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HORMONAL INDUCTION OF TWINNING IN FARMED RED DEER (*CERVUS ELAPHUS*): COMPARATIVE MORTALITY AND GROWTH OF TWINS AND SINGLES TO WEANING

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ABSTRACT

Multiple births were induced in 20 hinds subjected to a progesterone plus gonadotropin treatment to advance the breeding season by about 4 to 5 weeks. In total, over 2 years, 92 hinds were treated and run with melatonin-treated stags. Of the 59 hinds that calved to the induced oestrus, 39 produced single calves, 19 produced twins and one produced triplets. Birth weight, gestation length, perinatal mortality and growth rate to weaning at *ca.* 20 weeks of age were compared for singles and twins. There was a significant effect of birth type on birth weight with twins being proportionately 0·30 lighter than singles (6·27 and 8·94 kg; $P < 0.001$), with a significant interaction between the birth weight of a calf and the sex of its co-twin ($P < 0.05$); males born as co-twins to females were proportionately 0·18 heavier than their co-twin females ($P < 0.06$), while in contrast twin male sets were substantially lighter than twin female sets. Gestation lengths were similar for both singles and twins, with significant negative relationships between gestation length and birth weight for both singles and twins, with a 2-day decrease in gestation length for a 1-kg increase in mean birth weight. There was a strong quadratic relationship between perinatal survival and birth weight; the expected mortality rates at 5, 8 and 11 kg birth weight derived from the relationship were 0·39, 0·11 and 0·39 respectively, indicating reduced probability of survival at both low and high birth weights. The regression relationships between weaning weight or growth rate to weaning and birth weight indicate that at the same birth weight, twin-reared calves were about 5 kg lighter than singles at 20 weeks, while an extra 1 kg weight at birth was associated with an extra 2 kg weight at 20 weeks.

KEYWORDS: growth rate, PMSG, progesterone, red deer, twinning.

INTRODUCTION

BIOLOGICAL efficiency, defined as meat production per unit of food energy input, is strongly influenced by reproductive rate in a red deer farming system (Fennessy, 1982; Fennessy and Thompson, 1989). However, with a near maximal fertility rate achievable in farmed red hinds in New Zealand (more than 95% of hinds being pregnant in well managed herds in reported studies; Asher and Adam, 1985) there is little opportunity for increasing reproductive rate other than by an increase in multiple births.

Studies of wild populations of red deer have indicated that multiple pregnancies are rare (with a rate of less than 1·5%; Kroning and Vorreyer, 1957; Mitchell, 1973; Clutton-Brock, Guinness and Albon, 1982; Challies, 1985). The incidence of double ovulations in farmed red deer at Invermay has also been low, with only two of 48 hinds observed at laparoscopy having two *corpora lutea* (Fisher, Fennessy, Henderson, Newman and Manley, 1989). Consequently, the low incidence of natural twinning in red deer means that the only options for increasing reproductive rate in farmed red deer are genetic selection (as with cattle, see Morris, 1984) or hormonal induction of double ovulations.

This paper reports data from two experiments in separate years where multiple births were induced in some hinds given progesterone and gonadotropic stimulation, a hormonal treatment designed to advance the breeding season by about 4 to 5 weeks compared with the normal calving time at Invermay (Moore and Cowie, 1986). The details of the various synchronization treatments will be reported separately. In this paper the data for birth weight, gestation length, mortality, and growth rates of single- and twin-born red deer to about 20 weeks of age are compared. A popular version of some of this work has been published (Moore, 1987).

MATERIAL AND METHODS

Experimental treatments

The data from two experiments are reported. In 1986, 56 hinds were treated to advance the breeding season and in 1987, 36 hinds were treated; 24 of these 36 had been treated in 1986. The red hinds were 2 to 8 years of age at the time of treatment. The hinds used in 1986 had either failed to rear a calf or were weaned of their calves prior to progesterone treatment, whereas those used in 1987 were lactating with calves at foot from the start of the experiment until weaning on 3 April.

