TESTING THE LIBIDO OF BREEDING SIRES
TO PREDICT MATING PERFORMANCE IN THE FIELD
AND THE
HORMONAL CONTROL OF MALE SEXUAL BEHAVIOUR

by

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ABSTRACT

The incidence of sexually low-response rams was determined in a group of 31 young Ile de France stud rams by means of three libido tests. Eight rams (25,8%) were classified as being sexually low-response. The proportion of rams showing normal sexual behaviour did not increase over the three tests. As testing progressed the incidence of mounting behaviour increased significantly. The increase in serving activity was non-significant. Following flock mating two low-response rams had not marked any ewes and showed preference for homosexual company. Five of the eight low-response rams had a common sire and it is suggested that sexual behaviour may be influenced genetically.

A group of 20 month-old beef bulls (n=54) representing nine breeds were evaluated for libido using five serving capacity (SC) tests (bulls tested in groups) followed by one libido score test (bulls tested as individuals). *Bos indicus* bulls displayed significantly lower SC than bulls from exotic breeds. Bulls underwent a definite learning process over the five SC tests. The increase in SC was not significant for all breeds. The use of oestrous teaser females held no advantage over the use of properly restrained non-oestrous females. Agonistic behaviour did not depress SC (individual exceptions were noted). Growth rate did not have a consistent effect on SC. Activity on the libido score test was highly significantly correlated to SC.

The influence of night or day and the presence or absence of an observer on the SC of 20 month-old *Bos indicus* bulls (n=18) was evaluated over 12 SC tests. Bulls showed no preference for nocturnal sexual behaviour and were not hindered by personnel scoring SC. The improvement in SC with progression of the tests suggested some form of adaption to pen test conditions and/or learning experience. Serving behaviour was however inconsistent from test to

test indicating that restrained females do not represent the ideal stimulus. Agonistic interaction depressed SC. Subsequent pasture mating behaviour was significantly related to SC. Some of the factors influencing pasture behaviour are discussed. High SC bulls achieved a conception rate that was 10,8% higher than that of the low SC bulls (difference non-significant).

The relationship between bull libido (libido score test) and conception rate following a 21 day breeding season was investigated over two seasons. The average bulling percentages were 2,9 and 2,5% in the first and second seasons respectively. High libido bulls had no advantage over medium libido bulls in both seasons. Bulls achieved acceptable conception rates (> 70%) in both years and it is suggested that the commonly recommended bulling percentage of 4% is conservative.

In an investigation into the hormonal control of sexual behaviour in the ram the technique of active immunization against specific steroid hormones was used in an attempt to highlight the possible central action of dihydrotestosterone (DHT) on mounting behaviour. The central aromatization of testosterone gives rise to DHT and oestradiol (E_2). Immunization against E_2 and a combination of oestrone (E_1) and E_2 in testosterone propionate (TP) implanted wethers reduced mounting behaviour to levels seen in control animals (no exogenous hormone). Results suggest that DHT has no or limited central nervous action. The effect of DHT appears to be largely via its sensitization of the penis which enhances mounting behaviour in DHT+E_2 implanted wethers to levels higher than those recorded in E_2 implanted wethers but similar to the activity recorded for wethers receiving TP implants.

DECLARATION

I hereby declare that the results contained in this thesis are from my own original work and have not been previously submitted by me in respect of a degree at any other university

J.S. Crichton
Tongaat
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CONTENTS

GENERAL INTRODUCTION

In order to ensure the profitability of red meat production it is necessary to obtain optimum rates of reproduction (Lishman, Paterson & Beghin, 1984). With this in mind research into techniques for the control of reproduction in the female and management regimes designed to ensure their conception has to some extent enjoyed preference over work on male reproduction and reproductive efficiency. With the emergence of the AI industry, knowledge with regard to the production and quality of semen has increased rapidly. More recently attention has shifted to include male libido as a factor influencing reproductive rate and therefore profitability.

Work on libido, in bulls and rams, has largely centred on devising pen or yard tests of short duration to predict sexual behaviour under conditions of natural mating. Tests for bulls, in their present form, were initiated by Chenoweth and Osborne (1975) and Blockey (1976) while those for rams would appear to arise from the work of Mattner, Braden and Turnbull (1967). Although results with regard to performance on such tests and subsequent conception rates have at times showed a positive relationship (Blockey, 1978; Lunstra, 1980; Rival & Chenoweth, 1983), they have also been equally contradictory (Winfield & Cahill, 1978; Christensen, Seifert & Post, 1982).

In bulls, contradictory ideas exist as to the relative importance of learning on sexual behaviour in the first few exposures to teaser females (Lunstra, 1980; Blockey, 1981c) and it has been suggested that libido and growth rate may be unfavourably related (Ologun, Chenoweth & Brinks, 1981).

Concomitant with investigation into the behavioural aspects of libido, interest was stimulated in the hormonal mechanisms which control behaviour. In domestic animals work has largely concentrated on the ram and the role of the testosterone and oestradiol, has been investigated (D'Occhio & Brooks, 1976, 1980; Mattner, 1976). However, the manner in which dihydrotestosterone exerts its control on behaviour remains unclear.

In the work to be described here a number of investigations were conducted using rams and bulls. The incidence of sexually low-response rams in a typical flock was determined and their subsequent flock mating behaviour evaluated. This investigation was intended to highlight the need for a more complete understanding of the endocrinology of sexual behaviour and the role of dihydrotestosterone was investigated in a subsequent study. An evaluation of libido in a group of young bulls was carried out, the effect of learning and various parameters of growth on libido was examined. This investigation was followed by one in which the factors influencing zebu bull libido were examined more closely. Lastly an attempt was made to clarify the relationship between libido score and conception rate at veld mating.

CHAPTER 1

IDENTIFICATION OF SEXUALLY LOW-RESPONSE RAMS USING A LIBIDO TEST

INTRODUCTION

Generally, producers assume that a ram will perform satisfactorily when joined with a flock of ewes (Mickelsen, Paisley & Dahmen, 1982). This is however not necessarily the case and Cahill, Blockey and Parr (1975) suggested that ram behaviour could contribute to differences in flock fertility. Mattner, Braden and Turnbull (1967) observed great variation in the number of ewes a ram will serve in a day. This implies that rams should be evaluated before being mated to ewes and a libido test has been devised by Mattner, Braden and George (1971). Although the quantitative relationship between the serving activity of rams during pen mating tests and flock fertility is generally poor (Cahill et al., 1975; Kilgour & Wilkins, 1980; Kilgour & Whale, 1980), the latter group of workers suggested that such tests are useful for the identification of rams capable of serving large numbers of ewes in the flock situation.

Of particular concern to animal scientists is the occurrence of inactive or sexually low-response rams (Pretorius, 1967) which have the potential to wreak havoc on flock fertility. Mattner, Braden and George (1973) investigated the behaviour of such inactive rams in flock matings after being subjected to pen tests. Le Roux and Barnard (1974) and Zenchak (1976) sought to determine whether previous social experience could influence the incidence and behaviour of sexually inhibited rams. Despite a relative wealth of research data on such rams, farmers are largely ignorant of their occurrence and impact on flock reproductive rates.

The trial to be described was embarked on in an attempt to create awareness amongst farmers of the problems associated with ram mating behaviour and to determine the need for further research into the hormonal mechanisms involved in sexual behaviour of the ram.

PROCEDURE

Thirty-one Ile de France stud rams with no previous sexual experience were libido tested. The rams had been weaned from their dams at eight weeks of age, thereafter they were run in all male groups on cultivated pastures. There was no certainty that rams were never grazed in camps adjacent to those containing ewes. Two age groups were represented, those being born in the autumn (n = 10) and the spring (n = 21) of 1982. Their average age $\pm$ S.E. at the onset of the test was 455,2 $\pm$ 6,7 and 352,9 $\pm$ 11,0 days respectively.

Rams were subjected to three 10 minute libido tests (Le Roux and Barnard, 1974) with a recently-mated oestrous ewe. The tests were spaced four days apart. Twenty ewes were synchronized for each test using intravaginal progesterone sponges[1] inserted for eight days followed by a 10 mg intra-muscular injection of prostaglandin $F2\alpha$[2] for the first test and 600 IU of PMS[3] for the second and third tests. Ewes were injected at sponge removal. Tests were carried out approximately 45 hours after injection. Oestrous ewes were identified and mated by two rams fitted with harness and marking crayons. Ewes which became receptive between 36 and 45 hours post injection were used in the tests as were those which became receptive subsequent to the commencement of testing.

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1 Repromap, UpJohn Co.

2 Lutalyse, UpJohn Co.

3 Fostim, UpJohn Co.

The tests were carried out in a 9 m² pen[4] the sides of which were solid. **Figures 1** and **2** give an indication of the test environment.

The following aspects of sexual behaviour (adapted from those used by Zenchak, 1976) were evaluated and recorded using a small tape recorder:[5]

a) Investigation of the ewe's genital organs;

b) Staring at the ewe's head;

c) Tongue flicking, sometimes associated with vocalisation (grumbling);

d) Kicking the foreleg, ram stands alongside the ewe facing her head and kicks up at her flank. Kicking is often associated with nudging of the ewe's hindquarters using the chest or shoulder;

e) Intention to mount - muscles tense, the body weight shifts to mount but no mount occurs;

f) Attempted mount, the ram rises on his hindlegs, no contact is made with the ewe;

g) Mounting - the ram rises on his hindlegs making contact with the ewe;

h) Pelvic thrusting or penile seeking movements (**Figure 1**);

i) Service, characterised by a deep pelvic thrust associated with intromission and ejaculation.

