reproductive performance were evaluated following short-term selection within full-sib families for increased 8-week body weight in two replicates of four lines of mice: two ovine metallothionein–ovine growth hormone (oMt1a-oGH) transgene-carrier lines, one from a high-growth background (TM) and one from a control background (TC), and two non-transgenic lines, one from each of these genetic backgrounds (NM and NC, respectively). A fifth line (CC), not containing the transgene, served as a randomly selected control. The initial frequency of the oMt1a-oGH transgene construct in the TM and TC lines was 0·5. The frequency of transgenic females sampled at generations 7 and 8 of selection was 84·0% and 6·1% in the TC and TM lines, respectively. No significant female infertility differences were detected between transgene-carrier and non-transgenic lines or between transgenic and non-transgenic mice within carrier lines, whereas high-growth background lines had a higher infertility than control background lines ($P < 0{\cdot}05$). Correlated responses in the TC transgene-carrier line were suggestive of reduced reproductive performance as indicated by increased post-implantation mortality ($P < 0{\cdot}05$), number of dead fetuses plus implants ($P < 0{\cdot}05$), and loss of fetuses from day 16 to parturition ($P < 0{\cdot}001$). For the first two traits, the negative correlated responses were accounted for by the reduced performance of transgenic compared with non-transgenic females. Embryos carrying the transgene may also have a lower viability. In contrast, the NC non-transgenic line did not exhibit reduced reproductive performance for these traits. The low frequency of the transgene in the high-growth background TM line was associated with reduced fitness and a lower additive effect for 8-week body weight compared with the control background TC line.

1. Introduction

Incorporation of growth hormone transgenes has resulted in positive effects on growth, feed efficiency and body composition traits in many lines of mice (Palmiter *et al*., 1982; Hammer *et al*., 1985; Shanahan *et al*., 1989; Pomp *et al*., 1992). However, adverse responses on reproductive performance have been observed with many of these transgenes. Fertility problems were reported in lines of mice containing mouse metallothionein–bovine growth hormone (Mt-bGH) transgenes, phosphoenolpyruvate carboxykinase–bovine growth hormone (PEPCK-bGH) transgenes and mouse metallothionein–human placental growth hormone variant (Mt-hGH °V) transgenes (Naar *et al*., 1991). Hammer *et al*. (1985) also reported fertility impairments in female mice carrying the Mt-bGH transgene, and Cecim *et al*. (1995) found suppressed fertility, evidenced by decreased mating and pregnancy rates, in PEPCK-bGH transgenic females. Rat growth hormone transgenes have been associated with negative reproductive performance as well (Nagai *et al*., 1992). Bartke *et al*. (1988) also found that female mice containing mouse metallothionein–human growth hormone (Mt-hGH) transgenes were sterile, presumably due to inadequate luteal function. However, mice harbouring the phosphoenolpyruvate carboxykinase–human growth hormone (PEPCK-hGH) transgene were capable of

* Corresponding author. e-mail: gene_eisen@ncsu.edu

https://doi.org/10.1017/S001667239900436X Published online by Cambridge University Press

producing numerous litters (Milton *et al*., 1992). Orian *et al*. (1989) also found that mouse metallothionein–ovine growth hormone (Mt-oGH) transgenic females were fertile.

Chronic growth hormone expression has been recognized as a factor leading to the sterility of mice with many transgenic constructs (Bartke *et al*., 1988; Cecim *et al*., 1995). In contrast, expression of the sheep metallothionein 1a–sheep growth hormone (oMt1a-oGH) transgene can be regulated. Addition of zinc ions to the drinking water stimulates growth hormone increases in the sera, while withdrawal of the supplementation returns growth hormone concentrations to basal levels within 24 h (Shanahan *et al*., 1989). No detrimental reproductive effects were observed in oMt1a-oGH female mice mated before transgene activation (Murray & Pomp, 1995). Murray & Pomp (1995) also found that oMt1a-oGH female mice whose transgene was inactivated by withdrawal of zinc supplementation 2 weeks prior to mating were fertile. Siewerdt *et al*. (1999) first evaluated the growth and reproductive responses of short-term selection for large 8-week body weight in oMt1a-oGH transgenic lines of mice with different selection backgrounds. The objective of the present study was to determine whether the presence of a growth hormone transgene affected correlated responses in female reproductive performance following selection for increased body size in mice.

Materials and methods

(i) *Lines of mice*

The selection experiment has been described by Siewerdt *et al*. (1999). Briefly, two replicates of four lines were selected within full-sib families for increased 8-week body weight: two oMt1a-oGH transgene-carrier lines, one with a high-growth selected background (TM) and one with a control line background (TC), and two non-transgenic lines, one from each of these genetic backgrounds (NM and NC, respectively). The initial gene frequency of the oMt1a-oGH transgene construct in the TM and TC lines was 0·5. Two replicates of a fifth line (CC), derived from the control line background and not containing the transgene, served as a randomly selected control. Each replicate line consisted of 16 full-sib families per generation. The term transgene-carrier line, used for TC and TM, indicates that the transgene is segregating in the line but individuals within the line may be transgenic (homozygous or hemizygous) or non-transgenic.

