Growth hormone and fertility in oMt1a-oGH transgenic mice

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Female mice carrying a regulatable growth hormone transgene (oMt1a-oGH) are subfertile when the transgene is actively expressed. This study was designed to characterize subfertility caused by increased concentrations of growth hormone. In particular, this study aimed to: (i) determine the effects of transgene activation and inactivation on mating, conception, maintenance of pregnancy, ovulation rate, litter characteristics and embryonic survival at day 17 of pregnancy, (ii) characterize oestrous cyclicity in transgenic versus wild-type female mice, and (iii) correlate corticosterone concentrations with transgene expression and reproductive performance. Transgenic and wild-type female mice were allocated randomly to one of four treatment groups at weaning: (i) transgenic female mice that always express the transgene, (ii) transgenic female mice that never express the transgene, (iii) transgenic female mice that express the transgene for up to 8 weeks of age and (iv) non-transgenic wild-type female mice receiving the transgene stimulus

until 8 weeks of age. Activation followed by inactivation of the transgene resulted in an increased incidence of remating, resulting in an extended interval to establish pregnancy in comparison with all other treatment groups. Transgenic mice that always expressed the transgene and those that expressed the transgene for up to 8 weeks of age had lower pregnancy rates and higher ovulation rates compared with mice from other treatment groups. Both embryonic survival and the duration of the oestrous cycle did not differ among treatment groups. Active expression of the transgene resulted in an increase in the plasma concentration of corticosterone, which was associated with reduced fertility. These data indicate that the presence of a high growth hormone concentration impedes the establishment and maintenance of pregnancy. Increased plasma corticosterone concentrations may interfere with implantation as well as potentiate leptin resistance, which has been reported previously in studies with these mice.

Introduction

Although heterologous GH (GH) has been used in livestock to enhance desirable traits for economic purposes (Etherton *et al.*, 1986; Campbell *et al.*, 1989; Chapula and Galligan, 1989; McGuffey *et al.*, 1991a; Stelwagen *et al.*, 1993; Buskirk *et al.*, 1996; Chapula *et al.*, 1996), many studies have demonstrated reduced reproductive performance in cows supplemented with bovine somatotrophin (bST), a synthetic bovine GH (McGuffey *et al.*, 1991b; Downer *et al.*, 1993; Oldenbroek *et al.*, 1993; Esteban *et al.*, 1994; Buskirk *et al.*, 1996; Chapula *et al.*, 1996). In addition, humans with acromegaly, a disorder characterized by high circulating concentrations of GH, display menstrual disorders and impotence (Jadresic *et al.*, 1982). Multiple transgenic mouse lines expressing heterologous GH also display a variety of reproductive deficits (Bartke *et al.*, 1988; Cecim *et al.*, 1995a,b,c; Murray and Pomp, 1995; Pomp *et al.*, 1995).

The ability to control the ovine metallothionein 1a

promoter (oMt1a)-ovine GH (oGH) construct makes it a useful tool for the study of reproduction during developmental periods of interest. The oMt1a promoter, which possesses a low basal expression that does not enhance growth in animals with a non-induced transgene (Shanahan *et al.*, 1989), can be induced by the addition of ZnSO₄ to the drinking water, which in turn drives expression of oGH. Oberbauer *et al.* (1997) reported average plasma oGH concentrations of 2279 ng ml⁻¹ for activated transgenic animals, whereas oGH concentrations for non-induced transgenic mice were only 66 ng ml⁻¹. Pomp *et al.* (1995) observed that oMt1a-oGH female mice actively expressing the transgene are subfertile, and that failure of early pregnancy is associated with premature regression of the corpus luteum (Murray and Pomp, 1995). In addition, these females display multiple copulatory plugs over a short period of time without showing evidence of pseudo-pregnancy, indicating failure of pregnancy due to luteal 'insufficiency' (Pomp *et al.*, 1995).

The present experiments were undertaken to elucidate further the effects of GH on the reproduction of oMt1a-oGH female mice. The first objective of this study was to examine the effects of activation and inactivation of the transgene

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stimulus on the ability of the oMt1a-oGH female mice to conceive and carry pregnancy to term. Assessing the time required to establish and maintain pregnancy after transgene inactivation may provide an additional insight into the mechanism of GH inhibition on fertility. In addition, alterations in reproductive performance in response to GH or GH withdrawal, including ovulation, litter characteristics and embryonic survival as assessed on day 17 of pregnancy were also examined. The study also aimed to correlate oestrous cyclicity with transgene activation and inactivation, and to determine how circulating corticosterone concentrations vary with transgene expression and reproductive performance. Other studies have reported that high concentrations of corticosterone are associated with expression of other GH constructs (Cecim *et al.*, 1991, 1996) and Cecim *et al.* (1996) reported a decrease in glucocorticoid secretion from the adrenal glands when they are removed from the high GH environment. Plasma corticosterone concentrations are of interest because of their ability to suppress oestrous cyclicity (Brann and Mahesh, 1991), LH secretion (Baldwin, 1979; Suter and Schwartz, 1985; Kamel and Kubajak, 1987; Brann and Mahesh, 1991), sexual behaviour (de Catanzaro and Gorzalka, 1980; de Catanzaro *et al.*, 1981a,b; de Catanzaro, 1987) and pregnancy (Spaziani and Szego, 1958; Schlough, 1971).

