Evaluation of a vaccine to control bull behaviour

J.G. JAGO, J.J. BASS[1] AND L.R. MATTHEWS

Animal Behaviour and Welfare Research Centre, AgResearch, Ruakura Agricultural Centre,
Private Bag 3123, Hamilton, New Zealand.

ABSTRACT

To determine the effect of Vaxstrate®, an anti-GnRH vaccine, on the development of bull behaviour, 90 Friesian bull calves were either castrated at 2 months of age (steers, S), immunised against GnRH at 2, 2.5, 4 and 7.5 months of age (immunocastrates, I_2), at 4, 4.5 and 7.5 months of age (I_4), at 7.5 and 8 months of age ($I_{7.5}$), or left intact (bulls, B). Immunocastration delayed the prepubertal increase in testosterone. There were no differences in behaviour between immunocastration treatments. Mounting behaviour and paddock damage for immunocastrates and steers were lower than for bulls until 13 months of age, after which there was no difference between immunocastrates and bulls, and both were higher than steers. Bulls had a higher frequency of agonistic behaviour than both immunocastrates and steers; there was no difference between immunocastrates and steers until 16 months of age, when immunocastrates scored higher than steers. Immunisation against GnRH before puberty temporarily delayed the development of sexual and agonistic behaviour of young bulls and could provide a practical alternative to traditional methods used to control bull behaviour.

Keywords: bull; behaviour; immunocastration; GnRH; testosterone.

INTRODUCTION

Sexual and aggressive behaviour of bulls can cause damage to pasture, fences, and handling facilities and can result in serious injury to bulls and handlers. The prepubertal castration of bulls dramatically reduces the expression of sexual and aggressive behaviour (Price and Tennessen, 1981; Baker and Gonyou, 1986) and associated management difficulties. However, castration also reduces weight gain when growth promoters are not used (Field, 1971; Seideman *et al.,* 1982). Immunisation against gonadotrophin-releasing hormone (GnRH), commonly known as immunocastration, has been proposed as an alternative to surgical castration of cattle (Robertson *et al.,* 1979). Prepubertal immunocastration reduces testosterone concentrations for up to 4 months (Finnerty *et al.*, 1994), but maintains growth rates at levels similar to intact bulls and superior to surgical castrates (Robertson *et al.,* 1982; Adams and Adams, 1992; Finnerty *et al.*, 1994). The effects of immunocastration on growth and testes function have received considerable attention from researchers, but there is little information on the behavioural effects of immunisation against GnRH (Robertson *et al.,* 1979; Finnerty *et al.,* 1996). In previous studies we found that immunocastration at 9 months of age (peri-pubertal), had little effect on mounting behaviour of bulls, both at pasture and during lairage before slaughter at 24 months of age (Jago *et al.,* 1995). Therefore, the aim of this study was to determine aspects of the on-farm behaviour of bulls immunised against GnRH at three prepubertal ages, and compare this with the behaviour of surgical castrates and intact bulls.

MATERIALS AND METHODS

Animals and Treatments

Ninety 8- to 10-week-old, late September-born, Friesian bull calves were blocked by liveweight and randomly assigned to one of nine groups (n = 10/group; 2 groups/treatment except for steers). Bulls either remained intact (bulls, B), were castrated on d 0 at 2 months of age (steers, S), immunised against GnRH at 2 (d 0), 2.5, 4, and 7.5 months of age (immunocastrates, I_2), immunised against GnRH at 4(d 70), 4.5 and 7.5 months of age (I_4), or immunised against GnRH at 7.5(d 168) and 8 months of age ($I_{7.5}$). At primary and subsequent (booster) immunisations, each animal was administered 5 ml of Vaxstrate® (Peptide Technology, N.S.W, Australia; Hoskinson *et al.*, 1990) subcutaneously at two sites dorsally on the neck. From 4 months of age (d 70) animals were drafted into their groups and remained physically but not visually separated from other groups for the remainder of the trial. All groups were randomly rotated around 60 paddocks (0.25 or 0.33 ha in size) and were given access to feed *ad libitum* except during the winter months (d 209 to 300) when hay and silage were offered to each group daily. The experiment ended when the cattle were 18 months of age (d 478).