Farmers often contend that pen tests represent an artificial environment and believe that rams which do not mate in pen tests will do so when joined to ewes under more natural

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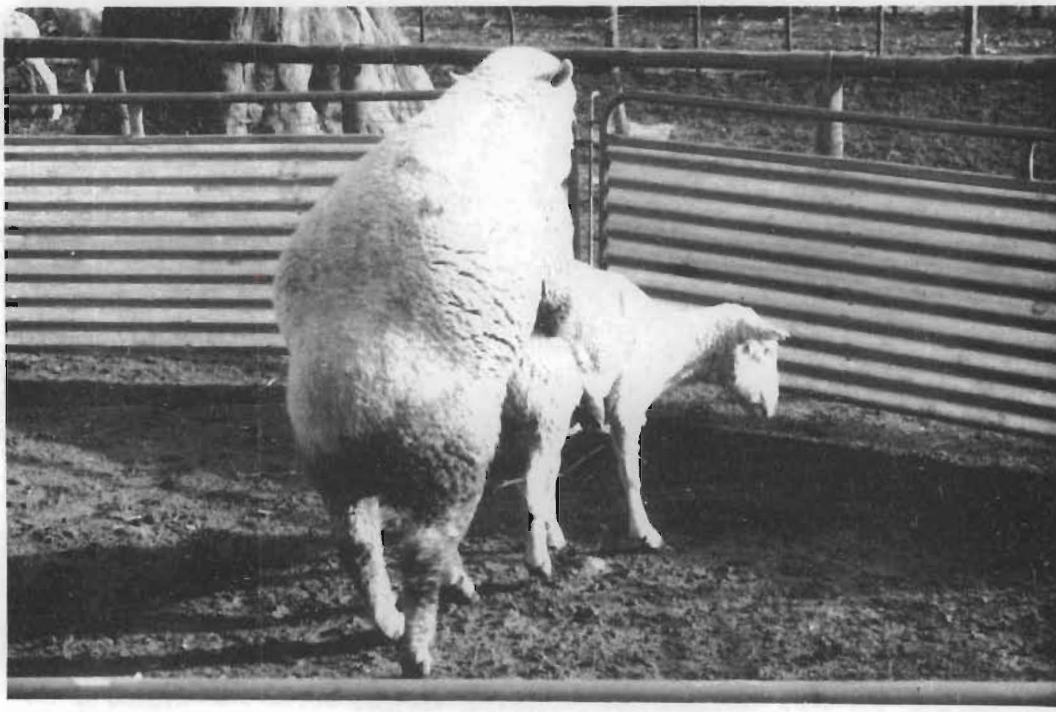
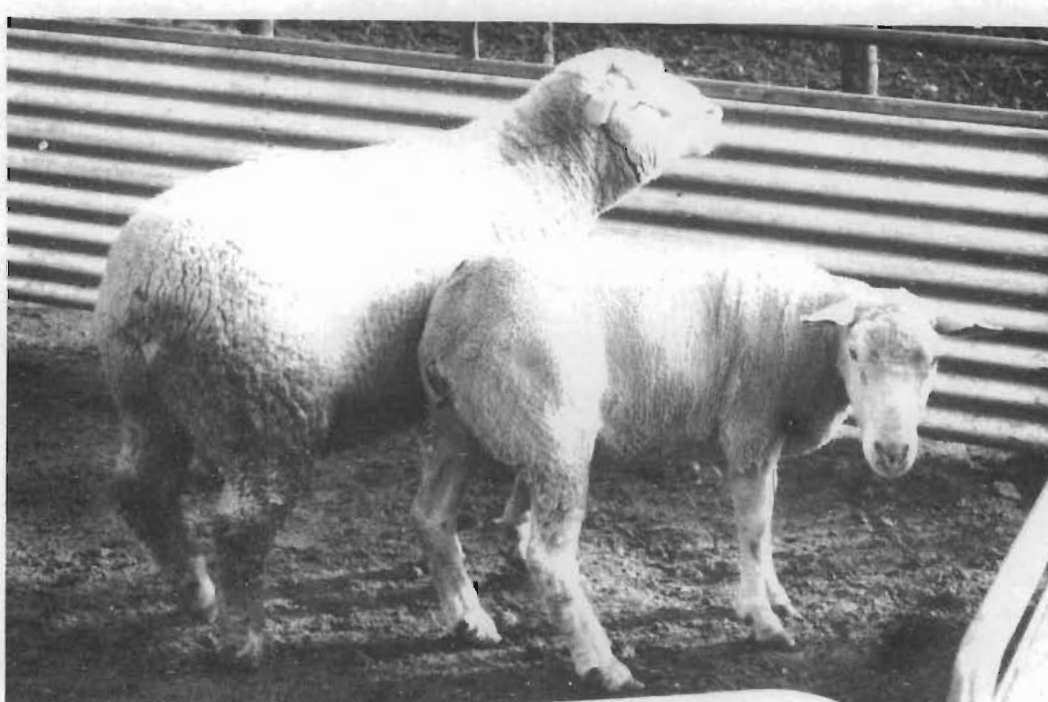
[4] Poldenvale portable handling facilities were used

[5] Aiwa, SLSS, TP-M7

Figure 1 A ram displaying pelvic thrusting/seeking movements prior to ejaculation

Figure 2 A ewe in characteristic oestrous stance with a ram positioned alongside





conditions. Thus, those rams which were classified as sexually low-response (those which failed to exhibit normal sexual behaviour toward an oestrous ewe) on the basis of the pen tests were allowed an opportunity to mate with ewes under pasture conditions three months after the pen tests. Each ram was allowed access to five oestrous synchronized (intravaginal progesterone sponges for eight days, 10 mg prostaglandin F2 α at sponge withdrawal) maiden ewes for five days. The rams were fitted with a harness and marking crayon. The ewes were checked daily for crayon markings. Those rams which still had not mated at the end of this period were placed in a group, with 18 maiden ewes (synchronised as before) for eight days. Daily observations were made as before. The rams were fitted with different colour crayons.

RESULTS

The number of rams showing different levels of sexual activity over the three libido tests is given in **Table 1**.

Table 1 The distribution of rams according to various levels of sexual activity over three libido tests

	Sexual Activity							
	Inactive		Mounting		Serving			
					One Service		Two or more Services	
Test number	n	%	n	%	n	%	n	%
1	9	29,0	2	6,5	12	38,7	8	25,8
2	8	25,8	1	3,2	12	38,7	10	32,3
3	9	29,0	1	3,2	10	32,3	11	35,5

Eight rams (25,8 per cent) were classified as sexually low-response following the three tests. One of the eight made a vague mounting attempt in test one but failed to show sexual activity during the remaining two tests. Of the remaining 23 rams, two which were sexually inactive in test one, served in tests two and three while another ram which served in the first test, attempted but failed to serve in test two and was sexually inactive in test three.

The level of mounting behaviour increased significantly ($P < 0{,}05$) from the first to the third test (**Table 2**). Serving activity on the other hand showed no significant increase.

Table 2 The incidence of different components of sexual behaviour in 31 rams over three libido tests

Sexual Behaviour Trait	Test number*			Level of significance
	1	2	3	
1. Genital investigation	269 [a]	241 [a]	282 [a]	
2. Staring	97 [a]	160 [b]	165 [b]	5%
3. Tongue flicks	141 [a]	138 [a]	240 [b]	10%
4. Foreleg kicks	57 [a]	61 [a]	85 [a]	
5. Intention to mount	9 [a]	22 [b]	24 [b]	10%
6. Mounts	12 [a]	24 [ab]	36 [b]	5%
7. Services	28 [a]	32 [a]	33 [a]	

* Within rows, means without common superscripts are significantly different

The incidence with which rams showed intention to mount more than doubled from the first to the second test and remained at a similar level for test three. The increase was only significant at the 10 per cent level. Staring behaviour increased significantly ($P < 0,05$) from test one to test two and stabilised in test three. Tongue flicking and kicking of the foreleg changed in a similar fashion over the three tests. Both remained at the same level during tests one and two, and increased in test three (the increase for tongue flicks was significant at the 10 per cent level). The incidence of genital investigation remained constant.

Linear regression analysis revealed a tendency for age (days) to influence the total number of services compeleted in the three tests. All the low-response rams were from the younger age group (spring born). Further investigation showed that five of the eight low-response rams had a common sire (HRW 148) which was on loan from another stud. Ten of the 31 rams tested were sired by HRW 148. The small numbers of offspring from the other sires did not facilitate a statistical comparison of sires.

Seven of the eight low-response rams (one had been culled on the basis of conformation) were subjected to the five-day pasture mating. Two rams (2159 and 2074) did not mark any ewes, while one ram (2117) marked a single ewe indistinctly on her flank. Following the second pasture mating, rams 2159 and 2074 appeared not to mate, and ram 2117 marked two ewes.