In both experiments, oestrus was induced in the hinds by the use of intravaginal controlled internal drug-releasing devices (CIDR) containing progesterone (0·09 w/w CIDR-S with 340 mg progesterone; Alex Harvey Industries, Hamilton, New Zealand). The CIDRs were withdrawn after 4 or 12 days in 1986 (date 27 February) and after 4 or 5 days in 1987 (date 7 or 8 March). At CIDR withdrawal, the hinds received an intramuscular injection of pregnant mare's serum gonadotropin (PMSG, Folligon, Invervet (Aust) Pty Ltd, Lane Cove, NSW, Australia; 350 i.u. per hind in 1986 and 300 i.u. per hind in 1987). At the time of CIDR withdrawal/PMSG treatment, the hinds were weighed and allocated to one of six stag groups in 1986 or one of four stag groups in 1987. Stags were treated with melatonin (two implants of Regulin®, Regulin Ltd, Melbourne, Australia on three occasions at monthly intervals from early December) to advance their breeding season. Individual melatonin-treated red stags were joined with the hinds either 24 or 48 h after CIDR withdrawal/PMSG treatment in 1986 and after 1 or 32 h in 1987. To record mating dates, coloured grease was applied to the abdomen and groin region of the stags and the hinds observed daily for signs of grease marking indicative of mating. The melatonin-treated stags were removed from their mating groups in early April and replaced by untreated red stags which were subsequently removed in mid May.

Hind management

The hinds were grazed on improved pastures (perennial ryegrass-white clover dominant) at medium to high allowances except over winter (late May to early September) when they were penned in a mature pine plantation. During winter they were given wilted pasture silage at about 0·8 of the estimated maintenance requirement of red hinds outdoors (Fennessy, Moore and Corson, 1981); the shelter provided by the trees effectively reduced the maintenance requirement by an estimated 0·15 to 0·20.

Animal recording

About 10 days before the date of expected calving the hinds were checked for their stage of udder development, and those hinds expected to calve to the induced oestrus run separately. The hinds were carefully monitored over calving (Moore, 1985) and newborn calves marked with coloured sprays for identification. The calves were weighed (to the nearest 0·1 kg) 12 to 36 h after calving, and this weight recorded as birth weight. Calves were given permanent eartags at 3 to 4 days of age. A post mortem was performed on each dead calf to determine the cause of death. The calves were weighed at about 12 weeks of age and at weaning at 20 to 21 weeks of age (mid March).

Statistical analyses

Gestation lengths, birth weights and live weights at 12 and 20 weeks were analysed by least squares, with an adjustment for year, before fitting factors expressing sex, rank and their interaction. Weight of dam and its interactions were then added to the models, but in no case were found to be significant; they were therefore eliminated. Regression analysis with backward elimination was used to investigate relationships with gestation lengths and growth rates from birth to 12 weeks and from 12 to 20 weeks as dependent variables on other recorded variables, including sex and rank factors and their interactions in the initial model. A generalized linear model with binomial error distribution and logit link function was used to analyse the relationship between perinatal survival and birth weight.

RESULTS

Calving performance

As a result of the treatments, 33 hinds in 1986 (mean pre-mating live weight of 98·9 (s.d. 5·2) kg) and 26 hinds in 1987 (109·7 (s.d. 7·9) kg) calved to the induced oestrus with 12 hinds producing twins and one triplets in 1986 and seven hinds producing twins in 1987. In 1986, 21 of the remaining 23 hinds calved to later cycles and in 1987, nine of the remaining 10 did so. For all analyses, the data for the set of triplets (males of 3·9, 4·0 and 5·8 kg at birth and a mean of 31·8 kg at 12 weeks) and that for a set of twins from an extremely heavy hind (a male twin of 10 kg and a female of 6·8 kg at birth from hind 741 at 136 kg live weight or 2·7 s.d. units above the mean weight for the whole 1987 group) and that for a single calf which was suckled by at least two hinds have been omitted. Birth weight data were obtained for all twins and 15 of 20 singles in 1986 and 18 of 19 singles in 1987. All mismothered calves, stillbirths and calves dying within 48 h of birth have been included in perinatal mortality.