Plasma Testosterone Concentration

A blood sample was collected from each animal every two weeks from d 0 to 196, and then every three weeks until the end of the experiment. The plasma was frozen at -20°C until analysed for plasma testosterone concentrations (Jago *et al.*, 1995). The intra-assay (n = 11) coefficient of variation (CV) was 20.9 and 24.1 % for two control samples

[1] Growth and Meat Science Group, AgResearch, Ruakura Agricultural Centre, Private Bag 3123, Hamilton, New Zealand.

containing 5.0 ng/ml and 10.5 ng/ml testosterone, respectively, and the inter-assay CV was 15.8 and 14.2 % for the same two samples, respectively. The sensitivity of the assay was 0.2 ng/ml.

Behavioural Assessment

Bull Challenge Test (BCT): The behavioural responses of entire bulls, steers and immunocastrates, to unfamiliar bulls of a similar age, were observed at seven ages during a BCT, ie. at approximately 7, 8.5, 10, 11.5,13, 14.5 and 16 months of age. On the day before a test each of the nine groups was drafted into two sub-groups (n = 5). The groupings were the same at each age of testing, and were initially determined by blocking the animals in each group by liveweight and then randomly allocating animals to the two sub-groups. At each age the BCT were performed over two days with the 18 groups of animals randomly allocated across the days. The tests took place in a 50 m x 55 m paddock. Adjacent to one end of this paddock was a 50 m (the width of the test paddock) x 5 m grassed area where five entire bulls ("foreign bulls") of a similar age and unfamiliar to the test animals were positioned. A wire mesh fence with wooden supports separated the foreign bulls from the test bulls. The test animals were released into the test paddock and their behavioural responses directed towards both the foreign bulls and other animals in their sub-group were recorded for 30 minutes using continuous behaviour sampling (Martin and Bateson, 1986). The following behaviours were recorded: mounts, attempted mounts, flehmen, mount intention, pawing, horning the ground, head pushing, bunt, fight, fence pace, long approach and short approach, and are defined in Table 1. After each BCT, the test animals were removed from the paddock and another subgroup introduced, and after every fifth BCT the foreign bulls were replaced. A score was calculated for each group by summing the frequency of all behaviours (listed above) displayed by individual animals during the BCT, and taking an average of these, to give a score for each group.

Pasture Damage: Damage to pasture in the form of holes dug by pawing and patches worn by persistent group mounting behaviour (trodden patches) was recorded from 7 (d 167) to 16 (d 453) months of age. The number of small holes (< 1 m^2, score = 1), large holes (•1 m^2, score = 2) and trodden patches (worn areas •4m^2, score = 2) were recorded, and a score assigned to each group after it had grazed a paddock. The scores were summed to give a total paddock damage score for each month. This score was divided by the number of days in that month to give a daily paddock damage score for each month. Holes were filled in after a group had grazed a paddock.

Male-Male Mounting: From 7 months of age (d 182) the male-male mounting behaviour of animals was estimated using a graduated leg wear score similar to that reported by McKenzie (1983). Wear to skin and hair on the medial aspect of the front legs at the elbow was scored (ranging from 0 for no wear, to 4 for abraded skin) each time the animals were blood sampled, while held in a raceway.

TABLE 1: Definitions of behaviours recorded during the bull challenge test.

Behaviour	Definition
Mount intention	head and shoulders are raised and the weight is shifted to the rear as if the animal is about to mount, but at least one front hoof remains on the ground.
Attempted mount	both front feet simultaneously leave the ground, but the animal does not become firmly positioned on the mountee's rump.
Mount	the animal lifts its forelegs off the ground and rests the chest on the body of another animal. A mount can be on the rear, head or side of an animal.
Flehmen	the animal raises its head, outstretches its neck and curls the upper lip, usually after sniffing urine from another animal.
Bunt	the animal lowers its head, then using the head sharply strikes another animal on the flank, head, neck or body.
Head pushing	the animal pushes its head against the head of another animal in a forceful manner.
Fight	this begins as head pushing but is more aggressive with both animals bracing their bodies, often resulting in the animals pushing each other off balance or across the ground.
Pawing the ground	dragging the front foot across the ground, followed by a throwing back movement, resulting in dirt being flung into the air.
Horning the ground	rubbing the side of the head on the ground usually in an existing hole dug by pawing.
Fence pace	the animal walks or runs parallel to the fence between the test paddock and the foreign bulls. A fence pace is deemed to have ended when the animal is stationary for more than 4 seconds.
Short approach	the animal walks from < 5m away from the foreign bull fence directly to the foreign bull fence.
Long approach	the animal walks from > 5m away from the foreign bull fence directly to the foreign bull fence.