DISCUSSION

The incidence of low-response rams

The 25,8 per cent which were classified as sexually low-response following the pen tests is in close agreement with the work of Mattner, Braden and George (1973) who found that 25,3 per cent of 75 sixteen to seventeen month old

Merino rams had not mounted following three 20 minute tests. In contrast Le Roux and Barnard (1974) reported that 56 per cent of Karakul rams (n = 9) raised in monosexual isolation until 18 months of age remained inactive following five 10 minute libido tests. Earlier Pretorius (1967) had found that one third of 36 two year old Merino rams (raised in isolation from ewes) showed no heterosexual interest following 20 ten minute libido tests.

Characteristic behaviour by low-response rams

The low-response rams exhibited abnormal behaviour in the form of an "odd" baaing sound, agitation (running around the pen or circling the ewe), attempts to leave the pen, staring through the cracks in the pen and avoiding eye contact with the ewe. Any genital investigation engaged in was generally brief and without purpose, other forms of courtship behaviour (staring and tongue flicking) were seldom displayed. Where courtship behaviour was displayed it tended to be erratic and followed no sequence. Zenchak (1976) reported similar behaviour in his study. Low-response rams exhibited "erratic oscillating displays of approach and avoidance behaviour toward the ewe". Rams appeared to be in a conflict situation and the avoidance behaviour was interpreted as a "fear" response.

Preference for homosexual company

Zenchak and Anderson (1980) reported that low-response rams engaged in significantly higher levels of sex-like behaviour with their pen-mates (monosexual groups) than did normal rams. This confirms an earlier observation made by Pretorius (1967). In this study, the low-response rams which had not marked any ewes during the first pasture mating tended to isolate themselves when placed in a pen with ewes. Ram 2159 actively courted and mounted the two other rams. Those low-response rams which had marked ewes displayed normal heterosexual interest.

On a number of occasions during the first pasture mating rams 2159 and 2074 escaped from their paddocks to be with rams in the adjacent camps. This did not happen during the last pasture mating when the rams were placed as a group with ewes. During the summer mating season which followed, an attempt was made by the stud breeder to use rams 2159 and 2074 in single-sire flocks, again they broke through fences to escape the ewes. It is apparent that such rams prefer homosexual company.

Sexual behaviour at first exposure to oestrous ewes

Pretorius (1967) reported that one third of Merino rams showed heterosexual interest on first exposure to oestrous females (following monosexual isolation from birth). Le Roux and Barnard (1974) reported similar findings with Karakul rams. In contrast 71 per cent of the rams in this study either mounted or served ewes in their first test (**Table 1**). Perhaps isolation had been more effective in the first two reports. Winfield and Makin (1978) suggested that very limited exposure to, or contact with, ewes such as may occur through the fence when ewes are grazed in adjacent paddocks is sufficient to overcome the effects of strict monosexual rearing. The possibility that the rams in this study experienced such contact cannot be excluded.

Learning sexual behaviour

Dy'rmundsson and Lees (1972) reported a consistent gradual increase in mating dexterity for Clun Forest ram lambs over six fortnightly introductions from the age of five months (142 days) to eight months (223 days). All rams had mated following their sixth introduction. Interpretation of these results is confused by the fact that some rams had not achieved physiological puberty (detachment of penile adhesions and release of viable sperm) at first introduction. Physiological puberty preceded full sexual maturity (first successful copulation) to a variable extent. Thus some rams learnt to copulate more rapidly than others, following attainment of physiological puberty. Pretorius (1967) reported that the proportion of rams mounting increased rapidly over the first four tests while the proportion copulating increased over 13 tests.

Rams in the present study did not undergo such a drastic learning process, probably because a far greater proportion of rams showed sexual interest during their first test. The proportion of rams mounting but not copulating remained more or less static over the three tests (**Table 1**), while the proportion copulating showed a non-significant increase from test one to two (64,5 versus 71,0 per cent) and decreased slightly in test three (67,8 per cent). The proportion of rams which were inactive did not show any great changes over the three tests. The fact that most of the inactive rams showed sexual interest during the subsequent pasture mating suggests that these rams needed more than three exposures to oestrous ewes in order to overcome their inexperience. It is worthwhile noting that the overall level of mounting activity increased significantly over the three tests (**Table 2**). Surprisingly the level of serving activity showed a non-significant increase.

The slow learning process which the rams tested by Pretorius (1967) underwent and the high percentage of rams which remained inactive following 20 tests would suggest that the longer exposure to oestrous ewes is delayed, the greater the problems associated with libido and mating dexterity in first and subsequent exposures.

The fact that five of the original eight sexually low-response rams had a common sire implies that the ability to learn sexual behaviour may be influenced genetically. Indeed, Mattner <u>et al</u>. (1973) found a higher proportion of low-response rams in one strain of Merinos.

It is apparent therefore that not all rams determined to be sexually low-response on the basis of a few pen tests remain so following flock mating. However, such rams should be regarded with suspicion in view of the likelihood of depressed conception rates due to their poor mating dexterity and libido (Mattner <u>et al</u>., 1973). Furthermore, the implication that the development of normal sexual behaviour may be determined partly on a genetic basis suggests that low-response rams should be culled without consideration being given to other superior traits.

CHAPTER 2

THE EVALUATION OF LIBIDO IN YOUNG BEEF BULLS

INTRODUCTION

Individual bulls show considerable variation in their ability to settle cows earlier in the season and over the whole breeding season (Neville, Smith & McCormick, 1979). This could be the result of differences in semen quality and quantity and in bull libido. Blockey (1978) demonstrated that bull libido can influence first oestrus conception rates.

Bull libido may be defined as the potential intensity of sexual behaviour measured relative to other bulls in terms of frequency and spacing of mounts, intromissions and successful copulations (Hafez, 1962, cited by Hentges, 1973).

Two methods for testing bull libido have evolved namely, the libido score technique (Chenoweth & Osborne, 1975) and the serving capacity test (Blockey, 1981c). The latter was designed to predict veld mating performance and has been used extensively in Australia to test large numbers of bulls.

According to Blockey (1981c) sexually inexperienced two-year-old-bulls, with few exceptions, display their inherent serving capacity on their first test. The findings of Lunstra (1980) were somewhat contradictory, with yearling bulls requiring at least two 30 minute tests before their sexual activity was stabilised.

The potential for injury to bulls as a result of falls or fighting (in the serving capacity test bulls are tested in groups) as well as the relatively high cost of the tests cannot be ignored. Thus any physical or physiological parameter which may be readily determined and which accurately predicts libido would eliminate the need for libido testing per sê. Various workers have investigated the relationship between libido and such parameters. Libido has been found to be unrelated to plasma luteinizing hormone and testosterone levels (Bindon, Hewetson & Post, 1976; Blockey & Galloway, 1978). Neither was it related to scrotal circumference or seminal characteristics (Chenoweth, 1983). It would therefore appear that at present libido testing is the only satisfactory means of identifying inferior bulls. The importance of such tests is accentuated in the light of the relatively high coefficient of heritability for libido (0,59 ± 0,16, Chenoweth, 1983).

Recent evidence which suggests that bulls which attain superior rates of growth in the feedlot may in fact have inferior libido (Ologun, Chenoweth & Brinks, 1981) has alarming implications.

The initiation of the phase D_2 bull performance test by a prominent Natal livestock co-operative in conjunction with the National Beef Performance Testing Scheme provided an opportunity to investigate the incidence of poor libido in young beef bulls representing diverse breeds. The importance of the element of learning in sexual behaviour was examined as was the relationship between libido and various parameters, average daily gains (ADG) being of particular interest.

PROCEDURE

Fifty-four young beef bulls representing nine breeds of cattle were evaluated for libido at the culmination of a year-long performance test. Bulls had been accepted at a minimum of five and a maximum of nine months of age. During the first five months bulls were fed maize silage plus an energy lick (12/5/82 until 8/10/82), a summer of grazing on kikuyu pasture plus a salt/phosphate lick followed (8/10/82 until 11/3/83), the test ended with a period in the feedlot on a complete ration. The duration of this last period was determined by the bull's breed-type. It was terminated when bulls reached "working" condition, thereafter their condition was maintained until they were sold. The bulls of the British beef type ended the feedlot phase first followed by the dual purpose and then the zebu types. As each breed-type group finished its feedlot phase, it was evaluated for libido.

Bulls were subjected to both the serving capacity (SC) test (Blockey, 1981c) and the libido score method (Chenoweth & Osborne, 1975).

Serving capacity test

Serving capacity may be defined as the number of services a bull completes in 30 minutes. Bulls were subjected to five SC tests spaced two to three days apart (see **Appendix A1** for the test schedule). Four of the tests made use of oestrous induced teaser females, in the remaining test females were not induced into oestrous. Within a given breed (as far as numbers allowed) bulls were divided into groups of three balanced for body mass. At the start of testing bulls were an average age of 628,8 ± 24,4 (S.E.) days (approximately 21 months).

Each group of bulls was allowed access to two restrained, ovariectomised, oestrous induced heifers for 30 minutes following 30 minutes of sexual stimulation (this involved observation of the sexual activity of the previous group on test). Bulls which had been culled for various reasons formed "stimulator" groups which were allowed to mate for 30 minutes prior to the first test on any given day. The order in which groups were tested was randomised over the five tests.

The following activities were recorded using a small tape recorder:[6]

a) Disorientated mounts, those mounts made at 90 degrees to the cow's backline.

b) Abortive mounts, mounts in which the bull mounts in line with the long axis of the cow, service is not completed, intromission may be achieved (**Figure 3**).

c) Complete service, the bull mounts, gains intromission and ejaculates, ejaculation being characterized by a convulsive pelvic thrust (**Figure 4**).

d) Agonistic interactions, interactions had either no definite outcome or resulted in a win or lose (one bull drives off another, immediate retaliation does not occur), disrupted mounts formed another aspect of interaction (**Figure 5**).

