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Puberty in Angus bulls from lines selected for heifers' age at puberty

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ABSTRACT

In reproductive studies of juveniles, puberty has to be defined and decisions made on how to record it in both sexes. Our recording criterion for age at puberty in heifers is the date (and hence age) of first behavioural oestrus. This is a heritable trait under New Zealand grazing conditions, with a realised heritability estimate from a long-term AgResearch selection experiment of 0.27 ± 0.04 . Data published in Edinburgh by R. B. Land suggest that male puberty traits in mammals are also inherited and are related to female puberty traits, but the difficulty is in measuring puberty in males. Our indicator in the selection experiment mentioned above is scrotal circumference (SC), where single SC records had a heritability of 0.40 ± 0.03 and a repeatability over time of 0.67 ± 0.01 . Our objective in the present study was to determine whether another indicator might be semen quality, derived from a serial assessment of semen samples over the likely puberty period, and to compare this with serial SC records. The selection experiment was established in 1984/85 with Angus cattle. Genetic selection was applied in one line ('AGE-') for lower age at puberty in heifers, and in a second line ('AGE+') for greater age at puberty in heifers. SC data were monitored each year in bulls from both lines. Young bulls from the 1997 and 1998 calf crops (25 per year) were also monitored for semen quality from about 9 to 12 months of age. Semen samples were obtained by ampullary massage, and quality was assessed on a warm microscope stage, using a 0-5 scale. Semen score was plotted against SC from means for each breeding line and month, and the correlations over both lines were 0.98 in 1997 and 0.86 in 1998. The AGE- line was superior to the AGE+ line by 0.81 semen score units ($P < 0.001$) and by 4.0 cm (14%) in SC ($P < 0.001$). Results showed that semen quality was associated with SC, and that both had responded to heifer puberty selection. It was easier to measure SC as the male puberty trait.

Keywords: cattle; puberty; selection; semen score; scrotal circumference.

INTRODUCTION

Age at puberty (AP) has direct importance to beef production systems where there is very limited feed supply and slow animal growth. Under many New Zealand systems, AP is not critical for yearling heifer mating, but we have been interested in any genetic correlations that may exist between puberty traits and later reproductive performance. AP and weight at puberty (WP) are heritable traits in grazing heifers (Morris *et al.*, 1992; 2000). Realised heritability estimates from a long-term selection experiment with single-suckled Angus calves, to breed two lines diverging in heifer AP (now in its sixth generation), are 0.27 ± 0.04 for AP and 0.44 ± 0.06 for WP (Morris *et al.*, 2000). The definition of AP in that study is the date (and hence age) of first behavioural oestrus. AP in heifers is a relatively easy criterion to measure, although not necessarily cheap. Data published in Edinburgh by Land (1973) suggest that male puberty traits in mammals are inherited and are related to female puberty traits, leading us to an interest in male puberty traits in cattle. The accepted definition of puberty in bulls is a threshold of 50 million total spermatozoa per ml,

with at least 10% motility (Wolf *et al.*, 1965), which is more difficult to measure than in heifers. A common proxy for AP in bulls is scrotal circumference measured at a fixed age, based on the observations of Lunstra *et al.* (1978) that SC at puberty was relatively constant at 27.9 cm (approx. s.d. 1.1 cm). The present study describes an evaluation of semen scores, taken over a time period covering the likely pubertal phase, in order to determine whether bulls' semen scores had changed in two breeding lines where genetic selection was for heifers' AP. We also investigated the relationship between semen score and SC over the puberty period.

MATERIALS AND METHODS

Ethics

This experiment was carried out using a trial design approved by the Ruakura Animal Ethics Committee (RAEC #2399).

Animals

Early phases of the puberty selection experiment were described in a previous Conference paper (Morris & Wilson, 1997).