Statistical Analyses

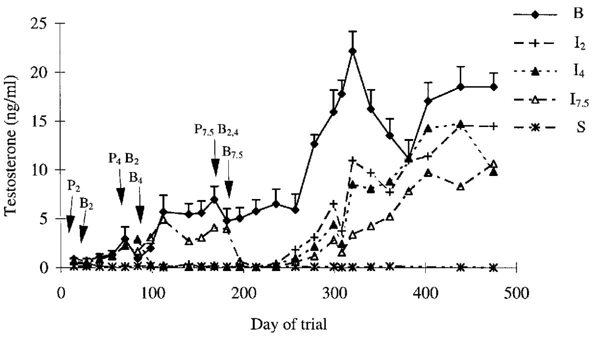
Data were analysed by ANOVA (Genstat; 5.3, Lawes Agricultural Trust, Rothamstead Experimental Station), unless otherwise stated. Where significant ($P < 0.05$) treatment effects were detected, comparisons of means were made using Student's t-test (Bhattacharyya and Johnson, 1977). Error terms were estimated using the variation between replicates when the variance ratio (between replicate standard error/within replicate standard error) was > 1. When the above variance ratio was < 1, the combined within and between replicate variation was used to estimate the error term. Bull challenge test scores and measures of testosterone concentration (d 0 to d 257) data had heterogeneous variance and were log (x + 1) transformed before analysis. Because of the large number of zeros for leg wear scores, these data were re-categorised as either 0 or > 0 and the proportions with non-zero legwear scores were compared using the Pearson Chi-square test with continuity correction (Conover, 1980). One animal from each of treatments I_2, I_4 and $I_{7.5}$ sustained an injury or died on d 403, 433 and 474 respectively, and were removed from the experiment.

RESULTS

Plasma Testosterone Concentrations

Plasma testosterone concentrations were < 0.5 ng/ml in 55 of the 60 immunised animals 28 d after their respective primary immunisations, and decreased to < 0.5 ng/ml in the remaining five animals by 46 d post primary immunisation. Mean plasma testosterone concentrations remained < 0.5 ng/ml until d 257 (about 10.5 months of age), when they began to increase for all immunised treatment groups, such that by d 382 they were similar to entire bulls (Figure 1). Although all immunocastrates had reduced plasma testosterone concentrations following immunisation, there were large differences between individuals in the duration of time that testosterone concentration remained < 1.0 ng/ml following their respective final booster immunisations (range: 1 to 10 months).

FIGURE 1: Mean (+ SEM for entire bulls only) plasma testosterone concentration from d 0 for entire bulls (B), bulls immunised against GnRH at 2, 2.5, 4 and 7.5 months of age (I_2), at 4, 4.5 and 7.5 months of age (I_4), and 7.5 and 8 months of age ($I_{7.5}$), and steers (S). Arrows indicate times of primary (P) and booster (B) immunisations, with subscripts denoting treatments immunised. The SEM is indicated for bulls only to improve clarity of the figure. SEM for the other treatments were smaller than for the bulls.