A second observer kept a separate written record of sexual activity. Bulls were identified by means of numbers painted on their hindquarters and shoulders.

Heifers were restrained by means of service crates. The crates were adjustable, adjustments being made for the length and width of the heifer (see **Appendix A2** for a plan of the crates). Adjustment for length was made in such a way as to allow the bull ready access to the heifers hind-quarter while adjustment for width limited movement from side to side (**Figures 6** and **7**).

Fourteen Simmentaler X Hereford and Hereford X Afrikaner type heifers were ovariectomised <u>per</u> flank 40 days before the first test. The heifers were approximately 20 months of

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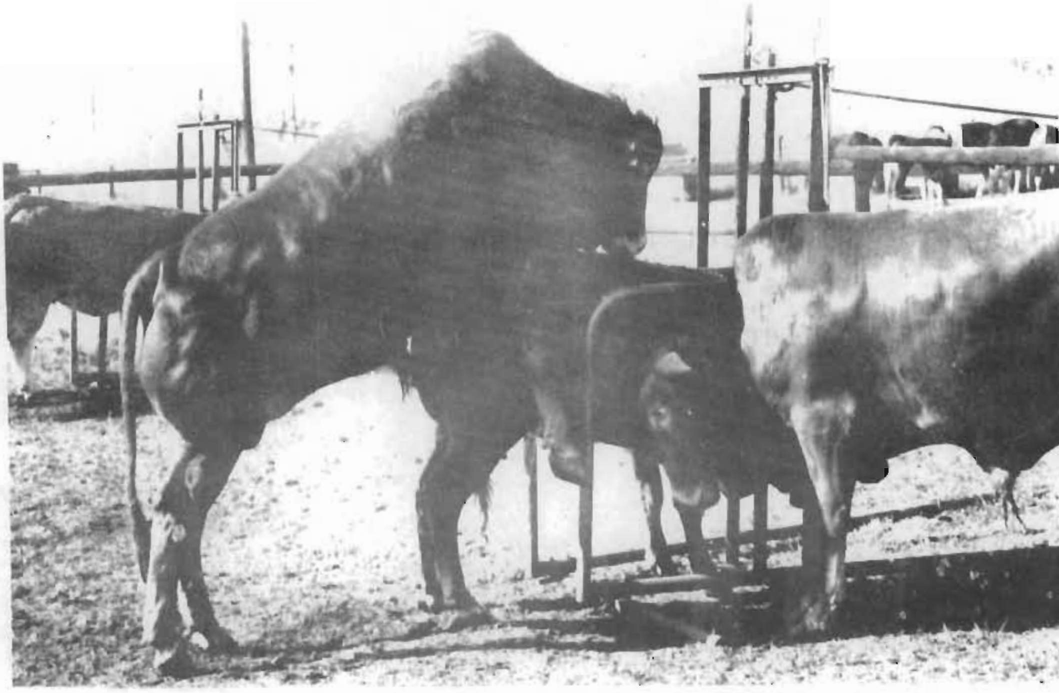
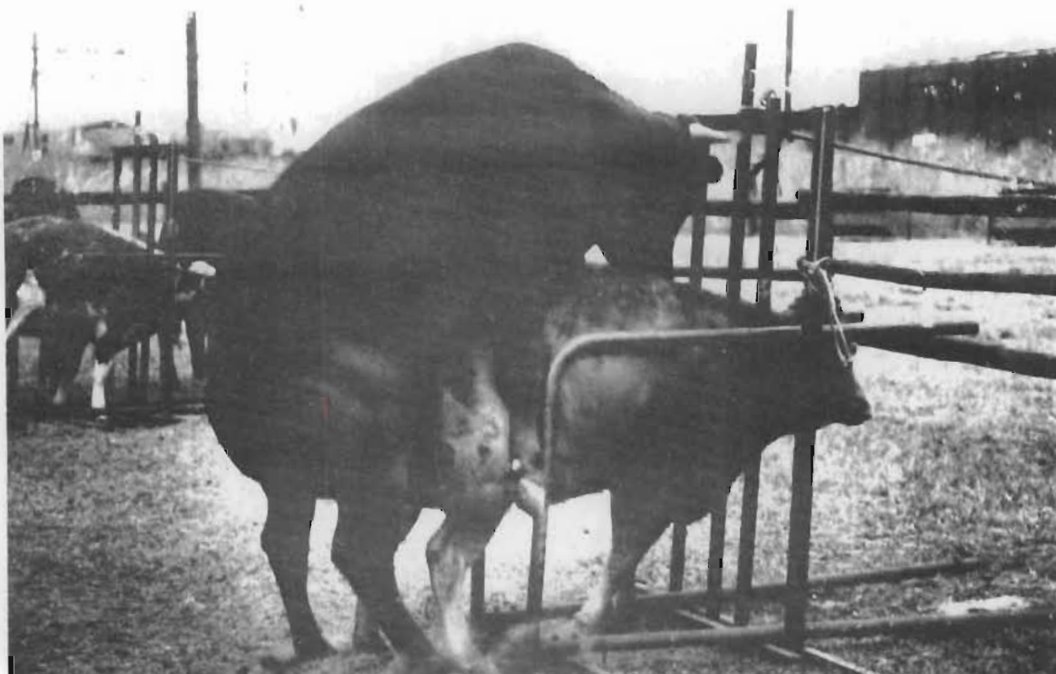
[6] Aiwa, SISS, TP-M7.

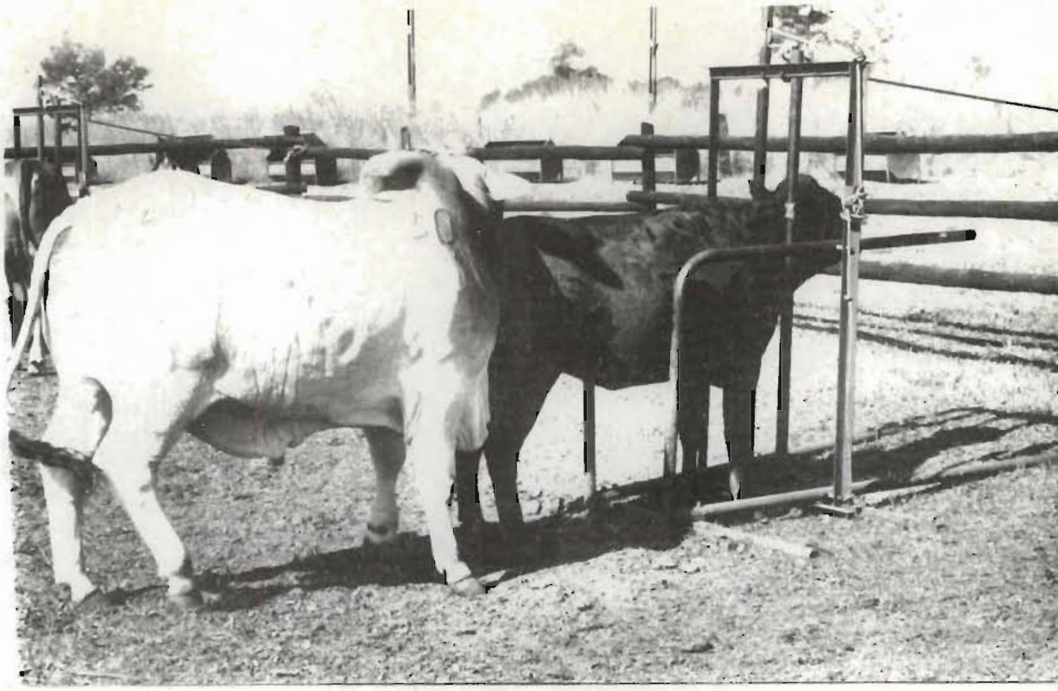
Figure 3 An Afrikaner bull in the process of making an abortive mount

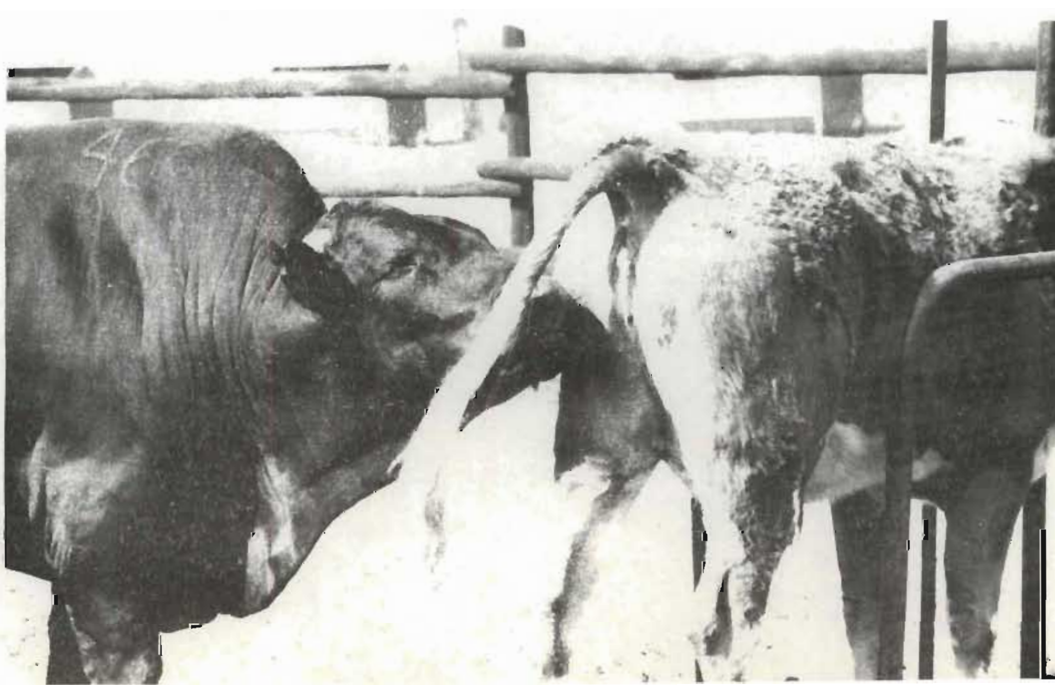
Figure 4 The characteristic pelvic thrusting action displayed during ejaculation