Briefly, the foundation Angus stock in 1984/85 came from a prior experiment (1964-81) where lines had been selected for weight or weight-gain traits (Carter *et al.*, 1990), followed by 3 years of re-randomisation by line-crossing (calvings 1982-84). Alongside an unselected control line, three puberty lines were then set up in 1984/85, selected for increased age at puberty in heifers (AGE+ line), reduced age at puberty in heifers (AGE- line), or increased scrotal circumference, with the last two lines being merged at the 1992 matings (forming a new AGE- line, with continued selection applied for early puberty in heifers).

The lines grazed on pastures which were predominantly a mix of ryegrass and white clover, with some supplementation in periods of feed shortage (silage and hay but no concentrates). All lines grazed together except at single-sire mating time. Mating began at the same time each year, for an 8-week period in all lines of breeding females (including yearling heifers), with chinball-harnessed selection-line bulls. Artificial insemination was used in the control line for the first eight years (using a semen panel of 37 bulls bred before the puberty selection began), with natural mating thereafter. Each selected or control sire was joined with an age-balanced sample of females from his own line. Generally 4 to 5 bulls were mated per line. About 120 females were joined per line each year until 1991, and 60-80 per line from 1992 onwards.

Measurements and routine analyses

Calves were tagged and recorded to dam within 24 hours of birth. Date at first behavioural oestrus was monitored by staff about twice-weekly in heifers from 8 to 17 months of age, with the assistance of paint marks from chinball-harnessed vasectomised bulls (except during the mating period from 14 to 16 months of age, when entire bulls were used). Age at first oestrus (AFO) was calculated from this date and the date of birth, and the AFO data were then transformed to an underlying scale (standardised age at first oestrus, SFO) with a standard deviation of unity, as if AFO was an ordered categorical response (Gianola & Norton, 1981); this was necessary because analyses to rank candidate sires were required part-way through the season when many heifers still had not reached puberty, and the standardisation process assisted with removal of some of the bias. Weight at first behavioural oestrus (WFO) was interpolated from monthly weights and first oestrus date. Scrotal circumference (SC) was measured with a flexible tape on all bulls every month, from 8 to 13 months of age. Cow and calf weights were recorded routinely. Breeding values (BV) for AFO,

SFO, WFO and SC were obtained from restricted maximum likelihood analyses (Gilmour *et al.*, 2002), over all years of data combined, with an animal model and a full relationship matrix; BVs for SFO and SC were obtained from a two-trait model, including a repeated-record treatment of monthly SC data. The BVs for the selection objective, SFO, were then used to make selection decisions each year in both sexes. Data on AFO, WFO and SC are now available for all calf crops through to birth year 2005.

Semen scores

1996 crop: Pilot trial

A total of 18 yearling bulls, comprising equal numbers from the AGE- and AGE+ lines, were monitored for semen score on four occasions, from 4 August 1997 (average age 11 months) to 23 October 1997. Preputial hair was clipped away close to the skin, prior to collection. Ampullary massage was applied to each bull *per rectum*, and semen was collected into a funnel attached to a warmed plastic tube. The semen sample was scored immediately on a warm microscope stage, using a 0-5 scale (described below). One operator (A.M. Day) carried out all the animal manipulations. The only bulls omitted from semen collection were those not well-enough grown for the operator to insert his left hand through the anus (circumference around the knuckle on the operator's hand = 24 cm). Semen scores were as follows: 0, no sperm; 1, no motile sperm; 2, few motile sperm; 3, general motility, but low sperm concentration; 4, dense sperm concentration, but little or no wave motion; 5, dense sperm concentration, with pronounced wave motion.

1997 and 1998 crops

Having established the feasibility of the procedure with the 1996 crop, bulls from the next two calf crops of yearlings in the AGE- and AGE+ lines were each measured on four occasions in the same way. The range of dates (and thus ages) was increased for the 1997 and 1998 crops, relative to the pilot trial: from 17 June (average age 9 months) through to 30 September 1998, and from 13 May (average age 8 months) to 3 September 1999.