TABLE 1
Mean birth weight (kg) of calves by birth type, sex of calf and sex of co-twin

Sex	Singles (no. of calves)	Twins (no. of calves): Male Male	Male Female	Female Female	s.e.d.
Male (M)	9·10 (20)	5·78 (10)	6·78 (10)		0·512†
Female (F)	8·65 (13)		5·74 (10)	6·89 (6)	0·592‡
s.e.d.	0·414	0·592‡	0·512†		

† s.e.d. for comparison of co-twins and of male twins.
‡ s.e.d. for comparison of MM with FF twin sets and of female twins.

Birth weights

There was a significant effect of birth type on birth weight with twins being proportionately 0·30 lighter than singles at birth (6·27 and 8·94 (s.e.d. 0·276) kg; $P < 0·001$). There was a significant interaction between the birth weight of a calf and the sex of its co-twin (F [1,63] = 4·62; $P < 0·05$). In this respect, males born as co-twins to females were proportionately 0·18 heavier than their co-twin females (1·04 kg; $P < 0·06$), while, in contrast, twin male sets were 0·16 lighter than twin female sets (1·11 kg; $P < 0·10$) while male singles were only 0·05 heavier than female singles (0·45 kg). The regression of birth weight on dam live weight was small and not significant (0·045 (s.e.d. 0·034)) and was therefore dropped from the overall model. The adjusted values for birth weights and the appropriate s.e.d. values from the model are presented in Table 1. The overall model accounted for 0·60 of the variance with a residual s.d. for birth weight of 1·14 kg.

Gestation length

The mean gestation lengths by sex and birth type are presented in Table 2. Neither sex of the calf nor birth type had any significant effect on gestation length and the apparent interaction with single males having shorter gestations than single females but twin males having longer gestations than twin females was not significant (difference of 5·7 (s.e.d. 3·38) days).

The regression relationships for gestation length on birth weight (mean value for twins) were parallel for single and twin calves with the gestation length for a single being 4·5

TABLE 2
Mean gestation lengths (days) by sex and birth type

Sex	Singles (no.)†	Twins (no.)†	s.e.d.
Male	232·4 (20)	236·4 (5)	2·10
Male/Female		233·8 (10)	
Female	234·5 (13)	232·7 (3)	2·70
s.e.d.	1·51	3·04	

† No. of records, twin sets in the case of twins; the data are restricted to animals with birth weight data.

(s.e.d. 1·72; $P < 0{\cdot}01$) days longer than for a twin set of the same mean birth weight (MBW). The regression relationships were:

$$\text{gestation length:} \begin{cases} \text{singles} = 251{\cdot}9 - 2{\cdot}09 \text{ (s.e. } 0{\cdot}50) \text{ MBW} \\ \text{twins} = 247{\cdot}4 - 2{\cdot}09 \text{ (s.e. } 0{\cdot}50) \text{ MBW.} \end{cases}$$

The relationship accounted for proportionately 0·24 of the variance with a residual s.d. of 3·61 days and indicates that for every extra 1 kg of individual birth weight, parturition was advanced by about 2 days.

Perinatal mortality

The overall perinatal mortality was 20%, the same rate for both males (8/42) and females (7/33). Mortality among twins was 25% (9/36) compared with 15% (6/39) among singles. Of the nine deaths among twins, five were born dead and four were due to starvation following mismothering. In contrast, of the six deaths of single calves, only one followed mismothering while three were born dead or died soon after birth and two died from accidents. Excluding the two deaths due to accidents, the mortality among singles was 10%. Since post-mortem examinations did not reveal disease conditions in any calves, it seems most likely that those calves born dead or dying soon after calving died from complications related to the birth process. The small twin calves which were mismothered were weak and lacked vigour.