Materials and Methods

Animals

Mice were produced by mating MG101H mice in a CCX (C57/BL6 × CBA) background, homozygous for an oMt1a-oGH fusion gene (Shanahan *et al.*, 1989), to CCX wild-type mice. The resulting offspring were hemizygous for the oMt1a-oGH construct. At 9–11 days of age, transgenic and wild-type CCX mice (derived from CCX × CCX crosses) were toe-notched for identification purposes. At 21 days of age, mice were weaned, weighed and separated by genotype, and then housed in groups of 3–7 animals per cage. Mice had *ad libitum* access to feed (Purina Mouse Chow diet 5001, St Louis, MO) and drinking water. Activation and inactivation of the transgene were achieved by the presence or absence of 25 mmol ZnSO₄ l⁻¹ (Sigma Chemical Inc.) in the drinking water, respectively. Removal of the zinc stimulus resulted in a decrease in the circulating GH concentrations to basal values within 24 h (Shanahan *et al.*, 1989). Body weights were recorded each week from weaning up to 8 weeks of age for all animals. A greater number of transgenic female mice was assigned to each treatment because of the known variability in reproduction in transgenic female mice.

Animals were housed under conditions of constant day length (14 h light:10 h dark), temperature (70°F) and average humidity (55%) in an AAALAC-approved laboratory animal colony following NIH guidelines for animal care.

Fertility study

Results of other studies have demonstrated that few oMt1a-oGH female mice actively expressing the transgene at the time of mating can maintain pregnancy (Murray and Pomp, 1995; Pomp *et al.*, 1995). The time required by transgenic female mice, after transgene inactivation, to establish and maintain pregnancy was determined by allocating at weaning, 56 transgenic and 22 wild-type female mice randomly to one of four treatment groups: (i) transgenic female mice that received zinc supplementation from 3 weeks of age to day 17 of pregnancy (TG ON); (ii) transgenic female mice that did not receive zinc supplementation (TG OFF), (iii) transgenic female mice that receive zinc supplementation from 3 to 8 weeks of age (TG ON/OFF); and (iv) wild-type female mice that received zinc from 3 to 8 weeks of age (WT). At 8 weeks of age, female mice were mated to CD1 males of proven fertility. The females were housed with the males for 14 days, during which time the presence or absence of a vaginal copulatory plug was evaluated each day. The day of mating (day 0) was determined by the presence of a copulatory plug. At day 17 after the last copulatory plug was observed, female mice were killed and the numbers of corpora lutea, live fetuses and resorption sites were counted.

Oestrous cyclicity

Most of the female mice (TG and WT) in the fertility study were cyclic at the time of exposure to males; however, transgenic females remated several times and had lower pregnancy rates. In an attempt to determine whether heterologous GH induced any alterations in the oestrous cycle, oestrous cycles were monitored over a 2 week period. At weaning, 36 transgenic and 13 wild-type female mice were allocated to one of four treatment groups: (i) transgenic female mice that received zinc supplementation from 3 to 10 weeks of age (TG ON), (ii) non-induced transgenic female mice (TG OFF), (iii) transgenic female mice that received zinc supplementation from 3 to 8 weeks of age (TG ON/OFF), and (iv) control mice that received zinc supplementation from 3 to 8 weeks of age (WT). From 8 to 10 weeks of age, female mice from all groups were subjected to vaginal saline lavage each day to assess oestrous cyclicity. The types of cell observed and estimated stage of the oestrous cycle were recorded each day for all animals.

Corticosterone concentrations

At weaning, 221 transgenic and 68 wild-type mice were allocated to one of four treatment groups: (i) transgenic female mice that received the zinc stimulus from 3 weeks of age to completion of the experiment (TG ON), (ii) non-induced transgenic female mice (TG OFF), (iii) TG female mice that received zinc supplementation from 3 to 8 weeks of age (TG ON/OFF), and (iv) control mice that received zinc supplementation from 3 to 8 weeks of age (WT).

A subset of female mice from each treatment group was killed each day between 8:00 and 8:30 h for 8 days beginning at 8 weeks of age (day 0). This time frame was chosen because the TG ON/OFF female mice used in the fertility study showed an interval of approximately 8 days before pregnancy was established after initial housing with males. Animals were housed in groups of 1–2 per cage and were decapitated within 1 min of initial disturbance of the cage. Blood was collected directly into funnels draining into ice-cold EDTA tubes (Fisher, Santa Clara, CA). Blood was centrifuged at 2500 *g* for 30 min, and plasma was collected and stored at -70°C until assayed. Corticosterone concentrations were measured by radioimmunoassay using ^{125}I (ICN Pharmaceuticals, Costa Mesa, CA). Five assays were required to evaluate all the plasma samples. Treatment and day samples were assigned randomly to different assays. The intra-assay and interassay coefficients of variation were 4.45% and 10.30%, respectively. The detection limit of the assay was 28.65 ng ml $^{-1}$.