Figure 5 A disrupted mount, one aspect of agonistic interactions









age and weighed 350 kg. Subsequent to ovariectomy the heifers were handled as often as possible. Handling entailed being driven through a crush periodically and being caught in neck clamps for the feeding of maize meal. As a result most heifers accepted with relative calm being restrained in service crates. Those heifers which showed themselves to be excessively tense during restraint were mildly sedated using Xylazine.[7]

For the purpose of testing, the heifers were subdivided into three groups (two with five, one with four heifers). Tests were spaced in such a way as to allow heifers at least 11 days rest before reuse. Six cull dairy cows were used as teasers for the non-oestrous tests. During testing, teaser females which became unco-operative were replaced by fresh ones.

Heifers were induced into oestrus using the following hormone regime (Lunstra, 1980):

a) Day one, 50 mg progesterone.[8]

b) Day two, 20 mg progesterone.

c) Day three, 4 mg oestradiol cypionate.[9]

All injections were intramuscular and were administered at 10h00 each day. Heifers showed standing oestrus approximately 20 hours later (**Figure 8** depicts a heifer strongly in heat being investigated by a bull).

During their first few SC tests some of the British beef bulls appeared to be affected by a sudden spell of hot weather. A Stevenson screen was subsequently erected and environmental termperatures were recorded before each test.

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[7] Rompun, Bayer

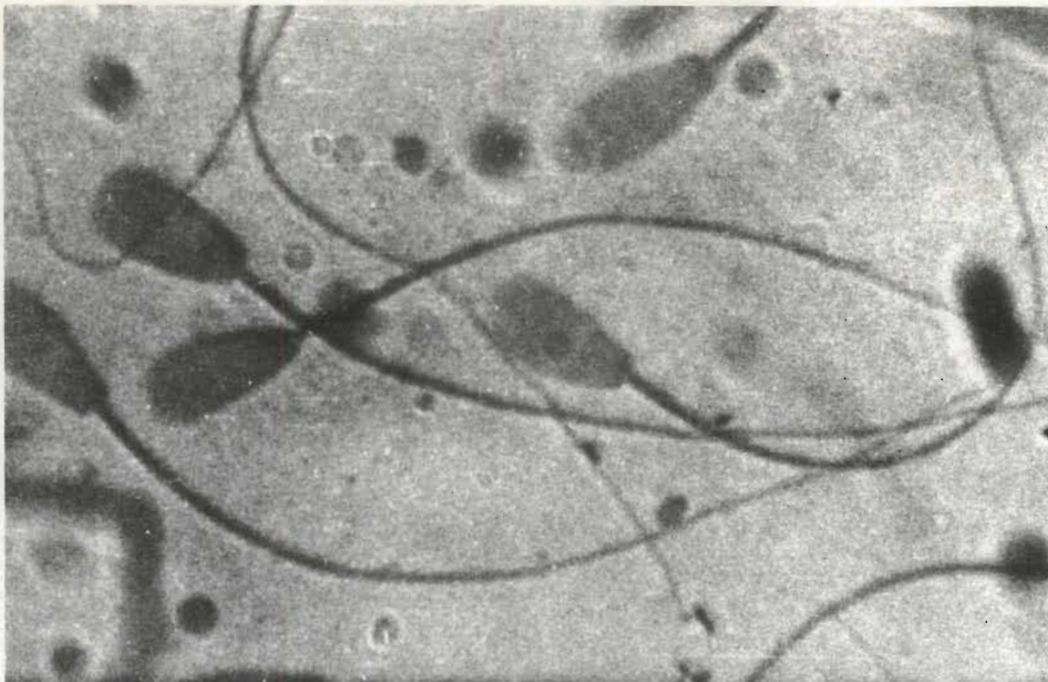
[8] Depogest, Centaur

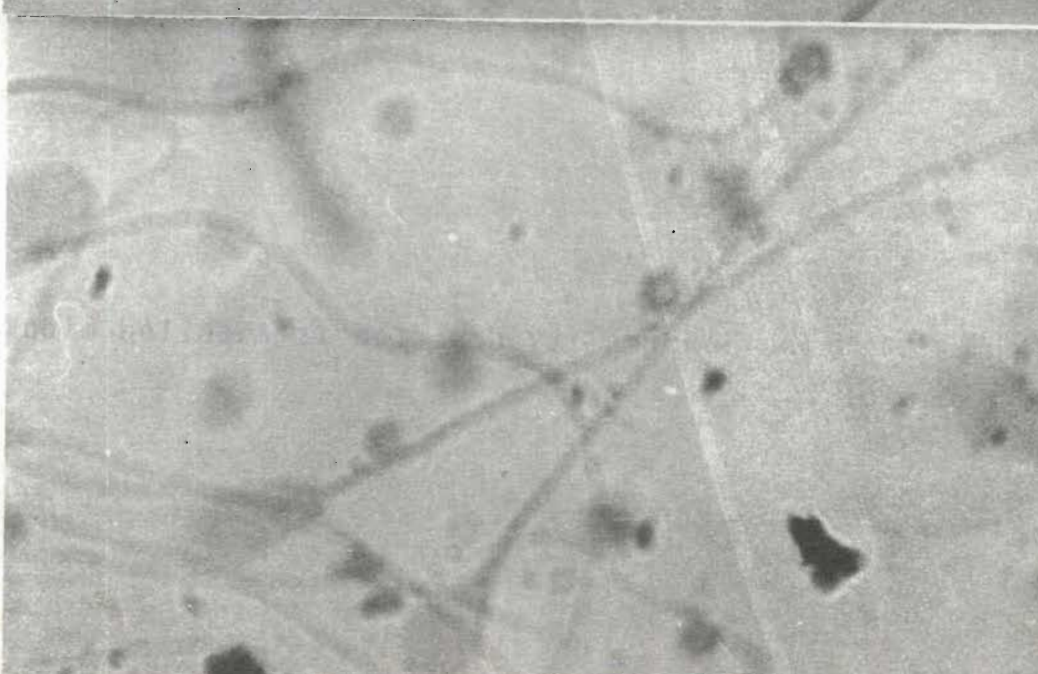
[9] ECP, UpJohn Co.

Figure 12 A double-headed sperm, top left

Figure 13 The sperm situated centrally displays a thickened midpiece, a coiled tail and a head defect

Figure 14 The abnormality depicted is a coiled midpiece





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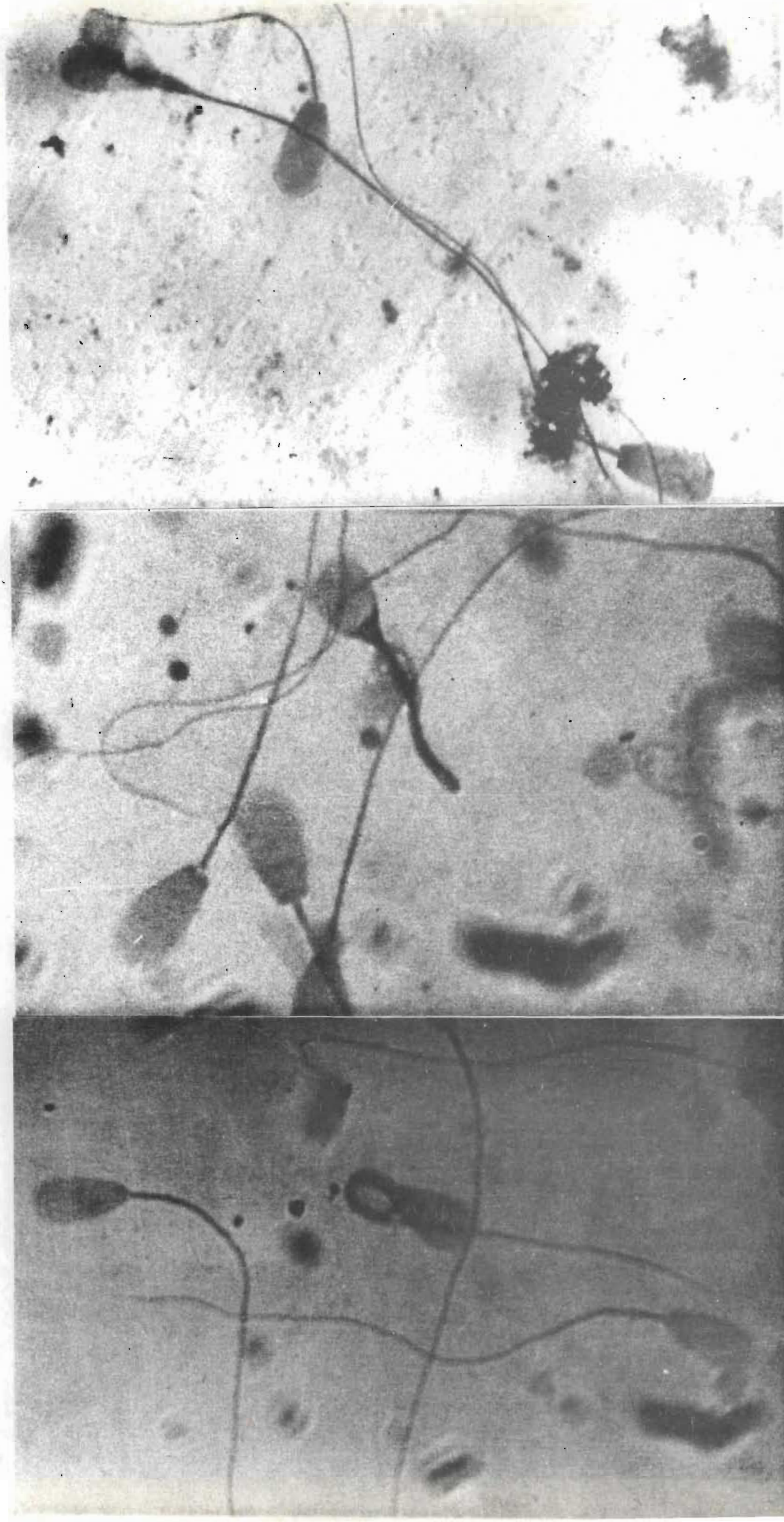