A logit regression analysis indicated that the probability of survival (P_s) was not related to birth type *per se* but was strongly related to birth weight (B) with both linear and quadratic terms significant ($P < 0{\cdot}05$) in the equation:

$$\text{logit } (P_s[B]) = -9{\cdot}63 + 2{\cdot}93 \text{ (s.e. } 1{\cdot}37)B - 0{\cdot}183 \text{ (s.e. } 0{\cdot}091)B^2.$$

The relationship indicated that the probability of a calf surviving was reduced at both low and high birth weights. The expected mortality rates at 5, 8 and 11 kg birth weight derived from the relationship were 39%, 11% and 39% respectively.

Live weight and growth rate

The mean live weights of the calves at 12 and 20 weeks by sex, birth and rearing type are presented in Table 3. The marginal means pointed to several underlying factors influencing live weights. For example male calves were proportionately 0·07 ($P < 0{\cdot}05$) and 0·10 ($P < 0{\cdot}01$) heavier than females at 12 and 20 weeks, while twin-reared calves were 0·20 ($P < 0{\cdot}01$) and 0·17 ($P < 0{\cdot}01$) lighter than singles at these ages. The parameter estimates from the regressions on birth weight are presented in Table 4. There were significant effects of rearing type, sex of calf and birth weight on the live weight of

TABLE 3

Mean live weights (kg) of calves at 12 and 20 weeks by sex, birth type and rearing type

	Singles (no.)†	Twins (no.)† TT	Twins (no.)† TS	s.e.d.‡
Weight at 12 weeks				
Male	42·3 (17)	33·3 (9)	40·3 (5)	1·49
Female	39·4 (11)	32·2 (13)		1·50
s.e.d.	1·41	1·57		
Weight at 20 weeks				
Male	61·7	50·6	58·2	2·04
Female	56·0	47·5		2·05
s.e.d.	1·93	2·15		

† No. of calves; TT = twin reared as twin; TS = twin reared as single.
‡ s.e.d. for singles compared with twin/twin.

TABLE 4

Parameters (± s.e. of the estimate) for the linear regression relationships of the form y *(live weight at 12 or 20 weeks)* = a + a_r + a_s + bB†

	Live weight (kg) at: 12 weeks	20 weeks
a	26·5 (2·80)	43·6 (4·23)
b	1·77 (0·32)	1·99 (0·49)
a_r	−4·21 (1·06)	−5·28 (1·59)
a_s	−1·74 (0·81)	−3·98 (1·22)
R^2 (adjusted)	0·730	0·658
Residual s.d.	2·86	4·32

† a_r and a_s are the parameter estimates for the effect of twin birth type and female sex on live weight (for singles a_r = 0 and for males a_s = 0) and b is the regression coefficient on birth weight (B) where all values are in kg.

calves at both 12 and 20 weeks of age. There were no significant interactions between any of the recorded variables, nor were there any significant effects of dam live weight on progeny weight (the regression coefficients of 12 and 20 weeks live weight on dam live weight were −0·085 (s.e. 0·076) and −0·087 (s.e. 0·115) respectively. Dropping the birth weight term from the model had virtually no effect on these coefficients.

The regression relationships indicate that at the same birth weight, twin-reared calves were 4·2 and 5·3 kg lighter than singles at 12 and 20 weeks of age respectively. The differences between the sexes at the same birth weight increased by 2·24 (s.e. 1·46) kg from 1·7 kg at 12 weeks to 4·0 kg at 20 weeks. In addition an extra 1 kg weight at birth was associated with an extra 1·8 and 2·0 kg weight at 12 and 20 weeks respectively. The growth rate of twin-born calves whose co-twin died was indistinguishable from that of single-born calves.

The regression models for growth rate from birth to 12 weeks and from 12 to 20 weeks of age are presented in Table 5. The regression of growth rate on dam weight was small and not significant (−1·01 (s.e. 0·90) from birth to 12 weeks and −0·03 (s.e. 1·23) from 12 to 20 weeks) and therefore has been dropped from the overall models. Although the effects of rearing rank and birth weight on growth rate from 12 to 20 weeks were in the same direction as those from birth to 12 weeks, the effects were smaller and no longer attained significance. By contrast, the sex effect was greater at 20 weeks, as was also indicated by the live-weight analysis (Table 3).