Statistical analysis

For analyses and interpretation of the results, genotypes were considered as combinations of the presence or absence of the oMt1a-oGH transgene and the provision or lack of zinc supplementation in the fertility study. Fertility (percentage of pregnant females), fecundity traits and all other data (body weights, ovulation rate, number of live pups and resorptions) were analysed by least-squares analyses of variance procedures for unequal subclass numbers using PROC GLM of SAS (SAS). The percentage of pregnant mice was also subjected to Duncan's multiple-range test (SAS). Maintenance of pregnancy was defined as a female having at least one live pup at day 17 of gestation (Murray and Pomp, 1995; Pomp *et al.*, 1995). Days to pregnancy was defined as the number of days from the initial housing with the male until observation of a copulatory plug resulting in pregnancy. Corticosterone concentration results were evaluated by ANOVA using PROC GLM of SAS (SAS).

Fecundity traits have been defined by Murray and Pomp (1995) as: ovulation rate (OR) = number of corpora lutea; litter size (LS) = number of live fetuses at day 17; per cent overall embryonic survival = (LS/OR)(100); per cent pre-implantation embryonic survival = (implantation sites/OR)(100) and per cent post-implantation embryonic survival = (LS/total implantation sites)(100). The duration of the oestrous cycle was designated as the interval between two periods of pro-oestrus, punctuated by a period of dioestrus. Pro-oestrus was determined by the presence of epithelial cells, and dioestrus by the presence of leucocytes in the vaginal smear.

Results

Fertility study

Growth. The increase in body weight at 3–8 weeks of age did not differ between TG ON and TG ON/OFF female

Fig. 1. Body weight gains from 3 to 8 weeks of age in chronically activated oMt1a-oGH transgenic female mice (TG ON), non-induced transgenic female mice (TG OFF), transgenic female mice inactivated at 8 weeks of age (TG ON/OFF) and wild-type control mice (WT). Values are means $\pm$ SEM. Bars with different letters are significantly different ($P < 0.05$).

mice (Fig. 1). However, an increase in body weight was significantly less ($P < 0.05$) for WT and TG OFF mice than for the other transgenic groups (Fig. 1).

Reproductive traits. TG OFF and WT control mice did not differ from each other in reproductive traits. Pregnancy rate was determined among female mice that were observed to have mated at least once. Female mice in which a copulatory plug was not recorded but were pregnant were not used in any of the reproductive analyses. The percentage of pregnant mice for both TG ON and TG ON/OFF female mice was significantly lower ($P < 0.05$) than for TG OFF and WT mice (Table 1).

In the overall ANOVA, time to the first copulatory plug was significantly affected by treatment. However, the experimental design requires multiple comparisons of the four treatment groups and this dictates the use of more conservative type I error values, such as those used by the Bonferroni adjustment. In the face of the multiple comparisons, significant differences between treatment effects disappear thereby precluding a ranking of time to copulatory plug by treatment group (Table 1). Inactivation of the transgene increased the incidence of remating leading to a longer interval to pregnancy in TG ON/OFF female mice compared with all other treatment groups ($P < 0.05$) (Table 1). TG ON, TG OFF and WT mice that became pregnant did so within the first 3 days of introduction to males (Table 1). Of the 18 mice that became pregnant in the TG ON/OFF group, nine mice became pregnant after the second copulatory plug was observed, and one female after the third copulatory plug (data not shown).

Both TG ON and TG ON/OFF mice had two to three additional ovulations ($P < 0.05$) compared with either WT control or TG OFF mice (Table 1). The TG ON female mice tended to produce these 'extra' ovulations on the right ovary

Table 1. Reproductive data for oMt1a-oGH transgenic and wild-type female mice

Treatment	Females mated ¹	Females pregnant ²	Pregnant (%)	Days to first copulatory plug ³	Interval to pregnancy ⁴	Ovulation rate ⁵	Live fetuses ⁶
TG ON	14	3	21.4 ^a	2.33 ± 1.17	2.33 ± 1.17 ^a	11.67 ± 0.87 ^b	10.33 ± 0.98
TG OFF	15	14	93.3 ^c	2.57 ± 0.54	2.86 ± 0.66 ^a	8.57 ± 0.40 ^a	7.92 ± 0.45
TG ON/OFF	30	18	60.0 ^b	4.28 ± 0.48	7.89 ± 0.58 ^b	10.83 ± 0.35 ^b	8.72 ± 0.40
WT	22	22	100.0 ^c	2.50 ± 0.43	2.50 ± 0.43 ^a	8.86 ± 0.32 ^a	7.64 ± 0.36

Values are mean ± SEM.

Values with different superscripts within each column are significantly different ($P < 0.05$).

TG ON: chronically induced transgenic female mice; TG OFF: non-induced transgenic female mice; TG ON/OFF: transgenic female mice inactivated at 8 weeks of age; WT: wild-type control mice.

¹Number of females having exhibited at least one copulatory plug.

²Percentage of pregnant females shown to have mated.

³Days from initial housing with male to observation of the first copulatory plug.

⁴Days from initial housing with male to observation of copulatory plug resulting in pregnancy.