Data analyses

Records of SC and semen score for the AGE- and AGE+ bulls from the 1996-98 crops were analysed using the JMP least squares programme (SAS, 1995). The statistical model included effects for line, year, year by line interaction (if significant), and collection month. A covariate was also tested for the effect of date of birth within season on semen score.

RESULTS

Semen score and scrotal circumference

Table 1 shows the numbers of animals present in the study, by year and line, including those from which semen samples were not obtainable. There were significant differences in semen score between lines each year, and the higher scores were in the AGE- line ($P < 0.001$). The effect of date of birth on semen score was significant ($P < 0.01$), with a 30-day earlier date of birth being equivalent to a 0.72 ± 0.23 increase in semen score, during the pubertal period. There was a significant interaction of year by line ($P < 0.01$), but this was a scale difference between lines each year, rather than the result of re-ranking.

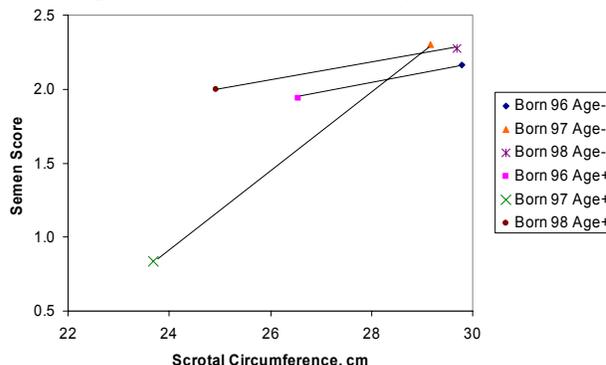
Table 1: Numbers of bulls included in the semen scoring study, and least square mean scores by year and selection line (AGE- minus AGE+).

Birth year	Line	Number ^a of bulls	Mean score	Difference ± S.E.
1996	AGE-	9	2.79	0.41 ± 0.26
	AGE+	9	2.38	
1997	AGE-	12	2.94	1.53 ± 0.26
	AGE+	13 (1)	1.41	
1998	AGE-	13 (1)	1.95	0.50 ± 0.28
	AGE+	12 (2)	1.45	
Total/Mean	AGE-	34 (1)	2.58	0.81 ± 0.15 , $P < 0.001$
	AGE+	34 (3)	1.76	

^aNumbers in brackets show those too small for ampullary massage: no scores.

Similarly to the semen score data for these three calf crops, SC was significantly greater in the AGE- than the AGE+ line. The realised heritability and repeatability of SC (combining all years with SC data) were 0.40 ± 0.03 and 0.67 ± 0.01 , respectively. The September (12-month) SC means by line were 32.7 and 28.8 cm, respectively, a difference of 3.98 ± 0.61 cm or 14% ($P < 0.001$). There were no significant effects of year, or of year by line interaction, on SC. Semen score was plotted against SC from means for each breeding line and month, and the correlations over both lines were 0.98 in 1997 and 0.86 in 1998. Figure 1 shows an example (July month) of the line means each year for semen score and SC, indicating a positive association. The largest slope was recorded in the 1997 bull calf crop. The increased SC (at fixed age) in the AGE- line was therefore associated with increased semen score at that age.

Figure 1: Mean semen score plotted against mean scrotal circumference by birth cohort: example showing data from the month of July.



Live weight

The mean live weight of all bulls on the date when the first ones were semen-scored after massaging was 229 kg for the 1996 crop, 199 kg for the 1997 crop, and 179 kg for the 1998 crop, with year effects ($P < 0.001$) partly reflecting the different ages at which the study began in each year. AGE- bulls were 15.0 ± 6.6 kg (7.7%, $P < 0.05$) heavier than the AGE+ bulls at this time. The mean live weight of all bulls which were large enough for the operator to massage on the first occasion each year ($n = 41$; mean = 213.7 kg) is shown in Table 2, compared with a mean live weight at the same time for those bulls first massaged at the second, third or fourth visits ($n = 23$; mean = 186.1 kg), and their average weight when first massaged (*i.e.* when providing their first semen sample) was 223.1 kg. Four other bulls were not massaged on any occasion (average weight = 137 kg: one AGE- bull and three AGE+ bulls; one from the 1997 crop and three from the 1998 crop). There was no significant effect of selection line on the mean live weight of the 64 bulls on the date when first massaged, although the tendency was towards heavier AGE- bulls (a 2.7% or 5.8 ± 7.0 kg difference).