(ii) *Sampling of mice*

The present experiment was initiated by sampling litters from replicate 2 of generation 7 and replicate 1 of generation 8 from each of the five lines. Litters were standardized at 1 day of age to eight pups (six females and two males). Pups were toe-clipped at day 12 for identification. Toes from pups in lines TC and TM were frozen at −20 °C for later polymerase chain reaction analyses of DNA to determine the presence or absence of the oMt1a-oGH transgene (Pomp *et al*., 1991). Transgenic animals were not assayed further to distinguish homozygous and hemizygous mice. In the base population, dominance effects were not found for the reproductive traits assayed (Seiwerdt *et al*., 1998). The genotypes of females were not determined until all the reproductive data were collected. Females were weaned at 3 weeks of age, housed three mice to a cage and weighed weekly from 3 to 10 weeks of age. All females received distilled drinking water containing 25 mM zinc sulphate from 3 to 8 weeks of age and tap water afterwards. Mice were given free access to feed and water. Purina Lab Chow 5001 (Purina Mills, St Louis, MO) containing 70 ppm zinc was provided to females upon weaning. Purina Mouse Chow 5015 (Purina Mills, St Louis, MO) containing 102·2 ppm zinc was fed during mating and gestation. Temperature (22 °C), humidity (55%) and light cycle (12 h light:12 h dark) were maintained in the laboratory.

(iii) *Collection of data*

At approximately 10 weeks of age, virgin females from each line were housed with non-transgenic males of proven fertility from an unrelated line (two females: one male per cage). This scheme may introduce heterosis in the progeny for embryo survival, but it was assumed that the heterosis effect was constant across all lines. Females were checked daily for the presence of a copulatory plug. At detection of a plug (day 0 of gestation), females were weighed and placed in a cage with no more than two other females. At day 16 of gestation, pregnant females were killed by cervical dislocation. Pregnant females in which copulatory plugs were not detected at day 0 were killed at an estimated day 16 of gestation. The right and left ovaries were dissected, and the number of corpora lutea in each ovary was recorded and totalled (TCL). The number of live fetuses (TF) and the number of dead fetuses plus implants (TD) were also recorded. Females in which plugs were not detected and females that mated but were clearly not pregnant were remated to different males. Females that showed no evidence of having mated were allowed to cohabit with the same male for approximately 3 weeks before being remated. Females were recorded as infertile approximately 3 weeks after remating. The number of females sampled per line for the various traits is given in Tables 1 to 3 and 5 and Figs. 1 and 2.

https://doi.org/10.1017/S001667239900436X Published online by Cambridge University Press

Table 1. *Mean percentages of infertile females by line and replicate and linear contrasts*

Line[a]	Replicate 1	Replicate 2	Mean	Contrast	χ^2
CC	8·7 (2/23)[b]	0·0 (0/26)	4·4	s[c]	4·80*
NC	0·0 (0/31)	0·0 (0/20)	0	t[d]	1·18
NM	12·9 (4/31)	19·2 (5/26)	16·1	s × t[e]	2·8
TC	6·9 (2/29)	10·3 (3/29)	8·6	Overall[f]	0·41
TM	20·0 (6/30)	6·9 (2/29)	13·5	NC − CC	0·73
				TC − CC	0·48

[a] CC, randomly selected control; the following lines were selected for large 8-week body weight: NC, non-transgenic line from control background; NM, non-transgenic line from high-growth background; TC, transgene-carrier line from control background; TM, transgene-carrier line from high-growth background.
[b] (Number of infertile females)/(Number mated) in parentheses.
[c] High-growth background lines versus control background lines. Contrast value is NM + TM − NC − TC.
[d] Transgene-carrier versus non-transgenic lines. Contrast value is TC + TM − NC − NM.
[e] Contrast value is NC + TM − NM − TC.
[f] Contrast value is NC + NM + TC + TM − 4C.
* $P < 0{\cdot}05$.

(iv) *Statistical analysis*

Pre-implantation embryo survival was calculated as $100 \times (TF + TD) \times TCL^{-1}$. Post-implantation embryo survival was determined as $100 \times TF \times (TF + TD)^{-1}$. Total embryo survival was estimated as $100 \times TF \times TCL^{-1}$. Because percentage data often tend towards non-normality, arcsine transformations were performed on pre-implantation, post-implantation and total embryo survival to satisfy the normality assumption of analysis of variance. Analyses were similar for actual values and the transformations; therefore, only actual survival values are presented. Data on pregnant females for the embryo survival traits as well as values for total live fetuses, total dead fetuses plus implants and total corpora lutea were analysed with PROC MIXED of SAS (1996). Weekly body weights, body weights at day of copulatory plug and the number of days from placement with a male to appearance of a copulatory plug were also analysed for pregnant females. The mixed model included the fixed effects of replicate-generation and line, the random effects of litter nested within line by replicate-generation subclasses, and a residual effect. Total corpora lutea were also analysed with the covariate 10-week body weight included in the model. Body weight at 10 weeks of age was used as a covariate instead of body weight at the time of the copulatory plug because the two weights were highly positively correlated and there were no missing values for the former trait.

Least-squares means were obtained for each trait and compared using four orthogonal contrasts: s, high-growth background lines (TM and NM) versus control background lines (TC and NC); t, transgene-carrier lines (TC and TM) versus non-transgenic lines (NC and NM); s × t, the interaction of selection background by transgenic line; and overall, the four selected lines versus the control line. Pre-planned correlated response contrasts were calculated as NC − CC and TC − CC. It was not possible to calculate analogous contrasts for NM and TM because a control from the high-growth background was not maintained. Traits were also analysed within lines TC and TM with the additional effects of transgene (present versus not present) and the interaction of line by transgene added to the model.

Line differences in percentage of infertile females were tested by maximum likelihood chi-square using PROC CATMOD in SAS (1996). Individual degree of freedom contrasts were conducted analogously to those described for the mixed model least-squares analysis of variance.

3. Results

(i) *Female infertility*

Percentages of infertile females and linear contrasts are presented in Table 1. The only significant contrast was due to females from the high-growth background (NM and TM) having a higher infertility rate than those from the control background (NC and TC). Within the transgene-carrier lines (TC and TM), the frequency of infertility was the same (11·1%) for transgenic and non-transgenic mice.