⁵Number of corpora lutea at day 17 of pregnancy.

⁶Number of live fetuses at day 17 of pregnancy.

Fig. 2. Duration of the oestrous cycle as assessed by vaginal lavage for chronically induced oMt1a-oGH transgenic female mice (TG ON), non-induced transgenic female mice (TG OFF), transgenic female mice inactivated at 8 weeks of age (TG ON/OFF) and wild-type (WT) control mice.

(data not shown). Although the treatment group was a significant determinant of number of fetuses produced at day 17, the variation within a group did not allow statistical ranking of the groups (Table 1). Resorption rates did not differ among groups. Overall embryonic survival, pre-implantation embryonic survival, and post-implantation embryonic survival did not differ among groups: 87.2%, 94.7% and 92.1%, respectively.

Oestrous cyclicity study

The duration of the oestrous cycle did not differ among the treatment groups (Fig. 2). In a number of vaginal smears collected from mice, leukocytes, epithelial and cornified cells were all present. This finding was displayed by a subset of mice in all treatment groups and at times, mice would remain at this stage for several days (data not shown).

Corticosterone concentrations

Treatment was a significant determinant of plasma corticosterone concentrations. Corticosterone concentrations were higher ($P < 0.0001$) in TG ON and TG ON/OFF female mice than in TG OFF and WT mice (Fig. 3). Plasma corticosterone was also significantly higher in TG OFF mice than in WT mice ($P < 0.0001$) (Fig. 3). Plasma corticosterone concentrations by treatment and day are shown (Table 2).

Discussion

The purpose of this study was to evaluate the effects of heterologous GH on the reproductive function of transgenic mice. The present study extends previous work on oMt1a-oGH transgenic mice by showing that reduced incidence of pregnancy in TG ON mice was not due to an alteration in oestrous cyclicity, and that transgene inactivation resulted in an increase in pregnancy rate relative to TG ON female mice. GH either directly or more likely through the associated increase in plasma corticosterone interferes with the establishment and maintenance of pregnancy.

Although TG ON and TG ON/OFF mice did not display an extended oestrous cycle, as reported in other GH transgenic mouse lines (Bartke *et al.*, 1988; Cecim *et al.*, 1995b, c), it is possible that the level of regulation of transgene expression (that is, the 'leakiness' of the promoter) may affect the duration of the oestrous cycle. Although the oMt1a promoter conveys fairly tight regulation of transgene expression (Shanahan *et al.*, 1989), constructs using a mouse MT promoter show constitutive overexpression of GH even in the absence of heavy-metal induction (Palmiter *et al.*, 1983; Orian *et al.*, 1989). Therefore, although the oMt1a-oGH mice display increased concentrations of GH for only a limited period during development, other transgenic GH mouse lines experience heterologous GH prenatally (Palmiter *et al.*, 1983). This prolonged and increased expression of GH may contribute to extended oestrous cycles.

Table 2. Corticosterone concentration in transgenic and wild-type female mice

Treatment ¹	Corticosterone (ng ml ⁻¹)								
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
TG ON	276.75 ± 43.27 (n = 8)	362.31 ± 43.27 (n = 8)	216.47 ± 43.27 (n = 8)	273.98 ± 43.27 (n = 8)	220.32 ± 43.27 (n = 8)	130.86 ± 40.79 (n = 8)	182.88 ± 43.27 (n = 8)	161.2 ± 43.27 (n = 8)	212.69 ± 43.27 (n = 8)
TG ON/OFF	350.61 ± 43.27 (n = 8)	306.81 ± 43.27 (n = 8)	302.25 ± 43.27 (n = 8)	236.98 ± 43.27 (n = 8)	148.48 ± 43.27 (n = 8)	126.74 ± 38.70 (n = 10)	106.18 ± 43.27 (n = 8)	218.04 ± 43.27 (n = 8)	216.94 ± 43.27 (n = 8)
TG OFF	131.79 ± 43.27 (n = 8)	177.43 ± 43.27 (n = 8)	88.90 ± 43.27 (n = 8)	137.11 ± 43.27 (n = 8)	118.23 ± 43.27 (n = 8)	60.60 ± 43.27 (n = 8)	112.66 ± 43.27 (n = 8)	139.32 ± 43.27 (n = 8)	92.65 ± 36.90 (n = 11)
WT	74.84 ± 43.27 (n = 8)	71.27 ± 43.27 (n = 8)	39.82 ± 43.27 (n = 8)	57.14 ± 43.27 (n = 8)	61.10 ± 54.73 (n = 5)	43.39 ± 43.27 (n = 8)	26.74 ± 43.27 (n = 8)	48.39 ± 43.27 (n = 8)	54.69 ± 46.26 (n = 7)

Day 0: mice at 8 weeks of age.

Values are mean ± SEM.

Numbers within parentheses indicate sample size.

TG ON: chronically induced transgenic female mice; TG ON/OFF: transgenic female mice inactivated at 8 weeks of age; TG OFF: non-induced transgenic female mice; WT: wild-type control mice.