Table 2: Bull live weights during the months of semen collection, and least square mean differences in weight by selection line (AGE- minus AGE+).

Trait	Effect	Live weight, kg	Live weight range, kg
Initial live weight	First day ^a ($n = 41$)	213.7 ± 3.6	166 to 289
	Later day ^a ($n = 23$)	186.1 ± 4.8	146 to 251
	AGE- minus AGE+	15.0 ± 6.6 , $P < 0.05$	
Weight when first massaged	First day	213.7 ± 3.6	166 to 289
	Later day	223.1 ± 6.5	172 to 292
	All bulls ($n = 64$)	217.0 ± 3.5	166 to 292
	AGE- minus AGE+	5.8 ± 7.0 , n.s.	

^aTime when ampullary massage was first possible.

DISCUSSION

Semen scores

There were insufficient bulls recorded for semen score here to yield data for a heritability estimate, but Gargantini *et al.* (2005) estimated a heritability value of 0.47 for age at puberty based on a bull's age when reaching a threshold of 50 million sperm per ml, with >10% motility (159 sires; 1.5 sons per sire). Literature estimates of heritability, from six groups of authors, had simple averages of 0.10 (range 0.01 to 0.22) for percent sperm motility, and 0.25 (range 0.07 to 0.47) for percent normal sperm, compared with estimates for SC from the same publications averaging 0.46 (range 0.36 to 0.57) (Christmas *et al.*, 2001; Kealey *et al.*, 2006; Knights *et al.*, 1984; Sarreiro *et al.*, 2002; Smith *et al.*, 1989; and Yilmaz *et al.*, 2004). These semen traits are thus heritable, although less so than SC, and average estimates of their genetic correlations with SC (available in four of the above six publications) were quite variable but slightly favourable, at 0.28 (range -0.04 to 0.56) for percent sperm motility, and 0.10 (range -0.36 to 0.33) for percent normal sperm. Brinks *et al.* (1978) reported correlations among sire-line means of 0.25 for SC with percent sperm motility, and 0.58 for SC with percent normal sperm.

Data in Figure 1 show a positive relationship between means for semen score and SC, for each birth cohort. Our results were consistent with the publications reviewed above, where semen traits were heritable, and positively associated with SC. Lunstra *et al.* (1978) reported a negative relationship on a between-breed basis between age at puberty in bulls and scrotal circumference in juveniles (7- and 10-month data). Genetic selection in our experiment was for divergence in heifers' age at puberty, which was associated not only with divergence in SC but also, from the present study, with divergence in semen score.

Live weight

The mean live weights in AGE- and AGE+ bulls at the first attempt at semen collection were significantly different, reflecting an AGE- line with slightly greater weight-for-age (7.7%), but not at the first successful collection. On the female side, AGE- heifers were younger at puberty (by 58 days, or approx. 15%) than AGE+ heifers, and there was a strong genetic correlation between SFO and WFO (0.83 ± 0.09 ; Morris *et al.*, 1992), but the genetic correlation was much closer to zero for SFO and 13-month weight (-0.22 ± 0.09 ; Morris *et al.*, 2000). Thus genes for age at puberty are not closely associated with those for growth,

and animals with earlier puberty are lighter at puberty mainly because they are younger.

CONCLUSIONS

Faced with a choice of measuring SC or determining semen scores on bulls for assessing puberty on a monthly basis, the cheaper and easier route is via measuring SC. The research has also validated the use of SC as a measure of puberty in bulls, genetically correlated with AP in heifers.

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