(ii) *Frequency of transgenic females*

All transgene-carrier line mice were hemizygous at generation 0 of selection. However, of those females included in the embryo survival analyses, 84·0% (42 of 50) of mice from line TC and 6·1% (3 of 49) of mice from line TM carried the transgene. The line difference in the incidence of transgenic female mice was

https://doi.org/10.1017/S001667239900436X Published online by Cambridge University Press

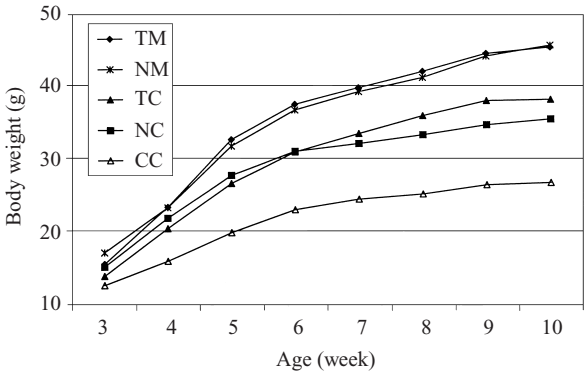

Fig. 1. Least-squares means of weekly body weights for each line. Sample size for each line is: CC, 46; NC, 51; NM, 45; TC, 50; TM, 49. CC, randomly selected control; the following lines were selected for large 8-week body weight; NC, nontransgenic line from control background; NM, transgenic line from high-growth background; TC, transgene-carrier line from control background; TM, transgene-carrier line from high-growth background.

significant ($\chi^2 = 60{\cdot}54$, d.f. $= 1$, $P < 0{\cdot}001$). Therefore, while statistical power is relatively high for testing line differences, it is low for testing the effects of transgene presence versus absence because of the disparate genotypic distributions within lines TM and TC.

(iii) *Female body weights*

Line had an effect ($P < 0{\cdot}001$) on female body weights throughout the 7-week period prior to mating (Fig. 1). High-growth background lines had larger ($P < 0{\cdot}001$) body weights than control background lines at each age (Table 2). Smaller differences existed between body weights of transgene-carrier and non-transgenic lines than between high-growth and control background lines. Selection background by transgene-carrier line interactions occurred for weeks 9 and 10 ($P < 0{\cdot}05$). The cause of the interaction was that TC mice were larger that NC mice ($P < 0{\cdot}01$), whereas the body weights of TM and NM mice did not differ significantly (Fig. 1). Large positive correlated responses were found for weekly body weights in lines TC and NC.

Body weight least-squares means for transgenic and non-transgenic mice in lines TC and TM are plotted against age in Fig. 2. Line TM was generally significantly larger than line TC from 4 to 10 weeks of age, and transgenic mice were significantly larger than non-transgenics from 7 to 10 weeks. Line by transgene interactions were not significant at any age.

(iv) *Days to and body weight at copulatory plug*

Least-squares means and orthogonal contrasts for the number of days to copulatory plug and body weight at

Table 2. *Orthogonal contrasts ± SE and correlated response contrasts ± SE involving lines[a] for weekly female body weights (g)*

Contrast	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
s^b	3·6 ± 0·8***	4·4 ± 1·2***	10·1 ± 1·4***	12·3 ± 1·4***	13·5 ± 1·4***	14·1 ± 1·5***	15·9 ± 1·5***	17·3 ± 1·5***
t^c	−2·8 ± 0·8**	−1·4 ± 1·2	−0·2 ± 1·4	0·7 ± 1·4	2·0 ± 1·4	3·5 ± 1·5*	3·7 ± 1·5*	2·4 ± 1·5
$s \times t^d$	−0·3 ± 0·8	1·5 ± 1·2	2·0 ± 1·4	0·9 ± 1·4	−0·8 ± 1·4	−1·9 ± 1·5	−3·0 ± 1·5*	−3·0 ± 1·5*
Overall[e]	11·2 ± 2·0***	25·1 ± 2·7***	39·6 ± 3·3***	44·4 ± 3·3***	47·2 ± 3·3***	52·1 ± 3·4***	55·7 ± 3·4***	57·9 ± 3·4***
NC − CC	2·5 ± 0·6***	5·9 ± 0·9***	7·9 ± 1·0***	8·1 ± 1·0***	7·7 ± 1·0***	8·2 ± 1·1***	8·4 ± 1·1***	8·8 ± 1·1***
TC − CC	1·3 ± 0·6*	4·5 ± 0·8***	6·8 ± 1·0***	8·0 ± 1·0***	9·1 ± 1·0***	10·9 ± 1·1***	11·6 ± 1·1***	10·9 ± 1·1***

[a] CC, randomly selected control; the following lines were selected for large 8-week body weight: NC, non-transgenic line from control background; NM, non-transgenic line from high-growth background; TC, transgene-carrier line from control background; TM, transgene-carrier line from high-growth background.
[b] High-growth background lines versus control background lines. Contrast value is NM + TM − NC − TC.
[c] Transgene-carrier lines versus non-transgenic lines. Contrast value is TC + TM − NC − NM.
[d] Contrast value is NC + TM − NM − TC.
[e] Contrast value is NC + NM + TC + TM − 4CC.
*$P < 0{\cdot}05$; **$P < 0{\cdot}01$; ***$P < 0{\cdot}001$.

https://doi.org/10.1017/S001667239900436X Published online by Cambridge University Press

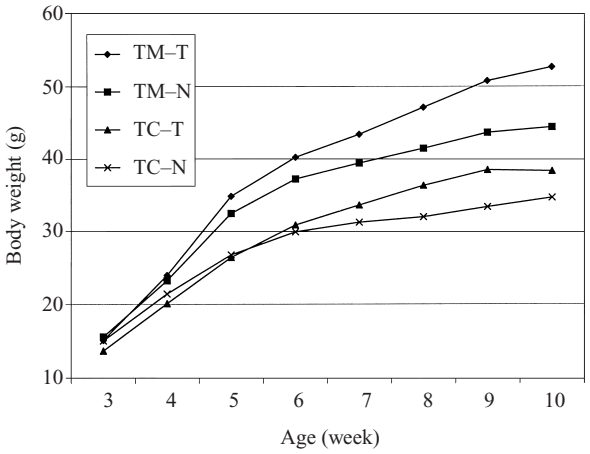

Fig. 2. Least-squares means of weekly body weights for transgenic (T) and non-transgenic (N) mice in lines TC and TM. Sample size for each subclass is: TC–N, 8; TC–T, 42; TM–N, 46; TM–T, 3. TC and TM are transgene-carrier lines from a control and high-growth background, respectively; both were selected for large 8-week body weight.

day of plug for each line are given in Tables 3 and 4, respectively.