TABLE 5

Parameters (± s.e. of the estimate) for the linear regression relationships of the form y *(daily growth rate in g/day from birth to 12 weeks and from 12 to 20 weeks)* = a + a_r + a_s + bB†

	Growth rates (g/day)	
	Birth to 12 weeks	12 to 20 weeks
a	315 (33·4)	306 (45·1)
b	9·1 (3·8)	4·1 (5·2)
a_r	−50·1 (12·6)	−19·2 (17·0)
a_s	−20·7 (9·6)	−40·1 (13·0)
R^2 (adjusted)	0·566	0·229
Residual s.d.	34·0	46·0

† a_r and a_s are the parameter estimates for the effect of twin birth type and female sex on live weight (for singles $a_r = 0$ and for males $a_s = 0$) and b is the regression coefficient on birth weight (B, kg).

DISCUSSION

Birth weights

As with other ruminant species, twin red deer were substantially lighter than singles at birth, although with the twins being proportionately 0·30 lighter, the difference was larger than the 0·15 to 0·25 generally reported for other species (cattle: Turman, Laster, Renbarger and Stephens, 1971; Smith, Pollak and Anderson, 1982; sheep: Burfening, 1972; Hinch, Kelly, Owens and Crosbie, 1983; black-tailed deer; Mueller and Sadleir, 1980). There are no obvious reasons for this apparent difference between species, although if the birth weight disadvantage to twins occurred in the wild, considerable selection pressure against twinning could be expected (Clutton-Brock *et al.*, 1982).

Among single calves the proportional 0·05 difference in birth weight between males and females was similar to that reported in other studies of farmed red deer (0·07 — Blaxter and Hamilton, 1980; 0·06 — Asher and Adam, 1985; 0·10 — Moore, Littlejohn and Cowie, 1988a) while the birth weights, apart from the Blaxter and Hamilton (1980) study, where the hinds were much lighter, were also similar. Unlike these studies, there was no significant relationship between birth weight and dam weight in the present work but the coefficient of 0·045 kg birth weight per kg dam live weight was similar to that reported in the other published studies.

The difference in birth weights between male and female co-twins was proportionately 0·18 compared with only 0·05 between the single male and female calves. This effect is similar to that reported in mixed-sex lamb twins (Burfening 1972). The twin effect probably reflects the differences in nutrient demand between males (with their substantially higher growth potential) and females and the resultant competition for nutrients *in utero*. Birth weight is highly correlated with post-natal growth rate as evidenced in studies of hybridization between wapiti (*Cervus elaphus manitobensis*) and red deer, where hybrid calves are proportionately about 0·50 heavier than red deer calves at birth, a similar difference to that recorded in post-natal growth rate (Milne, 1987; Moore and Littlejohn, 1989). The surprising component of the interaction, however, was the observation that sets of male twins were substantially lighter (0·16) than female twins. There are no obvious reasons for this result, but the question of whether it is a real effect or simply a problem of small numbers and genetic variability within the study herd awaits further research.

Gestation length

The shorter gestation period of 2·1 days of males compared with females in the present study, although not significant is similar to the significantly shorter gestation of wapiti × red deer hybrid males recorded by Moore and Littlejohn (1989). This is in contrast to the normal situation with ruminants where males generally have a longer gestation than females (1 to 2 days in cattle; see King, Seidel and Elsden, 1985). Further analysis revealed strong negative relationships between gestation length and birth weight, with calves with lower birth weights having longer gestations in marked contrast to other ruminant species; such a negative relationship was also recorded by Moore and Littlejohn (1989) with the wapiti × red hybrid males but not with females. In the domestic ruminant species there is generally a positive relationship between gestation length and birth weight (cattle: Bourdon and Brinks, 1982; Swan and Grasser, 1988; Burfening, Kress, Friedrich and Vaniman, 1978; sheep: Forbes, 1967). In the present work, a negative relationship was evident in both singles and twins, although at the same mean birth weight, twins had a significantly shorter gestation than singles, although twins with a higher total birth weight had a slightly longer gestation than singles. In both cattle and sheep, twins generally have a shorter gestation than singles (3 to 7 days in cattle: Turman *et al*., 1971; Anderson, BonDurant and Cupps, 1982; Diskin and Sreenan, 1985; Shelton, Simpson-Morgan and Summers, 1986; sheep: Forbes, 1967). Clearly, further study is needed with greater numbers of animals to investigate these apparent interrelationships, particularly because they contrast with relationships in other ruminant species.