Fig. 3. Plasma concentrations of corticosterone from day 0 to day 8 for chronically induced oMt1a-oGH transgenic female mice (TG ON), non-induced transgenic female mice (TG OFF), transgenic female mice inactivated at 8 weeks of age (TG ON/OFF) and wild-type (WT) control mice. Values are means $\pm$ sem. Bars with different letters are significantly different ($P < 0.0001$).

In addition, cytology of vaginal lavage showed multiple types of cell in mice of different genotypes. In mice that showed this cell distribution for several days, the proportion of each type of cell usually changed each day. It is possible that the heterologous GH may have contributed to this unique pattern of cells; however, a similar distribution of cells was observed in a number of wild-type animals, leading to the conclusion that it was a line effect of the mice used in this study.

Low pregnancy rates have been observed in a variety of GH transgenic mouse lines (Bartke *et al.*, 1988; Cecim *et al.*, 1995b; Murray and Pomp, 1995). Murray and Pomp (1995) demonstrated that chronic expression of the oGH transgene resulted in reduced pregnancy rates, despite the presence of blastocysts on day 3 of pregnancy and, therefore, concluded that lack of pregnancy was due to implantation failure. Embryonic survival rate in the present study indicates that once pregnancy is established, gestation appears to proceed normally. This finding is in contrast to reports on other GH transgenic mouse lines, in which a high incidence of post-implantation embryonic loss was reported (Naar *et al.*, 1991). Increased corticosterone secretion associated with transgene expression was also observed, confirming reports in other GH transgenic mouse lines (Cecim *et al.*, 1991, 1996). Chronically high concentrations of corticosterone interfere with implantation (Spaziani and Szego 1958; Schlough 1971). Corticosterone treatment inhibits the increase in capillary permeability in the uterus that is necessary for implantation to occur (Spaziani and Szego 1958; Schlough 1971), and Wong and Burton (1973) observed that the mouse placenta binds both progestins and corticoids in a competitive manner. Ogle (1974) hypothesized that in deer mice increased corticosteroid production results in pregnancy loss due to increased binding by the uterine and placental receptors of corticoids at the expense of progesterone. In support of this

finding, Pomp *et al.* (1995) found that administration of progesterone up to day 17 of gestation was necessary for oMt1a-oGH female mice to maintain pregnancy.

Adrenal progesterone contributes to the maternal pool of progesterone in rats (MacDonald and Matt, 1984). Fajer *et al.* (1971) observed that corticosterone secretion was markedly increased at the expense of progesterone in stressed rats. MacNiven and de Catanzaro (1990) reported that stress early in gestation resulted in the loss of pregnancy, although pregnancy could be rescued with progesterone or metyrapone, an inhibitor of adrenal 11 β -hydroxylase. MacNiven and de Catanzaro (1990) hypothesized that the reduction in available progesterone, attributable to increased corticosterone secretion associated with stress, was unable to sustain pregnancy. This suppression of progesterone may alter the progesterone:oestrogen ratio, which is important for implantation. Studies in both humans (Gidley-Baird *et al.*, 1986) and mice (Humphrey, 1968) have demonstrated that oestrogen dominance around the time of implantation reduces the incidence of pregnancy. Because of the variation in circulating corticosterone observed within treatment groups, TG ON and TG ON/OFF female mice that were able to establish and maintain pregnancy may have been able to do so because their individual corticosterone concentrations were low enough not to affect the progesterone:oestrogen ratio. Approximately 38% of TG ON and 74% of TG ON/OFF mice assessed for glucocorticoids had corticosterone concentrations within the range of those observed in wild-type mice, in which no impairment of fertility has been observed.

The extended interval to establish pregnancy in TG ON/OFF female mice was due to an increased incidence of remating, which is possibly the result of a combination of reduced circulating GH and increased corticosterone secretion. Presumably, increased corticosterone secretion is a result of direct or indirect actions of heterologous GH at one or more levels of the hypothalamic-pituitary-adrenal axis (Cecim *et al.*, 1991). It is unclear how transgene inactivation improves pregnancy rates. Although GH concentrations are markedly reduced within 24 h of transgene inactivation (Shanahan *et al.*, 1989), this time frame is insufficient to improve reproductive performance markedly. Heterologous GH attenuates GnRH-induced LH secretion (Chandrashekar *et al.*, 1988; Tang *et al.*, 1993), and it is possible that gonadotrophin secretion takes more than 24 h to return to normal concentrations after transgene inactivation. In contention with this finding is the apparent normal gonadotrophin function associated with ovulation. Alternatively, prolactin secretion induced by mating may be insufficient to maintain the corpus luteum. However, Pomp *et al.* (1995) reported that prolactin supplementation failed to rescue pregnancy in oMt1a-oGH mice. It is likely that in animals expressing the oGH transgene the associated increase in plasma corticosterone results in implantation failure due to inadequate progesterone secretion and consequently female mice continue to cycle and mate until pregnancy is established.