The effect of line was not significant for number of days to plug ($P > 0{\cdot}05$). Line did have a significant effect on weight at copulatory plug ($P < 0{\cdot}001$). High-growth background lines had larger body weights at day of copulatory plug than control background lines ($P < 0{\cdot}001$) whereas transgene-carrier lines did not differ from non-transgenic lines ($P < 0{\cdot}05$). The interaction of selection background and transgene-carrier line was significant ($P < 0{\cdot}01$). The basis for this interaction was that non-transgenic lines had greater weights at day of copulatory plug within the high-growth background but transgene-carrier lines had larger weights within the control background. Correlated responses were positive for weight at day of copulatory plug in lines NC and TC.

Analyses conducted for days to copulatory plug and weight at day of copulatory plug for lines TC and TM with the effect of transgene added to the model showed that effects of line, transgene and the interaction of line by transgene were not significant for days to plug ($P > 0{\cdot}05$). Line TM had larger body weights at day of plug than line TC ($46{\cdot}85 \pm 2{\cdot}07$ g vs. $36{\cdot}01 \pm 0{\cdot}86$ g; $P < 0{\cdot}001$), and transgenic mice had larger weights compared with non-transgenic mice ($43{\cdot}88 \pm 2{\cdot}06$ g vs. $38{\cdot}99 \pm 0{\cdot}84$ g; $P < 0{\cdot}05$). The interaction of line by transgene did not approach significance for weight at day of plug ($P > 0{\cdot}05$).

(v) *Ovulation rate and embryo survival traits*

Least-squares means of the embryo survival traits for each line are shown in Table 3 and linear contrasts are in Table 4. Selection background did not affect pre-implantation or total embryo survival ($P > 0{\cdot}05$), but mice of the high-growth background lines had a larger percentage of post-implantation embryo survival compared with mice of the control background lines. Transgene-carrier lines did not differ from non-transgenic lines for any of the embryo survival traits ($P > 0{\cdot}05$). The interaction of selection background and transgenic line was significant for pre-implantation embryo survival ($P < 0{\cdot}01$). Non-transgenic lines had greater pre-implantation embryo survival means within the high-growth background, but transgene-carrier lines had larger means within the

Table 3. *Least-squares means* ± *SE of days to copulatory plug* (*CP*), *body weight at day of CP and pre-implantation, post-implantation and total embryo survival*

Line[a]	Genotype	n_1[b]	Days to CP	Body weight at CP	n_2[c]	Pre-implantation embryo survival (%)	Post-implantation embryo survival (%)	Total embryo survival (%)
CC		35	2·8 ± 0·4	26·4 ± 0·9	46	92·9 ± 2·1	93·2 ± 1·7	86·0 ± 2·6
NC		38	3·5 ± 0·4	34·0 ± 0·8	51	90·2 ± 2·0	93·6 ± 1·6	84·4 ± 2·5
NM		29	2·9 ± 0·4	46·7 ± 0·9	45	95·0 ± 2·1	94·2 ± 1·7	89·5 ± 2·6
TC		38	2·9 ± 0·4	37·4 ± 0·8	50	95·1 ± 2·0	88·0 ± 1·6	84·0 ± 2·5
	Transgenic	30	2·7 ± 0·5	38·3 ± 0·8	42	95·5 ± 2·4	86·4 ± 1·8	82·7 ± 3·0
	Non-transgenic	8	3·2 ± 0·8	33·8 ± 1·5	8	93·9 ± 5·5	97·5 ± 3·9	91·9 ± 6·6
TM		28	3·7 ± 0·4	44·4 ± 0·9	49	89·2 ± 2·0	94·9 ± 1·6	85·0 ± 2·5
	Transgenic	1	3·4 ± 2·3	49·5 ± 4·0	3	94·9 ± 9·0	93·5 ± 6·2	89·6 ± 10·6
	Non-transgenic	27	3·8 ± 0·5	44·2 ± 0·9	46	88·7 ± 2·3	95·1 ± 1·7	84·7 ± 2·8

[a] CC, randomly selected control; the following lines were selected for large 8-week body weight: NC, non-transgenic line from control background; NM, non-transgenic line from high-growth background; TC, transgene-carrier line from control background; TM, transgene-carrier line from high-growth background.
[b] Sample size for days to CP and body weight at CP.
[c] Sample size for embryo survival.

https://doi.org/10.1017/S001667239900436X Published online by Cambridge University Press

Table 4. *Orthogonal contrasts ± SE and correlated response contrasts ± SE involves lines[a] for days to copulatory plug (CP), body weight at CP and pre-implantation, post-implantation and total embryo survival*