Bulls from each breed-type were subjected to a fertility examination following their second SC test. An ejaculate of semen was obtained by means of rectal massage and electro-ejaculation. Semen was evaluated for motility, density and percentage abnormal sperm after Roberts (1971). The testicles were palpated for abnormalities and scrotal circumference was measured. Semen smears were stained using the stain known commercially as Rapidiff. Evaluation was carried out under 1 000 x magnification using an oil emersion lens. Various fields were counted, field placings being made at random until 100 cells had been evaluated. In order to check the accuracy of scoring, a number of slides were taken to the local veterinary laboratory to be evaluated independently. Scores coincided closely. Some of the typical abnormalities identified are shown in **Figures 9 to 14.**

Data from the performance test and measurements of body length and height were available for use in statistical analysis.

The data was analysed using a statistical package.[10] The effect of breed of bull and number of exposures (sexual experience or time) on mating behaviour was analysed using a split-plot design. Successive observations on the same animal were treated as sub-plots in the design. The effect of age, body mass, scrotal circumference, length, height, sperm motility, percentage abnormal sperm and the various parameters of growth on SC were evaluated using multiple regression techniques. Linear regression analysis was used to investigate the influence of agonistic interactions and ambient temperature on sexual activity.

Libido score method

Following completion of all the SC tests all bulls were

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[10] Genstat on a Sperry Univac (1 100/70) computer

evaluated for libido as individuals. Each bull was given one such test. The bulls were sexually stimulated for at least 10 minutes before being allowed 10 minutes access to an ovariectomised oestrous induced heifer restrained in a service crate. Bulls were evaluated according to the following scoring system (Chenoweth, 1981):

a) 0 = showed no sexual interest.

b) 1 = sexual interest shown only once.

c) 2 = positive sexual interest in female more than once.

d) 3 = active pursuit of the female with persistent sexual interest.

e) 4 = one mount or mounting attempts, no service.

f) 5 = two mounts or mounting attempts, no service.

g) 6 = more than two mounts or mounting attempts, no service.

h) 7 = one service followed by no further sexual interest.

i) 8 = one service followed by sexual interest including mounts or mounting attempts.

j) 9 = two services followed by no further sexual interest.

k) 10 = two services followed by sexual interest, including mounts, mounting attempts or further services.

In order to extend the upper limit of the scoring system, consideration was given to the actual number of services a bull completed. Chenoweth, Brinks and Nett (1979) suggested that the ability of the test to predict pregnancy rates at a veld mating would be improved by such a change since a large proportion of bulls achieve a 10 score.

The extent to which the individual test predicted SC was determined using linear regression analysis.

RESULTS

Serving capacity test

Breed significantly influenced mounting activity (mounts which did not result in ejaculation). Bulls from the Brahman breed displayed the lowest level of mounting activity and were significantly less active than bulls from the Hereford, Sussex, Afrikaner (all $P < 0,05$) and Simmentaler ($P < 0,01$) breeds. Across all breeds the Afrikaner had the highest level of mounting. Within the zebu breeds the Afrikaner had a significantly higher level of mounting activity than the Brahman and Drakensberger bulls.

A comparison of breed-type revealed that the British beef and dual purpose types displayed significantly higher levels of mounting activity than the zebu types ($P < 0,05$). The difference between the British beef and dual purpose types was negligible.

Exclusion of the Santa Gertrudis, Brahman and Afrikaner breeds revealed that mounting activity showed a strong tendency to decrease with time (almost significant at the five per cent level).

The analysis of variance table for the effect of breed and time on SC (**Table 3**) showed that the effect of sexual experience/time was strongly apparent. Serving capacity increased over the first four tests and remained at a similar level for test five (**Figure 15**).

Table 3 Split-plot analysis of the effect of breed and time (number of exposures) on SC

SOURCE OF VARIATION	D.F.	S.S.	M.S.	F	SIGNIFICANCE
Between animals	53	806,367			
Breed	8	270,441	33,805	2,838	P< 0,05
Residual (a)	45	535,926	11,910		
Within animals	216	351,000			
Time	4	49,200	12,300	9,624	P< 0,001
Breed x Time	32	71,703	2,241	1,754	P< 0,05
Residual (b)	180	230,079	1,278		
TOTAL	269	1157,367			

Table 4 The average breed-type and breed serving capacities over five tests

BREED	N	TYPE	MEAN SERVING CAPACITY BREED[1]	MEAN SERVING CAPACITY TYPE[2]
1. Sussex	5		2,96	
2. Red Angus	4	British beef	2,65	2,06[a]
3. Hereford	9		1,29	
4. Simmentaler	14	Dual purpose	2,64	2,59[a]
5. South Devon	4		2,40	
6. Drakensberger	9		1,07	
7. Santa Gertrudis	2	Zebu	0,50	0,59[b]
8. Brahman	5		0,00	
9. Afrikaner	2		0,00	

[1] Significant differences between breed means, * = P<0,05, ** = P< 0,01 2,4, 5>8*; 4>3*; 1,4 6,9*; 1>8**.

[2] Means without common superscripts are significantly different (P < 0,01).

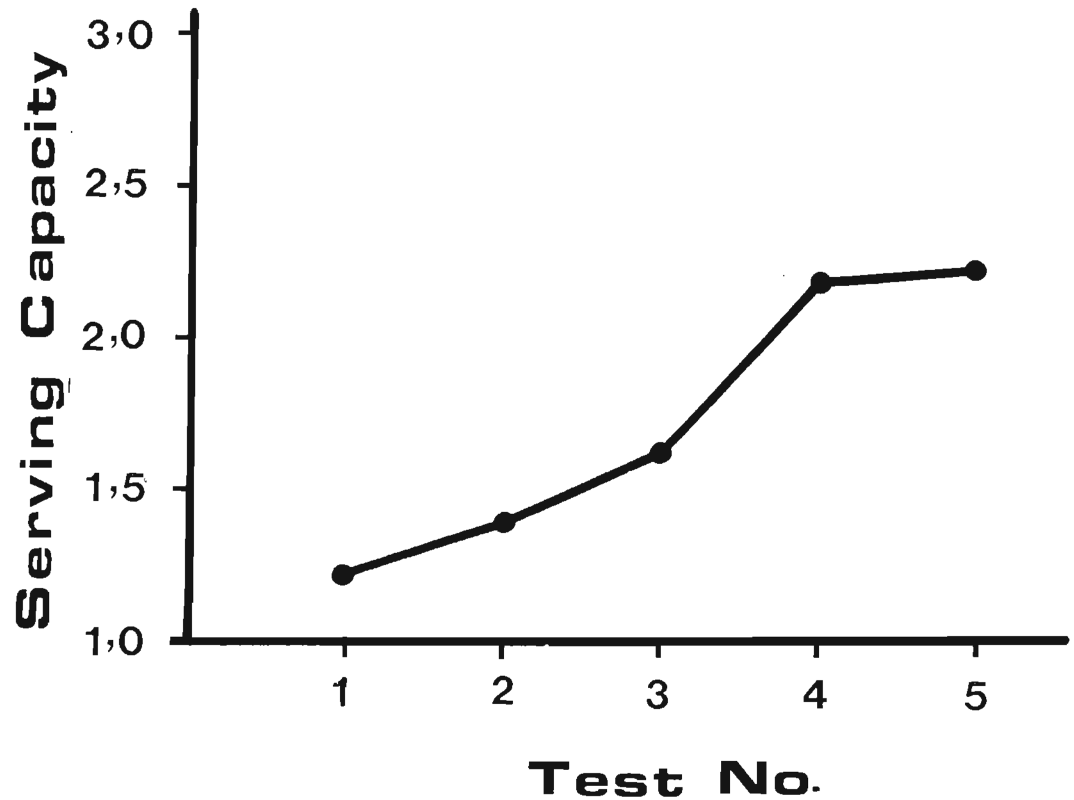

Figure 15 The improvement in serving capacity (SC) over five tests, expressed as the mean number of services completed in 30 minutes (n = 54)

The small number of bulls in some breeds limits the value of interbreed comparison (**Table 4**). A comparison of breed-types (**Table 4**) revealed that the British beef and dual purpose breeds achieved significantly higher SC scores than the zebu types ($P < 0,01$). The dual purpose types displayed a tendency to out-perform the British beef types ($P < 0,1$), no doubt a result of the poor performance of bulls from the Hereford breed (**Table 4**).