Increased concentrations of corticosterone may also contribute to leptin resistance, which has been associated with impaired fertility. Dexamethasone, a synthetic glucocorticoid, upregulates leptin gene expression both *in vitro* (Murakami *et al.*, 1995; Slieker *et al.*, 1996) and *in vivo* (de Vos *et al.*, 1995), and may contribute to a condition of leptin resistance. Previous work from our laboratory has confirmed leptin resistance in TG ON/OFF mice (Runstadler *et al.*, 2001). Dexamethasone administration leads to an increase in hypothalamic expression of neuropeptide Y in the arcuate nucleus (Wilding *et al.*, 1993). During a normal oestrous cycle, high neuropeptide Y expression is necessary for the pro-oestrus LH surge to occur (Kalra, 1993). However, chronically high expression of neuropeptide Y is associated with infertility (Catzefflis *et al.*, 1993; Pierroz *et al.*, 1995). Thus, in a leptin-resistant state potentiated by glucocorticoids, it is likely that leptin is less able to downregulate neuropeptide Y expression, resulting in suppression of GnRH (Pierroz *et al.*, 1995) and subsequent reduction in fertility. In contention with this theory is the increased number of ova shed spontaneously in both the TG ON and TG ON/OFF mice, which would indicate normal function of both LH and FSH, at least in transgenic animals that were able to establish and maintain pregnancy.

The present study reported an increased ovulation rate for oMT1a-oGH mice expressing the transgene, as has been observed in this (Murray and Pomp, 1995; Pomp *et al.*, 1995) and other GH lines (Naar *et al.*, 1991). It is unclear whether GH exerts its effects on ovulation through hepatic IGF-I, acting on the ovary directly or through local ovarian production of IGF-I, which amplifies ovarian responsiveness to gonadotrophins (Geisthovel *et al.*, 1990). IGF-I synergizes with FSH to stimulate aromatase activity (Adashi *et al.*, 1985a,b; Erickson *et al.*, 1989), which results in increased oestradiol production important for ovulation (Ando *et al.*, 1994).

Although chronic expression of the transgene suppresses fertility, withdrawal of the transgene stimulus significantly increases pregnancy rates in TG ON/OFF mice, reflecting the ability of reproductive function to improve after transgene inactivation. In conclusion, increased corticosterone secretion, associated with transgene expression, impairs fertility by interfering with implantation, resulting in repeated matings until implantation can be established. In addition, corticosterone may induce leptin resistance, which can also impair fertility. Further study is required to assess progesterone and oestrogen concentrations after mating to validate implantation failure due to reduced progesterone secretion. Studies are currently being undertaken to correlate leptin resistance with transgene expression and fertility.

References

- Adashi EY, Resnick CE, D'Ercole AJ, Svoboda ME and Van Wyk JJ (1985a) Insulin-like growth factors as intraovarian regulators of granulosa cell growth and function *Endocrine Review* **6** 400–420
- Adashi EY, Resnick CE, Brodie AMH, Svoboda ME and Van Wyk JJ (1985b) Somatomedin-C-mediated potentiation of follicle-stimulating hormone-induced aromatase activity of cultured rat granulosa cells *Endocrinology* **117** 2313–2320
- Ando M, Yoshimur Y, Iwashita M, Oda T, Karube M, Ubukata Y, Jinno M and Nakamura Y (1994) Direct ovarian effect of growth hormone in the rabbit *American Journal of Reproductive Immunology* **31** 123–132
- Baldwin DM (1979) The effect of glucocorticoids on estrogen-dependent luteinizing hormone release in the ovariectomized rat and on gonadotropin secretion in the intact female rat *Endocrinology* **105** 120–128
- Bartke A, Steger RW, Hodges SL, Parkening TA, Collins TJ, Yun JS and Wagner TE (1988) Infertility in transgenic female mice with human growth hormone expression: evidence for luteal failure *Journal of Experimental Zoology* **248** 121–124
- Brann DW and Mahesh VB (1991) Role of corticosteroids in female reproduction *FASEB Journal* **5** 2691–2698
- Buskirk DD, Faulkner DB, Hurley WL, Kesler DJ, Ireland FA, Nash TG, Castree JC and Vicini JL (1996) Growth, reproductive performance, mammary development and milk production of beef heifers as influenced by prepubertal dietary energy and administration of bovine somatotropin *Journal of Animal Science* **74** 2649–2662
- Campbell RG, Steele NC, Caperna TJ, McMurtry JP, Solomon MB and Mitchell AD (1989) Interrelationships between sex and exogenous growth hormone administration on performance, body composition and protein and fat accretion of growing pigs *Journal of Animal Science* **67** 177–186
- Catzefflis C, Pierroz DD, Rohner-Jeanrenaud F, Rivier JE, Sizonenko PC and Aubert ML (1993) Neuropeptide Y administered chronically into the lateral ventricle profoundly inhibits both the gonadotropic and the somatotropic axis in intact adult female rats *Endocrinology* **132** 224–234
- Cecim M, Ghosh PK, Esquifino AI, Began T, Wagner TE, Yun JS and Bartke A (1991) Elevated corticosterone levels in transgenic mice expressing human or bovine growth hormone genes *Neuroendocrinology* **53** 313–316
- Cecim M, Fadden C, Kerr J, Steger RW and Bartke A (1995a) Infertility in transgenic mice overexpressing the bovine growth hormone gene: disruption of the neuroendocrine control of prolactin secretion during pregnancy *Biology of Reproduction* **52** 1187–1192
- Cecim M, Kerr J and Bartke A (1995b) Effects of bovine growth hormone (bGH) transgene expression or bGH treatment on reproductive functions in female mice *Biology of Reproduction* **52** 1144–1148
- Cecim M, Kerr J and Bartke A (1995c) Infertility in transgenic mice overexpressing the bovine growth hormone gene: luteal failure secondary to prolactin deficiency *Biology of Reproduction* **52** 1162–1166
- Cecim M, Alvarez-Sanz M, van de Kar L, Milton S and Bartke A (1996) Increased plasma corticosterone levels in bovine growth hormone (bGH) transgenic mice: effects of ACTH, GH and IGF-1 on *in vitro* adrenal corticosterone production *Transgenic Research* **5** 187–192
- Chandrasekar V, Bartke A and Wagner TE (1988) Endogenous human growth hormone (GH) modulates the effect of gonadotropin-releasing hormone on pituitary function and the gonadotropin response to the negative feedback effect of testosterone in adult male transgenic mice bearing the human GH gene *Endocrinology* **123** 2717–2722
- Chapula W and Galligan DT (1989) Nutritional implications of somatotropin for lactating cows *Journal of Dairy Science* **72** 2510–2524
- Chapula W, Vecchiarelli B, Galligan DT *et al.* (1996) Responses of dairy cows supplemented with somatotropin during weeks 5 through 43 of lactation *Journal of Dairy Science* **79** 800–812
- de Catanzaro D (1987) Alteration of estrogen-induced lordosis through central administration of corticosterone in adrenalectomized-ovariectomized rats *Neuroendocrinology* **46** 468–474
- de Catanzaro D and Gorzalka BB (1980) Effects of dexamethasone, corticosterone, and ACTH on lordosis in ovariectomized and adrenalectomized-ovariectomized rats *Pharmacology Biochemistry and Behavior* **12** 201–206
- de Catanzaro D, Gray DS and Gorzalka BB (1981a) Effects of acute central and peripheral ACTH administration on lordosis behavior *Physiology and Behavior* **26** 207–213