Contrast	Days to CP	Body weight at CP (g)	Pre-implantation embryo survival (%)	Post-implantation embryo survival (%)	Total embryo survival (%)
s[b]	0·2 ± 0·8	19·7 ± 1·7***	−1·04 ± 4·1	7·5 ± 3·3*	6·0 ± 5·0
t[c]	0·2 ± 0·8	1·1 ± 1·7	−0·9 ± 4·1	−4·8 ± 3·3	−4·9 ± 5·0
s × t[d]	1·5 ± 0·8	−5·8 ± 1·7**	−10·8 ± 4·1**	6·3 ± 3·3	−4·1 ± 5·0
Overall[e]	1·8 ± 1·7	56·9 ± 3·8***	−2·0 ± 9·3	−2·0 ± 7·5	−4·3 ± 11·4
NC − CC	0·7 ± 0·5	7·6 ± 1·2***	−2·7 ± 2·9	0·4 ± 2·3	−2·4 ± 3·5
TC − CC	0·1 ± 0·5	11·0 ± 1·2***	2·2 ± 2·9	−5·2 ± 2·3	−2·8 ± 3·5

[a] CC, randomly selected control; the following lines were selected for large 8-week body weight: NC, non-transgenic line from control background; NM, non-transgenic line from high-growth background; TC, transgene-carrier line from control background; Tm, transgene-carrier line from high-growth background.
[b] High-growth background lines versus control background lines. Contrast value is NM + TM − NC − TC.
[c] Transgene-carrier lines versus non-transgenic lines. Contrast value is TC + TM − NC − NM.
[d] Contrast value is NC + TM − NM − TC.
[e] Contrast value is NC + NM + TC + TM − 4CC.
*$P < 0·05$; **$P < 0·01$; ***$P < 0·001$.

Table 5. *Least-squares means ± SE of total corpora lutea, adjusted total corpora lutea, total live fetuses and total dead for each line*

Line[a]	Genotype	*n*	Total corpora lutea	Adjusted total corpora lutea[b]	Total live fetuses	Total dead[c]
CC		46	13·3 ± 0·4	14·7 ± 0·6	11·6 ± 0·5	0·77 ± 0·19
NC		51	16·2 ± 0·4	16·6 ± 0·4	13·7 ± 0·5	0·92 ± 0·18
NM		45	16·4 ± 0·4	15·4 ± 0·5	14·8 ± 0·5	0·87 ± 0·19
TC		50	13·2 ± 0·4	13·2 ± 0·4	11·2 ± 0·5	1·42 ± 0·18
	Transgenic	42	12·9 ± 0·5	13·5 ± 0·5	10·7 ± 0·6	1·60 ± 0·19
	Non-transgenic	8	14·6 ± 0·9	15·8 ± 1·0	13·2 ± 1·3	0·29 ± 0·42
TM		49	15·7 ± 0·4	14·8 ± 0·5	13·5 ± 0·5	0·66 ± 0·18
	Transgenic	3	15·7 ± 1·4	13·8 ± 1·5	13·8 ± 2·1	1·03 ± 0·69
	Non-transgenic	46	15·7 ± 0·4	15·1 ± 0·5	13·5 ± 0·6	0·63 ± 0·18

[a] CC, randomly selected control; the following lines were selected for large 8-week body weight: NC, non-transgenic line from control background; NM, non-transgenic line from high-growth background; TC, transgene-carrier line from control background; TM, transgene-carrier line from high-growth background.
[b] Adjusted for 10-week body weight by covariance analysis.
[c] Dead fetuses plus implants.

control background. Line NC had no significant correlated responses in embryo survival whereas TC had a negative correlated response in post-implantation embryo survival.

Least-squares means for the embryo survival traits of TC and TM mice with and without the transgene are shown in Table 3. Effects of line, transgene and the interaction of line by transgene were not significant ($P > 0·05$) for any of these traits.

Least-squares means of total corpora lutea, adjusted total corpora lutea, total live fetuses and total dead fetuses plus implants for each line are given in Table 5 and contrasts are in Table 6. High-growth background lines had more corpora lutea ($P < 0·001$) and live fetuses ($P < 0·01$) than control background lines, and non-transgenic lines had larger means than transgene-carrier lines for both traits ($P < 0·001$). The interaction of selection background by transgenic line was significant for total corpora lutea ($P < 0·01$) but not for total live fetuses ($P > 0·05$). The interaction for total corpora lutea was caused by line TM means being greater than line TC means ($P < 0·01$), whereas NM and NC means did not differ. When number of corpora lutea was adjusted for 10-week body weight, selection background and the overall contrast were no longer significant ($P > 0·05$). However, non-transgenic lines still had more corpora lutea than transgene-carrier lines ($P < 0·001$), and the interaction was still significant. The positive correlated response in number of corpora lutea ($P < 0·001$) shown by line NC was reduced after adjustment for body weight. In contrast, the TC line had no significant correlated response in unadjusted corpora lutea count and a negative response after adjustment. Number of live

https://doi.org/10.1017/S001667239900436X Published online by Cambridge University Press

Table 6. *Orthogonal contrasts ± SE and correlated response contrasts ± SE involving lines*[a] *for total corpora lutea, adjusted total corpora lutea, total live fetuses and total dead*

Contrast	Total corpora lutea	Adjusted total corpora lutea[f]	Total live fetuses	Total dead[g]
s[b]	2·6 ± 0·8***	0·4 ± 1·0	3·4 ± 1·0**	−0·82 ± 0·37*
t[c]	−3·7 ± 0·8***	−3·9 ± 0·8***	−3·8 ± 1·1***	0·29 ± 0·37
s × t[d]	2·3 ± 0·8**	2·7 ± 0·8***	1·4 ± 1·0	−0·71 ± 0·37
Overall[e]	8·5 ± 1·8***	1·1 ± 2·8	7·0 ± 2·4**	0·80 ± 0·86
NC − CC	2·9 ± 0·6***	1·8 ± 0·7**	2·2 ± 0·8**	0·15 ± 0·27
TC − CC	−0·0 ± 0·6	−1·5 ± 0·7*	−0·4 ± 0·8	0·65 ± 0·27*

[a] CC, randomly selected control; the following lines were selected for large 8-week body weight: NC, non-transgenic line from control background; NM, non-transgenic line from high-growth background; TC, transgene-carrier line from control background; TM, transgene-carrier line from high-growth background.
[b] High-growth background lines versus control background lines. Contrast value is NM + TM − NC − TC.
[c] Transgene-carrier lines versus non-transgenic lines. Contrast value is TC + TM − NC − NM.
[d] Contrast value is NC + TM − NM − TC.
[e] Contrast value is NC + NM + TC + TM − 4CC.
[f] Adjusted for 10-week body weight by covariance analysis.
[g] Dead fetuses plus implants.
*$P < 0·05$; **$P < 0·01$; ***$P < 0·001$.

fetuses increased ($P < 0·01$) in line NC but remained unchanged in line TC.