The breed x time interaction was significant ($P < 0,05$, **Table 3**). Thus all breeds of bull did not show a significant improvement in SC as their sexual experience increased. The data in **Table 5** shows the mean SC for each breed for each of the five SC tests.

Table 5 The mean number of services completed by each breed for each of the five SC tests.

		SERVING CAPACITY TEST *				
BREED	N	1	2	3	4	5
Sussex	5	$2,20^{a}$	$4,00^{b}$	$2,40^{ac}$	$2,60^{ac}$	$3,60^{bc}$
Red Angus	4	$0,75^{a}$	$2,25^{ac}$	$2,00^{a}$	$4,50^{b}$	$3,75^{bc}$
Hereford	9	$0,33^{a}$	$1,11^{ab}$	$1,56^{bc}$	$1,22^{bc}$	$2,22^{c}$
Simmentaler	14	$2,57^{ab}$	$2,07^{a}$	$2,50^{ab}$	$3,21^{b}$	$2,86^{ab}$
South Devon	4	$2,25^{a}$	$0,50^{b}$	$2,50^{a}$	$3,25^{a}$	$3,50^{a}$
Drakensberger	9	$0,44^{a}$	$0,22^{a}$	$1,00^{ab}$	$1,89^{b}$	$1,78^{b}$
Santa Gertrudis	2	$0,00^{a}$	$1,50^{a}$	$0,00^{a}$	$0,50^{a}$	$0,50^{a}$
Brahman	5	0,00	0,00	0,00	0,00	0,00
Afrikaner	2	0,00	0,00	0,00	0,00	0,00

* Means without common superscripts within breeds are significantly different ($P < 0,05$).

All breeds excluding the Santa Gertrudis, Brahman and Afrikaner breeds increased their SC with increasing sexual experience. In the Simmentaler and South Devon breeds the increase was not significant (**Table 5**).

Lane, Kiracofe, Craig and Schalles (1983) determined serving efficiency as:

$$E = \frac{\text{No. Services}}{\text{No. Mounts + No. Services}}$$

A bull's mating dexterity may be expressed in terms of E. In view of the effect of time/sexual experience on SC, E was expected to increase with time. Serving efficiency was determined for all breeds excluding the Brahman, Afrikaner and Santa Gertrudis. The data was analysed using a split-plot analysis. Breed had no effect on E. Sexual experience had a highly significant effect on E. As for SC, not all breeds improved their efficiency significantly with increasing experience (the breed x time interaction was significant, P<0,05).

The use of non-oestrous teaser females had no significant effect on SC (**Table 6**).

Table 6 The effect of oestrous versus non-oestrous teaser females on SC (scores are the breed type means for each test).

BREED TYPE[2]	N	TEST NUMBER[1] 1	2	3	4	5
British beef	18	$0,94^{a}$	$2,17^{ab}$	$1,89^{ab}$	$\boxed{2,33^{b}}$	$2,74^{b}$
Dual purpose	18	$2,50^{a}$	$1,72^{a}$	$\boxed{2,50^{ab}}$	$3,22^{b}$	$3,00^{ab}$
Zebu	18	$0,22^{a}$	$\boxed{0,28^{a}}$	$0,50^{a}$	$1,00^{a}$	$0,94^{a}$

[1] The non-oestrous tests were 4,3 and 2 for the British beef, dual purpose and zebu breed types respectively.

[2] Means within breed types which do not have common superscripts are significantly different, P<0,05.

Mounting activity was significantly stimulated by all forms of agonistic interaction (disrupted mounts, wins, losses and interactions with no definite outcome). Serving capacity was not significantly influenced by agonistic activity. The performance of the Simmentaler breed decreased markedly, though non-significantly, in tests two and five (**Table 5**). Although those decreases were non-significant a linear regression analysis of SC with all forms of agonistic interaction for the Simmentaler breed for each test revealed a significant negative relationship in test five ($P < 0,05$) and a strong tendency in test two ($P < 0,1$). The performance of one South Devon bull was decreased to one service in test two (down from six in test one) by continual harrassment from another bull which did not serve. This significantly decreased the breed average for test two (**Table 5**).

Environmental temperature (range 10,5 to 26,5°C) had no influence on SC.

An initial multiple regression analysis of SC as influenced by age (days), mass (kg), scrotal circumference (cm), height (cm), length (cm), sperm motility, percentage abnormal sperm, ADGRP (grams, ADG over winter and summer, a predominantly roughage diet) and ADGF (grams, ADG in the feedlot, a predominantly concentrate diet) enabled selection of the variates with most influence on SC. These variates were included in a further analysis. The variates were as follows (r - value in parenthesis), scrotal circumference (0,2946), ADGRP (0,2462), length (0,2416) and ADGF (-0,1558).

The F - value for the regression was significant ($P < 0,05$). The only partial regression co-efficient to have a significant t - value was that for ADGF ($P < 0,05$). The coefficient for scrotal circumference was almost significant at the 5 per cent level (**Table 7**).

Table 7 The partial regression co-efficients (B) arising from the regression of SC on scrotal circumference, ADGRP, length and ADGF.

VARIATE	B	SEB	T	LEVEL OF SIGNIFICANCE
Constant term	-0,1232534E+02	6,702	1,839	-
Scrotal circumference	0,2306036E+00	0,118	1,960	NS
Length	0,5336945E-01	0,049	1,082	NS
ADGRP	0,2157414E-02	0,003	0,830	NS
ADGF	-0,2442679E-02	0,001	2,581	5%

The regression of SC on ADGF and scrotal circumference was significant (P< 0,05). The partial regression co-efficients for ADGF and scrotal circumference were both significant (P< 0,05 and P< 0,01 respectively).

Investigation of the data revealed that all variates included in the model presented in **Table** 7, with the exception of length, varied with breed-type (**Table 8**). The variates ADGW (ADG over winter) and ADGS (ADG over summer) were included in subsequent analyses instead of in their combined form (ADGRP). The correlation between ADGW and ADGS was poor (r = 0,0936).

In order to take into account maturity-type within and between breeds growth rates were expressed as relative values (daily gain relative to body mass at the end of any given phase of feeding). By the same token scrotal circumference was expressed as circumference/unit body mass. Furthermore, it was decided to exclude the zebu bulls from subsequent analyses in the light of a possible observer/ environment effect and as a result of the obvious differences in SC between exotic and zebu bulls.

Table 8 The breed-type means for scrotal circumference, body length, ADGRP, ADGW, ADGS and ADGF for British Beef, dual purpose and zebu types, 1, 2 and 3 respectively

Variate	1 (n)	2 (n)	3 (n)	Level of significance
Scrotal Circumference (cm)	37,72 (18)	40,89 (18)	38,85 (17)	1vs2**, 1vs3^{NS}, 2vs3*
Length (cm)	148,61 (18)	151,67 (18)	147,89 (18)	ALL NS
ADGRP (gm)	654,56 (14)	814,89 (18)	689,83 (18)	1vs2**, 1vs3^{NS}, 2vs3**
ADGW (gm)	1182,86 (14)	1246,11 (18)	997,78 (18)	1vs2^{NS}, 1vs3**, 2vs3**
ADGS (gm)	202,14 (14)	398,89 (18)	381,67 (18)	1vs2**, 1vs3**, 2vs3^{NS}
ADGF (gm)	1445,00 (16)	1750,56 (18)	1804,44 (18)	1vs2**, 1vs3**, 2vs3^{NS}

$P < 0,05$ is denoted by *, $P < 0,01$ is denoted by **, and ^{NS} denotes non-significance

All the variates were included in a stepwise regression analysis and none of them had a significant influence on SC (British beef and dual purpose bulls combined).

Separate stepwise regressions were also carried out for the dual purpose and British beef breeds. None of the variates in either regression had a significant effect on SC. For the British beef breeds scrotal circumference ($r = 0,4051$) was selected as the variate having the most infuence on SC (it accounted for 16,4 per cent of the variation in SC). For the dual purpose bulls ADGF and height ($r = -0,3479$ and $r = -0,2693$ respectively) were selected. A multiple regression analysis for SC on ADGF and height was almost significant at the 5 per cent level (together ADGF and height accounted for 55,3 per cent of the variation in SC).

Total SC over the five tests was highly significantly correlated with the number of services completed during the libido score test ($r = 0,664$).

DISCUSSION

Effect of breed-type on sexual activity

The Brahman and Afrikaner breeds did not complete service at any time during the five SC tests despite a willingness to mount (bearing in mind the small numbers of Brahmans (five) and Afrikaners (two) included in the analysis). Two Afrikaner bulls, one in a "stimulator" group and one which did not complete any SC tests due to intestinal ulcers but which partook in the individual test, completed service.