- de Catanzaro D, Knipping RP and Gorzalka BB (1981b) Antagonism of estrogen-induced lordosis by corticosterone in adrenalectomized-ovariectomized female rats and mice *Pharmacology Biochemistry and Behavior* **15** 761–766
- de Vos P, Saladin R, Auwerx J and Staels B (1995) Induction of *ob* gene expression by corticosteroids is accompanied by body weight loss and reduced food intake *Journal of Biological Chemistry* **270** 15 958–15 961
- Downer JV, Patterson DL, Rock DW *et al.* (1993) Dose titration of sustained-release recombinant bovine somatotropin in lactating dairy cows *Journal of Dairy Science* **76** 1125–1136
- Erickson GF, Garzo G and Magoffin DA (1989) Insulin-like growth factor-I regulates aromatase activity in human granulosa and granulosa lutein cells *Journal of Clinical Endocrinology and Metabolism* **69** 716–724
- Esteban E, Kass PH, Weaver LD, Rowe JD, Holmber CA, Franti CE and Troutt HF (1994) Reproductive performance in high producing dairy cows treated with recombinant bovine somatotropin *Journal of Dairy Science* **77** 3371–3381
- Etherton TK, Wiggins JP, Chung CS, Evock CM, Rebhun JF and Walton PE (1986) Stimulation of pig growth performance by porcine growth hormone and growth hormone-releasing factor *Journal of Animal Science* **63** 1389–1399
- Fajer AB, Holzbauer M and Newport HM (1971) The contribution of the adrenal gland to the total amount of progesterone produced in the female rat *Journal of Physiology* **214** 115–126
- Geisthovel F, Moretti-Rojas I, Rojas FJ and Asch RH (1990) Insulin-like growth factors and thecal-granulosa-cell function *Human Reproduction* **5** 785–799
- Gidley-Baird AA, O'Neill C, Sinosich MJ, Porter RN, Pike IL and Saunders DM (1986) Failure of implantation in human *in vitro* fertilization and embryo transfer patients: the effects of altered progesterone/estrogen ratios in humans and mice *Fertility and Sterility* **45** 69–74
- Humphrey KW (1968) The effects of some anti-oestrogens on the decidual reaction and delayed implantation in the mouse *Journal of Reproduction and Fertility* **16** 201–209
- Jadresic A, Banks LM, Child DF, Diamant L, Doyle FH, Fraser TR and Joplin GF (1982) The acromegaly syndrome *Quarterly Journal of Medicine* **202** 189–204
- Kalra SP (1993) Mandatory neuropeptide-steroid signaling for the preovulatory luteinizing hormone-releasing hormone discharge *Endocrine Reviews* **14** 507–538
- Kamel F and Kubajak CL (1987) Modulation of gonadotropin secretion by corticosterone: interaction with gonadal steroids and mechanism of action *Endocrinology* **121** 561–568
- MacDonald GJ and Matt DW (1984) Adrenal and placental steroid secretion during pregnancy in the rat *Endocrinology* **114** 2068–2073
- MacNiven E and de Catanzaro D (1990) Reversal of stress-induced pregnancy blocks in mice by progesterone and metyrapone *Physiology and Behavior* **47** 443–448
- McGuffey RK, Basson RP and Spike TE (1991a) Lactation response and body composition of cows receiving somatotropin and three ratios of forage to concentrate *Journal of Dairy Science* **74** 3095–3102
- McGuffey RK, Basson RP, Snyder DL, Block E, Harrison JH, Rakes AH, Emery RS and Muller LD (1991b) Effect of somidobove sustained release administration on the lactation performance of dairy cows *Journal of Dairy Science* **74** 1263–1276
- Murakami T, Iida M and Shima K (1995) Dexamethasone regulates *obese* expression in isolated rat adipocytes *Biochemical and Biophysical Research Communications* **214** 1260–1267
- Murray JD and Pomp D (1995) Interaction between reproductive performance and dietary energy levels fed to female oMT1a-oGH transgenic mice *Transgenics* **1** 553–563