Analysis within transgene-carrier line means (Table 5) showed that line TM had more corpora lutea than TC (15·68 ± 0·76 vs 13·77 ± 0·54, $P < 0·05$) whereas transgenic and non-transgenic mice did not differ significantly (14·32 ± 0·75 vs 15·12 ± 0·50), and there was no significant line by transgene interaction. The covariate 10-week body weight was significant ($P < 0·01$), and its addition to the model markedly affected the adjusted corpora lutea means compared with the unadjusted means. The difference between lines TM and TC was no longer significant (14·44 ± 0·83 vs 14·62 ± 0·59), and transgenic mice had fewer corpora lutea than non-transgenic mice (13·63 ± 0·75 vs 15·43 ± 0·50, $P < 0·05$). Line, transgene and line by transgene interactions were non-significant for total live fetuses.

The randomly selected background lines had a larger ($P < 0·05$) number of dead fetuses plus implants than did the high-growth background lines. No difference in the number of dead fetuses plus implants existed between transgene-carrier lines and non-transgenic lines, nor was the interaction of selection background by transgene-carrier line significant. Correlated responses for number of dead fetuses were not significant in line NC and were positive in line TC ($P < 0·05$).

Analysis of the number of dead fetuses plus implants for lines TC and TM with the effect of transgene presence versus absence included in the model showed that transgenic mice had a greater number of dead fetuses plus implants compared with non-transgenic mice (1·32 ± 0·36 vs 0·46 ± 0·23, $P < 0·05$). However, effects of line and the interaction of line and transgene were not significant ($P > 0·05$).

4. Discussion

The reduced frequency of transgenic females in the high-growth background transgene-carrier line TM contrasts with the increase in the control background line (TC). The basis for this difference is related to a reduced fitness of oMt1a-oGH transgenic mice; segregation ratios consistently show a significantly lower frequency of transgenic mice compared with expected values (Siewerdt *et al.*, 1999). However, in the high-growth background the additive effect on the selected trait, 8-week body weight, is much less, particularly in males, than in the control background (Siewerdt *et al.*, 1998). Therefore, the combination of reduced fitness and relatively small average effect of the transgene caused a reduction in its frequency. Sabour *et al.* (1991) reported that the decreased frequency of a rat growth hormone transgene in mice selected for high growth was due to lower fertility of females carrying the gene.

The present experiment was designed to compare the effects of selection for increased growth on female reproduction in the non-transgenic and transgenic carrier lines, the latter including both transgenic and non-transgenic mice. While the lower frequency of transgenic mice in the TM line reduced the power of the test, the goal also was to compare lines regardless of the frequency of the transgene.

(i) *Transgene-carrier versus non-transgenic line differences*

No evidence was found that the transgene-carrier and non-transgenic lines differed in female fertility rate. When measuring overall fertility, where male and

https://doi.org/10.1017/S001667239900436X Published online by Cambridge University Press

female effects could not be separated, no difference in fertility rate in the first seven generations of selection was found between transgene-carrier and non-transgenic lines (Siewerdt *et al*., 1999). Within transgene-carrier lines, female fertility did not differ between transgenic and non-transgenic mice that were mated 2 weeks after transgene inactivation by withdrawal of the zinc supplement. Murray & Pomp (1995) reported similar findings in mice having the same construct. In the TC and TM base population, transgenic females that had been mated 2 weeks following zinc withdrawal did not differ in fertility from transgenic females never receiving a zinc supplement (Eisen *et al*., 1995).

No differences were observed between transgene-carrier lines and non-transgenic lines or between transgenic and non-transgenic mice within lines TC and TM for pre-implantation, post-implantation or total embryo survival. Siewerdt *et al*. (1998) found that embryo survival traits did not differ across transgenic and non-transgenic genotypes within line TC or within line TM in the base population prior to the initiation of selection for 8-week body weight. Murray & Pomp (1995) found no differences in post-implantation embryo survival between inactivated oMt1a-oGH transgenic and non-transgenic mice; however, pre-implantation and overall embryo survival were greater among oMt1a-oGH transgenic mice.

A positive additive genetic effect of the oMt1a-oGH transgene on the number of corpora lutea was found in the base populations of line TC but not in the base of line TM (Siewerdt *et al*., 1998). Thus, the lower corpora lutea count in TC transgenic compared with non-transgenic mice in the present study was contradictory and may represent an interaction due to selection at other loci affecting ovulation rate.

Although the non-transgenic lines had a larger number of live fetuses than transgene-carrier lines, no differences in fetus numbers were evident between transgenic and non-transgenic mice within lines TC and TM. Siewerdt *et al*. (1998) found that the number of live fetuses did not differ between transgenic and non-transgenic mice within line TC or within line TM prior to the initiation of selection for increased body weight. However, Siewerdt *et al*. (1999) reported that at generation zero, non-transgenic females in lines NC and NM had a larger litter size at birth than never-activated transgenic females in lines TC and TM, respectively. It was hypothesized that transgenic genotypes may have a reduced fitness during early development.