Perinatal mortality

The overall rate of perinatal mortality in the singles in this study (10% excluding accidental deaths) was within the range recorded in other studies with red deer, both in farmed (7% — Blaxter and Hamilton, 1980; 7% — Moore, Littlejohn and Cowie, 1988b) and in wild populations (18% — Guinness, Clutton-Brock and Albon, 1978). Overall there was a very strong quadratic relationship between birth weight and survival, as is common in many other studies in different species (e.g. deer: Blaxter and Hamilton, 1980; lambs: Dalton, Knight and Johnson, 1980). Most of the deaths among twins in the present study were in calves of low birth weight (eight of nine deaths were calves of ≤6 kg birth weight), while among singles, they were mainly of higher than average birth weight. Poor viability of both relatively small and relatively large neonates is common in many species and these birth weight-related mortalities highlight the potential importance of management in ensuring adequate nutrition among twin-bearing hinds without over-feeding single-bearing hinds. Consequently, in the practical situation, a means of identification of twin-bearing hinds (e.g. real-time ultrasound scanning) could well be an important management tool assuming that the level of nutrition during pregnancy can make a significant impact on birth weight in this species.

Live weights and growth rate

The proportional difference of between 0·17 and 0·20 in live weights at 12 and 20 weeks between single and twin deer in the present study are similar to those recorded in both sheep (Jury, Johnson and Clarke, 1979) and cattle (Smith *et al*., 1982). Thus despite the low natural incidence of twinning in red deer, the hind has a considerable capacity to increase milk output adequately to rear twins. This additional capacity is also evident where red deer females have reared Canadian wapiti × red calves at rates of gain proportionately 0·50 higher than purebred red calves (Milne, 1987; Moore and Littlejohn, 1989). The lack of any relationship between growth rate of the sucking calf and dam live weight is in contrast to other studies (Blaxter and Hamilton, 1980; Moore *et al.*, 1988a) but may well reflect the importance of the genotype of the calf and its milk demand on the milk production of the hind.

The effect of rearing type on growth rate was much lower from 12 to 20 weeks than in the early sucking phase, which is to be expected in view of the declining influence of milk on calf growth as lactation progresses (Arman, Kay, Goodall and Sharman, 1974; Loudon, Darroch and Milne, 1984). The influence of birth weight *per se* had also diminished by the 12 to 20 week period of lactation, as expected, for the same reason.

The difference between male and female in live weights at 12 weeks is similar to that reported in other studies with red deer (Blaxter and Hamilton, 1980; Adam and Moir, 1987; Moore *et al.*, 1988a). The small increase in the difference between 12 and 20 weeks, reflected in both live weights and growth rates, is expected considering the very large difference in mature body size and the relative rates of gain (Moore *et al.*, 1988b) and patterns of growth of males and females (Suttie, 1987).

General

The differences in growth rates to weaning recorded for red deer in the present study are comparable with those recorded for the domesticated ruminant species, sheep and cattle. However, the difference in birth weights appears to be larger than that recorded for other species. This factor may well have considerable implications for the success of induced twinning in red deer, particularly when the relationship between survival and birth weight is considered. The incidence of freemartinism may also be an important consideration; in this respect two of the females from sets of unlike-sex twins examined for karyotype from the present study were freemartins (Stewart-Scott, Pearce, Moore and Fennessy, 1989). Clearly therefore, from a practical point of view, any production advantages of twinning are dependent on intensive management to ensure both good survival and good growth rates of twins (see Moore, 1987).

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