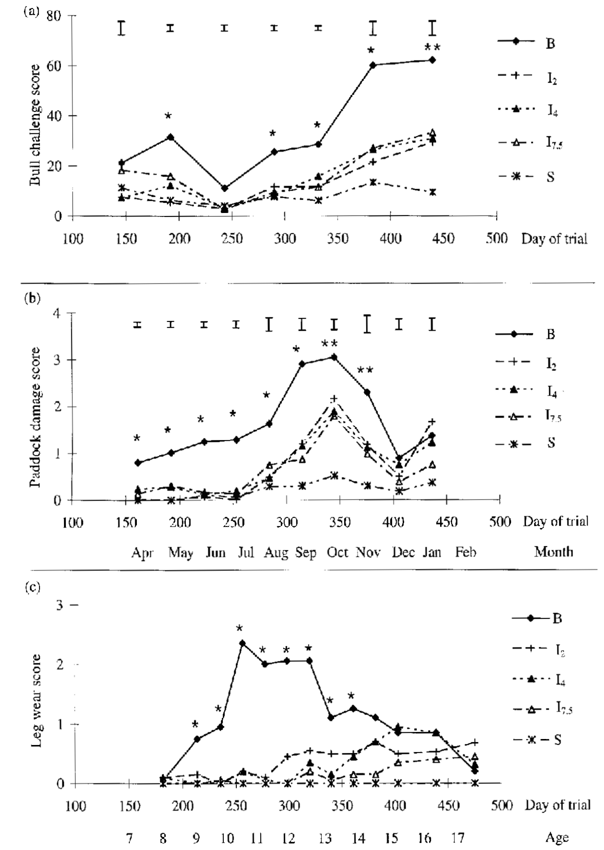


Behaviour

Bull Challenge Test: Over all tests, agonistic behaviour (pawing, horning the ground, head pushing, bunt, fight, fence pace, long approach and short approach), made up 91.0 % of the responses and sexual behaviour (mounts, attempted mounts, flehmen, mount intention) 9.0 % of responses observed. Figure 2a shows that at each age the entire bulls scored higher ($P < 0.05$ for tests two, four, five, six and seven) than all other treatments. There were no differences ($P > 0.05$) between the scores for I_2, I_4 and $I_{7.5}$ at any stage and all three were similar to steers until 16 months of age (d 440), when their behaviour scores increased while those for the steers remained consistent.

Paddock Damage: The paddock damage score for bulls increased steadily from April (7 months of age) to August (11 months of age), after which there was a steep increase during September and October then a decrease in November and December (Figure 2b). From approximately 7 months (d 167) through to 14 months (d 380) of age, the paddock damage score for bulls was higher ($P < 0.05$) than for all other treatments. All three immunocastration treatments followed a similar pattern, but at a lower ($P < 0.05$) level than for the bulls. Steers caused little pasture damage and scored less than 0.5 throughout the entire experiment.

FIGURE 2: Mean (a) bull challenge score, (b) paddock damage score, and (c) leg wear score for entire bulls (B), bulls immunised against GnRH at 2, 2.5, 4 and 7.5 months of age (I_2), at 4, 4.5 and 7.5 months of age (I_4), and 7.5 and 8 months of age ($I_{7.5}$), and steers (S). Also shown are age of animal and month of year. An asterisk (*) indicates that entire bulls scored higher ($P < 0.05$) than all other treatments, two asterisks (**) indicates that bulls scored higher ($P < 0.05$) than I_2, I_4 and $I_{7.5}$, which scored higher ($P < 0.05$) than steers. The pooled SED is illustrated above each set of data points in (a) and (b).



Male-Male Mounting: Leg wear scores > 0 did not occur on any animal until approximately 8 months of age. Figure 2c shows that between 9 and 10.5 months of age the entire bulls recorded a sharp increase in mean leg wear score, followed by a plateau between 11 and 12.5 months of age (d 250 and 330), then a gradual decline. The immunised bulls did not show a similar rapid rise, but gradually increased to be similar to bulls at 14 months of age (d 400). The steers did not score higher than 0 at any time.

DISCUSSION

This study has shown that prepubertal immunisation against GnRH delays the pubertal rise in testosterone and the development of sexual and agonistic behaviour typical of bulls, while castration abolishes any increase in testosterone and the expression of such behaviours.