The total number of dead fetuses plus implants did not differ between transgene-carrier lines and non-transgenic lines, but when the trait was analysed within lines TC and TM only, females that contained the transgene had a greater number of dead fetuses plus implants than non-transgenic females. The loss of embryos among transgenic mice may be due to a fitness problem associated with the transgene, which may also have led to the decrease in transgene frequency in line TM during selection (Siewerdt *et al*., 1999). Transgene frequency actually increased in line TC where the additive effect on 8-week body weight was much higher than in line TM (Siewerdt *et al*., 1999). Siewerdt *et al*. (1998) found no difference in the number of dead fetuses between transgenic and non-transgenic mice within line TC or within line TM prior to initiation of selection. In contrast to number of dead fetuses, live fetus number was not significantly different between transgene-carrier and non-transgenic lines.

(ii) *Selection background differences*

Although mice from the high-growth background had a larger percentage of infertile females than control background mice, the former group exhibited reproductive advantages in many of the traits. Selected background mice had higher corpora lutea means on an absolute basis, but corpora lutea numbers did not differ between high-growth and control background lines when the covariate 10-week body weight was included in the model. Total live fetuses and post-implantation embryo survival means were greater among high-growth background mice as well. Females from the high-growth background hBad fewer dead fetuses versus females from the control background. Siewerdt *et al*. (1999) also found that females from the high-growth background lines TM and NM had reproductive advantages over females from control background lines TC and NC. Previous studies have shown that selection for large body size increases ovulation rate, but prenatal survival and fertility rates are often reduced (Fowler & Edwards, 1960; Land, 1970; Barria & Bradford, 1981).

(iii) *Correlated responses in control background lines*

The non-transgenic lines in the control background (NC) had no significant correlated responses in female fertility, days to copulatory plug, embryo survival and number of dead fetuses plus implants. Positive correlated responses were found for number of corpora lutea and number of live fetuses. Adjusting corpora lutea number for body weight reduced the correlated response but did not eliminate it. An increased ovulation rate as a consequence of selection for large body weight is a frequent occurrence (Land, 1970) and has been ascribed to the allometric relationship between female body weight and surface area of the ovary (Durrant *et al*., 1980), which explains the reduced correlated response after adjustment for body weight.

Although the regression of litter size at birth as a deviation from control (CC) on generation number, was not significant in line NC (Siewerdt *et al*., 1999), a significant positive correlated response in litter size was evident by comparing the means of contemporary dams from generations 7 and 8 of the present study ($12{\cdot}72 \pm 0{\cdot}38$, $n = 73$ in NC vs $11{\cdot}59 \pm 0{\cdot}40$, $n = 67$, in CC; $P < 0{\cdot}05$). Therefore, the greater litter size in NC compared with CC can be attributed to the greater corpora lutea number. The reproductive performance of the NC line has not decreased during this short-term period of selection for increased body weight.

The transgene-carrier line in the control background (TC) exhibited no significant correlated responses in female fertility, days to copulatory plug, number of corpora lutea, number of live fetuses at day 16 and pre-implantation and total embryo survival. The basis for the lack of a positive correlated response in number of corpora lutea is that transgenic TC females had fewer corpora lutea than their non-transgenic sibs ($12{\cdot}94 \pm 0{\cdot}46$ vs $14{\cdot}59 \pm 0{\cdot}91$, $P < 0{\cdot}10$). This result differs from the base population of TC where there was a positive additive effect for number of corpora lutea (Siewerdt *et al*., 1998) and may represent an interaction between the transgene and the alleles favoured by selection for increased body weight that have had a pleiotropic effect on ovulation rate, as discussed earlier.

Evidence that reproductive performance of female TC mice has declined was the negative correlated response in post-implantation embryo survival and the positive correlated response in number of fetal deaths. Again, these changes in the TC line were attributed to transgenic females having an inferior uterine environment compared with non-transgenic females for post-implantation embryo survival ($86{\cdot}40 \pm 1{\cdot}77\%$ vs $97{\cdot}53 \pm 3{\cdot}88\%$; $P < 0{\cdot}01$) and number of dead fetuses plus implants ($0{\cdot}29 \pm 0{\cdot}42$ vs $1{\cdot}60 \pm 0{\cdot}19$; $P < 0{\cdot}01$), respectively. Further evidence that the TC line has a reduced reproductive performance comes from a comparison of 16 day fetus numbers and litter size at birth in contemporary TC females of generations 7 and 8 ($11{\cdot}17 \pm 0{\cdot}52$ vs $8{\cdot}67 \pm 0{\cdot}43$; a loss of $2{\cdot}50 \pm 0{\cdot}67$ fetuses; 22%; $P < 0{\cdot}001$) with females from the CC control line ($11{\cdot}55 \pm 0{\cdot}54$ vs $11{\cdot}59 \pm 0{\cdot}40$, essentially no loss). These data provide indirect evidence that TC females had a high fetal loss between day 16 of gestation and parturition or soon after parturition. The increased prenatal mortality is unlikely to be due to cumulative effects of inbreeding, which is expected to be only 6% in generation 8. Collectively, the reproductive performance of the TC line has generally declined, and at least part of the reduced performance may be due to the oMt1a-oGH transgene maintained in the population.

(iv) *Conclusions*

Initial evaluation of the oMt1a-oGH transgene as a useful model to incorporate into a selection program for increased growth suggested advantages from the standpoint of reproductive performance (Eisen *et al*., 1995; Siewerdt *et al*., 1998). The reduced frequency of oMt1a-oGH mice when selection for large 8-week body weight was applied in the high-growth background may be caused by reduced viability of the embryos carrying the transgene construct (Clutter *et al*., 1996; Siewerdt *et al*., 1999) and the additive effect of the gene being smaller in a high-growth background (Siewerdt *et al*., 1998). The present study suggests that the transgene-carrier line developed from a control line background exhibited a decline in reproductive performance when subjected to short-term selection for large 8-week body weight. Furthermore, the reduced performance was attributed to transgenic females having an inferior uterine environment compared with non-transgenic females, although embryos carrying the transgene are also a likely contributing factor.