- Naar EM, Bartke A, Majumdar SS, Buonomo FC, Yun JS and Wagner TE (1991) Fertility of transgenic female mice expressing bovine growth hormone or human growth hormone variant genes *Biology of Reproduction* **45** 178–187
- Oberbauer AM, Stern JS, Johnson PR, Horowitz BA, German JB, Phinney SD, Beermann DH, Pomp D and Murray JD (1997) Body composition of inactivated growth hormone (oMT1a-oGH) transgenic mice: generation of an obese phenotype *Growth, Development and Aging* **61** 169–179
- Oberbauer AM, Runstadler JA, Havel PJ and Murray JD (2001) Obesity and elevated plasma leptin concentration in oMT1a-oGH growth hormone transgenic mice *Obesity Research* **9** 51–58
- Ogle TF (1974) Effects of ACTH on ovarian histochemistry and maintenance of pregnancy in deermice *Biology of Reproduction* **11** 288–296
- Oldenbroek JK, Garssen GJ, Jonker LJ and Wilkinson JID (1993) Effects of treatment of dairy cows with recombinant bovine somatotropin over three or four lactations *Journal of Dairy Science* **76** 453–467
- Orian JM, Lee CS, Weiss LM and Brandon MR (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 RD, Norstedt G, Gelinas RE, Hammer RE and Brinster RL (1983) Metallothionein-human GH fusion genes stimulate growth of mice *Science* **222** 809–814
- Pierroz DD, Gruaz NM, d'Allèves V and Aubert ML (1995) Chronic administration of neuropeptide Y into the lateral ventricle starting at 30 days of life delays sexual maturation in the female rat *Neuroendocrinology* **61** 293–300
- Pomp D, Geisert RD, Durham CM and Murray JD (1995) Rescue of pregnancy and maintenance of corpora lutea in infertile transgenic mice expressing an ovine metallothionein 1a-ovine growth hormone fusion gene *Biology of Reproduction* **51** 170–178
- SAS (1988) *SAS/STAT User's Guide, Release 6.03* Statistical Analysis System Institute, Inc., North Carolina
- Schlough JS (1971) The effect of glucocorticoids on preimplantation capillary permeability in the rat *Fertility and Sterility* **22** 389–391
- Shanahan CM, Rigby NW, Murray JD, Marshall JT, Townrow CA, Nancarrow CD and Ward KA (1989) Regulation of expression of a sheep metallothionein 1a-sheep growth hormone fusion gene in transgenic mice *Molecular and Cellular Biology* **9** 5473–5479
- Slieker LJ, Sloop KW, Surface PL, Kriauciunas A, LaQuier F, Manetta J, Bue-Valleskey J and Stephens TW (1996) Regulation of expression of *ob* mRNA and protein by glucocorticoids and cAMP *Journal of Biological Chemistry* **271** 5301–5304
- Spaziani E and Szego CM (1958) The influence of estradiol and cortisol on uterine histamine of the ovariectomized rat *Endocrinology* **63** 669–678
- Stelwagen K, Grieve DG, Walton JS, Ball JL and McBride BW (1993) Effect of parturition bovine somatotropin in primigravid ewes on mammarygenesis, milk production, and hormone concentrations *Journal of Dairy Science* **76** 992–1001
- Suter DE and Schwartz NB (1985) Effects of glucocorticoids on secretion of luteinizing hormone and follicle-stimulating hormone by female rat pituitary cells *in vitro*. *Endocrinology* **117** 849–854
- Tang K, Bartke A, Gardiner CS, Wagner TE and Yun JS (1993) Gonadotropin secretion, synthesis, and gene expression in two types of bovine growth hormone transgenic mice *Biology of Reproduction* **49** 346–353
- Wilding JPH, Gilbey SG, Lambert PD, Ghatei MA and Bloom SR (1993) Increases in neuropeptide Y content and gene expression in the hypothalamus of rats treated with dexamethasone are prevented by insulin *Neuroendocrinology* **57** 581–587
- Wong MD and Burton AF (1973) Isolation and preliminary characterization of corticosterone-receptor complexes in mouse placental tissue *Biochemical and Biophysical Research Communications* **50** 71–79

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