The three ages at which bulls received a primary immunisation were chosen to coincide with changes in the major postnatal reproductive hormones occurring during the prepubertal period. The first was at 2 months of age, before the onset of significant testosterone production from the testes, the second at 4 months was timed to follow the increase in the amplitude of luteinising hormone pulses, but precede the initial prepubertal rise in testosterone, and the third at 7.5 months of age was timed to follow the initial prepubertal increase in testosterone production (Rawlings *et al.*, 1972; Amann and Walker, 1983). Despite the different ages at primary immunisation, and the variation in duration of suppressed testicular function, mean plasma testosterone began to increase at a similar age (10.5 months) for all immunised treatments and typical pubertal levels were attained by 14 months of age. This increase in testosterone was followed by a rise in the male-male mounting behaviour, agonistic behaviour and damage to pasture from pawing and persistent group mounting behaviour. Similarly, an increase in the expression of these behaviours by intact bulls occurred as testosterone concentration increased between 7 and 10 months of age. Together, these data indicate that testosterone is an important factor in the expression of bull behaviour but that normal hypothalamo-pituitary-gonadal (HPG) function in the prepubertal period (between 2 and 10 months of age) is not necessary for the development or later expression of these behaviours. This is in contrast to several other species including pigs and sheep, in which the absence of testosterone during specific periods between birth and puberty prevents the development of typical male behaviours (Ford and D'Occhio, 1989).

The suppression of behaviour following immunocastration of bulls in this study is consistent with the subjective behavioural assessment made by Robertson *et al.*, (1979), and the lower levels of sexual behaviour following prepubertal immunocastration reported by Finnerty *et al.*, (1996). Finnerty *et al.*, (1996) suggested that prepubertal immunocastration had a long term suppressive effect on behaviour, as differences between the behaviour of intact and immunised bulls were observed at 20 months of age, approximately 14 months after testosterone concentrations of immunocastrates had returned to the levels of intact bulls. This is in contrast with our results which indicate that the effects of immunocastration on male-male mounting behaviour and behaviours causing paddock damage reduce with time, but is consistent with the lower level of agonistic behaviour observed for immunocastrates at 17 months of age. It is known that agonistic behaviour develops after sexual behaviour (Reinhardt *et al.*, 1978; Hinch *et al.*, 1982/83; Appleby, 1986), and as a large proportion of the behaviour responses measured in the BCT were agonistic, it is possible that had the experiment continued, the difference between immunocastrates and bulls would have diminished. In addition, the lower levels of agonistic behaviour may be due to the animals that still had suppressed testosterone concentrations at the time of the last BCT, reducing the average level of activity of the group, or to a long term effect of immunocastration on behaviour.

The decrease in male-male mounting behaviour and paddock damage score that was observed for intact bulls between 12 and 17 months of age, may have been related to seasonal changes but probably not feed availability. These decreases occurred as the seasons changed from spring to summer and the increasing temperatures are likely to have been a contributing factor. However, they could also reflect age or maturity-related changes in behaviour. Price and Wallach (1991) reported a similar increase, followed by a decrease in male-male mounting behaviour amongst all-male groups, although these changes were observed earlier (mounting increased before 9 months and decreased between 10 and 15 months of age) than in our study. This difference may be due to the heavier liveweight relative to age and therefore more advanced maturity of the bulls in the study by Price and Wallach (1991).

In conclusion, prepubertal immunisation against GnRH delayed the development of bull behaviour. There appears to be no particular period, between the ages of 2 and 10 months of age, during which normal GnRH function is necessary to allow the expression of male behaviour in later life. Although immunocastration reduced homosexual mounting, agonistic behaviour in response to foreign bulls and damage to pasture for a period, it appears that multiple booster immunisations may be necessary to maintain low testosterone concentrations and suppress behaviour to the level of castrates for long periods.

ACKNOWLEDGMENTS

We would like to thank Ross Lasenby, Trevor Watson, Kaye Bremner, Jake Crockford, John Ingram, Matthew Holmes and Terry Bright for assistance with animal handling and behavioural observations and Dr Tim Trigg (Peptech Animal Health, N.S.W, Australia) for supplying the Vaxstrate®. Also Dr Cheryl O'Connor and Dr Andrew Fisher for their comments on the manuscript.

REFERENCES

Adams, T.E. and Adams, B.M. 1992. Feedlot performance of steers and bulls actively immunized against gonadotropin-releasing hormone. *Journal of Animal Science,* **70:** 1691-1698.

Amann, R.P. and Walker, O.A. 1983. Changes in the pituitary-gonadal axis associated with puberty in Holstein bulls. *Journal of Animal Science,* **57:** 433-442.