The research reported herein was funded by USDA National Research Initiative Competitive Grant no. 9403975 and the North Carolina Agricultural Research Service (NCARS), Raleigh, NC 27695–7643, USA. The use of trade names in this publication does not imply endorsement by the NCARS, nor criticism of similar ones not mentioned.

References

Barria, N. & Bradford, G. E. (1981). Long-term selection for rapid gain in mice. II. Correlated changes in reproduction. *Journal of Animal Science* **52**,739–747.

Bartke, A., Steger, R. W., Hodges, S. L., Parkening, T. A., Collins, T. J., Yun, J. S. & Wagner T. E. (1988). Infertility in transgenic female mice with human growth hormone expression: evidence for luteal failure. *Joul of Experimental Zoology* **248**, 121–124.

Cecim, M., Kerr, J. & Bartke, A. (1995). Effects of bovine growth hormone (bGH) transgene expression or bGH treatment on reproductive functions in female mice. *Biology of Reproduction* **52**, 1144–1148.

Clutter, A. C., Pomp, D. & Murray, J. D. (1996). Quantitative genetics of transgenic mice: components of phenotypic variation in body weights and weight gains. *Genetics* **143**, 1753–1760.

Durrant, B. S., Eisen, E. J. & Ulberg, L. C. (1980). Ovulation rate, embryo survival and ovarian sensitivity to gonadotrophins in mice selected for litter size and body weight. *Journal of Reproduction and Fertility* **59**, 329–339.

Eisen, E. J., Murray, J. D. & Schmitt, T. J. (1995). An ovine-growth-hormone transgene model suitable for selection experiments for growth in mice. *Journal of Animal Breeding and Genetics* **112**, 401–413.

Fowler, R. E. & Edwards, R. G. (1960). The fertility of mice selected for large or small body size. *Genetical Research* **1**, 393–407.

Hammer, R. E., Brinster, R. L. & Palmiter, R. D. (1985). Use of gene transfer to increase animal growth. *Cold Spring Harbor Symposia on Quantitative Biology* **50**, 379–387.

Land, R. B. (1970). Genetic and phenotypic relationships between ovulation rate and body weight in the mouse. *Genetical Research* **15**, 171–182.

Milton, S., Cecim, M., Li, Y. S., Yun, J. S., Wagner, T. E. & Bartke, A. (1992). Transgenic female mice with high human growth hormone levels are fertile and capable of normal lactation without having been pregnant. *Endocrinology* **131**, 536–538.

Murray, J. D. & Pomp, D. (1995). Interaction between reproductive performance and dietary energy levels fed to female oMt1a-oGH transgenic mice. *Transgenics* **1**, 553–563.

Naar, E. M., Bartke, A., Majumdar, S. S., Buonomo, F. C., Yun, J. S. & Wagner, T. E. (1991). Fertility of transgenic female mice expressing bovine growth hormone or human growth hormone variant genes. *Biology of Reproduction* **45,** 178–187.

Nagai, J., Sabour, M. P. & Benkel, B. (1992). Reproductive impairment in mice with the rat growth hormone transgene. *Journal of Animal Breeding and Genetics* **109**, 291–300.

Orian, J. M., Lee, C. S., Weiss, L. M. & Brandon, M. R. (1989). The expression of a metallothionein-ovine growth hormone fusion gene in transgenic mice does not impair fertility but results in pathological lesions in the liver. *Endocrinology* **124**, 455–463.

Palmiter, R. D., Brinster, R. L., Hammer, R. E., Trumbauer, M. E., Rosenfeld, M. G., Birnberg, N. C. & Evans R. M. (1982). Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. *Nature* **300**, 611–615.

Pomp, D., Murray, J. D. & Medrano, J. F. (1991). Single day detection of transgenic mice by PCR of toe-clips. *Mouse Genome* **89**, 279.

Pomp, D., Nancarrow, C. D., Ward, K. A. & Murray, J. D. (1992). Growth, feed efficiency and body composition of transgenic mice expressing a sheep metallothionein 1a–sheep growth hormone fusion gene. *Livestock Production Science* **31**, 335–350.

Sabour, M. P., Ramsy, U. & Nagai, J. (1991). Decreased frequency of the rat growth hormone transgene in mouse populations with or without selection for increased adult body weight. *Theoretical and Applied Genetics* **81**, 327–332.

SAS Institute (1996). *Changes and Enhancements*, release 6.12. Cary, NC: SAS Institute.

Shanahan, C. M., Rigby, N. W., Murray, J. D., Marshall, J. T., Townrow, C. A., Nancarrow, C. D. & Ward, K. A. (1989). Regulation of expression of a sheep, metallothionein 1a–sheep growth hormone fusion gene in transgenic mice. *Molecular and Cellular Biology* **9**, 5473–5479.

Siewerdt, F., Eisen, E. J. Conrad-Brink, J. S., & Murray, J. D. (1998). Gene action of the oMt1a-oGH transgene in two lines of mice with distinct selection backgrounds. *Journal of Animal Breeding and Genetics* **115**, 211–226.

Siewerdt, F., Eisen, E. J. & Murray, J. D. (1999). Direct and correlated responses to short-term selection for 8-week body weight in lines of transgenic (oMt1a-oGH) mice. In *Transgenic Animals in Agriculture* (ed J. D. Murray, G. B. Anderson, A. M. Oberbauer, & M. M. McGloughlin), pp. 231–250. Oxford: CAB International.