Appleby, M.C. 1986. Development of sexual and agonistic behaviour in bulls and steers. *Applied Animal Behaviour Science,* **15:** 190 (Abstr.).

Baker, A.M. and Gonyou, H.W. 1986. Effects of Zeranol implantation and late castration on sexual, agonistic and handling behavior in male feedlot cattle. *Journal of Animal Science,* **62:** 1224-1232.

Bhattacharyya, G.K. and Johnson, R.A.. 1977. Statistical concepts and methods. John Wiley & Sons, Ltd, New York.

Conover, W.J. 1980. Practical nonparametric statistics. (2nd edition). John Wiley & Sons, Ltd, New York.

Field, R.A. 1971. Effect of castration on meat quality and quantity. *Journal of Animal Science,* **32:** 849-858.

Finnerty, M., Enright, W.J., Morrison, C.A. and Roche, J.F. 1994. Immunization of bull calves with a GnRH analogue-human serum albumin conjugate: effect of conjugate dose, type of adjuvant and booster interval on immune, endocrine, testicular and growth responses. *Journal of Reproduction and Fertility,* **101:** 333-343.

Finnerty, M., Enright, W.J., Prendiville, D.J., Spicer, L.J. and Roche, J.F. 1996. The effect of different levels of gonadotropin-releasing hormone antibody titres on plasma hormone concentrations, sexual and aggressive behaviour, testes size and performance of bulls. *Animal Science,* **63:** 51-63.

Ford, J.J. and D'Occhio, M.J. 1989. Differentiation of sexual behaviour in cattle, sheep and swine. *Journal of Animal Science,* **67:** 1816-1823.

Hinch, G.N., Lynch, J.J. and Thwaites, C.J. 1982/83. Patterns and frequency of social interactions in young grazing bulls and steers. *Applied Animal Ethology,* **9:** 15-30.

Hoskinson, R.M., Rigby, R.D.G., Mattner, P.E., Huynh, V.L., D'Occhio, M., Neish, A., Trigg, T.E., Moss, B.A., Lindsey, M.J., Coleman, G.D. and Schwartzkoff, C.L. 1990. Vaxstrate® An anti-reproductive vaccine for cattle. *Australian Journal of Biotechnology,* **4:** 166-170.

Jago, J.G., Lasenby, R.R., Matthews, L.R., Trigg, T.E. and Bass, J.J. 1995. The effect of immunological castration on behaviour and growth of young bulls. *Proceedings of the New Zealand Society of Animal Production,* **55:** 190-191.

Martin, P. and Bateson, P. 1986. Measuring Behaviour. Cambridge University Press, Cambridge.

McKenzie, J.R. 1983. Effects of Zeranol implants on behaviour, growth rate, and carcass characteristics of Friesian bulls. *New Zealand Journal of Experimental Agriculture,* **11:** 225-229.

Price, M.A. and Tennessen, T. 1981. Preslaughter management and dark-cutting in the carcasses of young bulls. *Canadian Journal of Animal Science,* **61:** 205-208.

Price, E.O. and Wallach, S.J. 1991. Development of sexual and aggressive behaviours in Hereford bulls. *Journal of Animal Science,* **69:** 1019-1027.

Rawlings, N.C., Hafs, H.D. and Swanson, L.V. 1972. Testicular and blood plasma androgens in Holstein bulls from birth through puberty. *Journal of Animal Science,* **34:**435-440.

Reinhardt, V., Mutiso, F.M. and Reinhardt, A. 1978. Social behaviour and social relationships between female and male prepubertal bovine calves (*Bos indicus*). *Applied Animal Ethology,* **4:** 43-54.

Robertson, I.S., Wilson, J.C. and Fraser, H.M. 1979. Immunological castration in male cattle. *Veterinary Record,* **105:** 556-557.

Robertson, I.S., Fraser, H.M., Innes, G.M. and Jones, A.S. 1982. Effect of immunological castration on sexual and production characteristics in male cattle. *Veterinary Record,* **111:** 529-531.

Seideman, S.C., Cross, H.R., Oltjen, R.R. and Schanbacher, B.D. 1982. Utilization of the intact male for red meat production: A review. *Journal of Animal Science,* **55:** 826-840.