Differences in libido between bulls representing B. indicus and B. taurus breeds are well documented (Chenoweth, 1975; Chenoweth & Osborne, 1975; Chenoweth, 1981). Indeed, Chenoweth (1981) states that "zebu bulls have been reported to exhibit marked sexual sluggishness and a tendency only to mount cows in full oestrus". Chenoweth & Osborne (1975) reported that individual Brahman bulls (16 to 31 months of age) achieved libido scores equal to the best of other breeds (Afrikaner, Hereford and various crossbreeds). It was suggested that selection against deficient or delayed libido could improve reproductive performance in this breed.

The possibility that the zebu bulls in this study could still have been undergoing pubertal changes has been suggested. In work carried out by Fields, Burns & Warnick (1979) on Angus, Hereford, Brahman and Santa Gertrudis bulls from the age of 16 until the age of 20 months, Brahman and Santa Gertrudis bulls showing marked improvement in testes size and semen quality traits. More recently however, Fields, Hentges & Cornelisse (1982) reported that Brahman bulls reached puberty at 16 months of age. Puberty being defined as the developmental stage at which the ejaculate contained a minimum of 50×10^6 sperm with 10% motility. In the earlier study (Fields et al., 1979) the bulls had already achieved these minima at the start of the study. Work done by Post, Christensen & Seifert (1987) indicates that sexual behaviour in crossbred zebu bulls is repeatable at 24 and 48 months of age. Furthermore, the response of these bulls to GnRH, measured in terms of testosterone response over time, was also repeatable at 17 and 44 months of age. Thus, lack of sexual maturity is unlikely to have been a factor influencing sexual behaviour in the present study.

Houpt & Wolski (1982) are of the opinion that as a result of domestication, the aim of which is to select for easy breeders, bulls (raised in single sex groups) respond to a less specific series of sexual stimuli than those offered by an oestrous female. Exotic breeds seem more likely to mount inappropriate sexual objects than zebu breeds, an indication that the former breeds have a lower sexual threshold.

The poor performance of bulls from the Hereford breed can only be speculated upon. Their performance was with exception of one bull (average SC of 5,8 for five tests) disappointing.

Cade (1984) presented evidence in opposition to the theory that traits which determine fitness (specifically those related to reproduction and survival) have negligible genetic variation. The extremes in SC reported in the present and other studies (Blockey, 1981 a & c; Lunstra, 1980) lend support for Cade (1984) who maintained that reduction of additive genetic variance as a result of selection in a polygenic system will be slow and that variance should be maintained at a certain level in large populations. Furthermore, prediction of the reduction of variation assumes that selection remains at a constant level. However, this is not the case. The gene pool for the Hereford breed in South Africa is considered large enough to prevent such reduction in the additive genetic variation underlying reproductive traits (Paterson, personal communication). Furthermore, nine Hereford bulls from two studs can hardly be considered representative of the breed as a whole.

Observer/environment effect

That there are obvious breed-type differences cannot be disputed. However, in view of the Afrikaner and Brahman bulls' willingness to mount (the Afrikaner displayed the highest level of mounting activity across all breeds) it may be speculated that copulation did not occur or occurred erratically in the case of the Santa Gertrudis bulls as a result of an observer/environment effect. Males are more readily disturbed by environmental factors than females (Houpt & Wolski, 1982). Thus zebu bulls with their higher sexual threshold are more likely to be disturbed than are exotic bulls. The zebu bulls certainly seemed to be more aware of the observer than were other bulls.

Therefore, it would appear as if the Brahman and Afrikaner bulls overcame any inhibitions to the extent that they mounted, but stopped short of service. The erratic scores achieved by the two Santa Gertrudis bulls is perhaps indicative of their incomplete adaption to the test situation.

Element of learning in sexual behaviour

The significant increase in SC and E over the first four SC tests reinforces evidence for an element of learning in sexual behaviour. Lunstra (1980) and Lane et al., (1983) presented evidence for learning sexual behaviour in yearling beef bulls while Blockey (1981c) reported that the majority of 20 month-old bulls displayed their inherent SC on their first test.

Perhaps a distinction needs to be drawn between peak sexual activity and the need for bulls to overcome their initial inexperience and achieve a level of performance which is consistent relative to their conspecifics, particularly in the light of the high costs of testing.

Most breeds showed a gradual and consistent increase in SC over the first three tests (**Table 5**) which implied that the bulls underwent the learning process at a similar rate maintaining their rank relative to one another with few obvious exceptions. An examination of the correlation coefficients (Brahmans, Afrikaners and Santa Gertrudis excluded) for SC tests 1 versus 2, 2 versus 3, 3 versus 4, and 4 versus 5 (0,55; 0,69; 0,71 and 0,76 respectively, all $P < 0,01$) lends support to this view.

Blockey (1981c) stated that the successful testing of virgin bulls depends on proper sexual stimulation prior to testing. In the present experiment bulls observed sexual activity through a split-pole fence adjacent to the test area. Stimulation should have been adequate. Bulls became conditioned to the test situation and (in subsequent tests) were highly excited on arrival at the holding area.

Vigorous agonistic activity often occurred.

Based on the data from this experiment it is apparent that bulls need at least two, and in some cases three, tests before their SC relative to other bulls became stabilised.

The significant breed x time/sexual experience interaction (**Table 3**) was no doubt due largely to those zebu breeds which did not serve or served inconsistently and to a lesser extent to the non-significant increase displayed by the dual purpose bulls.

The use of oestrous versus non-oestrous teaser females

The results of this study (**Table 6**) confirm those from other studies (Chenoweth et al., 1979; Blockey, 1981b) which show that sexual behaviour is not influenced detrimentally by the use of non-oestrous teaser females.

Kiddy, Mitchell & Hawk (1984) demonstrated the existence of oestrous-related odours in vaginal fluids, vulvular and vestibular swabs, urine, milk and blood plasma. Jacobs, Sis, Chenoweth, Klemm, Sherry & Coppock (1980) highlighted the mechanism whereby urine or vaginal secretions are introduced into the vomeronasal organ just prior to Flehmen, the incidence of which increases sharply over oestrous (Hradecky, Sis & Klemm, 1983). On the other hand Nasholds & Simmons (1982) concluded that if oestrous urine did contain a pheromone, bulls could not identify it with a high degree of success.

Most other authors (Blockey, 1976, 1981c; Chenoweth, 1981, 1982, 1983) are of the opinion that visual cues are more important than olfactory in the detection of oestrus. Detection occurs mainly by means of the bulls' observation of mounting activity within the restless, mobile, sexually active group (SAG, Williamson, Morris, Blood, Cannon & Wright, 1972). The bull investigates cows within the SAG testing them for receptivity (Chenoweth, 1981). Immobility of the cow's hindquarters is the most important stimulus causing bulls to mount and serve a cow (Blockey, 1981b).

When only a few cows are on heat, proportionately more time is spent in olfactory investigation, while when more cows are on heat the SAG forms and visual cues become more important (Blockey, 1976).

The advantage of using non-oestrous teaser females is that induction of "standing" oestrus in sufficient numbers of females at the right time cannot readily be achieved and that ovariectomised females are not readily available (Blockey, 1981b). Thus non-oestrous females, properly restrained and presented, greatly facilitate ease of testing (Chenoweth <u>et al</u>., 1979). However, it is felt that wherever possible oestrous teaser females should be used since a non-oestrous cow is likely to be less co-operative and to offer more resistance to penile entry than are oestrous females which are physiologically and phsycologically prepared for sexual activity. Such considerations may be more important when testing zebu bulls which are more sensitive to environmental stimuli than are other breeds.

Effect of social interaction on sexual activity

Lane <u>et al</u>. (1983) reported that interference (whether as the aggressor or defendant) was positively correlated with the frequency with which bulls mounted heifers. The results from the present study confirm this.

Spacing between teaser heifers is one of the factors which could influence the level of agonistic activity and therefore bull performance. Lane <u>et al</u>. (1983) spaced heifers at 2 m intervals and found that individual evaluation was more efficient than group evaluation. Furthermore, they found that E was significantly negative correlated with being interfered with. Lunstra (1981) spaced heifers approximately 4 m apart and reported group evaluation to be more efficient than individual. In the present study heifers were spaced at least 4 m apart. Blockey (1981b & c) spaced cows approximately 5 m apart.

Blockey (1979) reported that the social dominance order (SDO) in bull groups of mixed age was stable and closely related to sexual activity. The opposite was true for groups of two year old bulls.

Ologun <u>et al</u>. (1981) reported that dominance value in yearling bulls (the ratio of individuals dominated to the total encountered) was negatively correlated with libido score and SC ($P<0,05$). However, they concluded that caution was necessary in the interpretation of their data since it was possible that the social heirarchies observed were weak and short-lived.

In the present study the extent to which bulls engaged in agonistic activity did not depress SC significantly. However, the fact that SC for the Simmentaler breed was significantly negatively correlated with all forms of agonism in test five and was almost significantly correlated in test two suggests that social interaction may have limited the full expression of sexual behaviour by some bulls and no doubt contributed to this breed's non significant increase in SC over time. The dual purpose breeds engaged in significantly higher levels of agonistic activity than other breed types. The low proportion of encounters won, tends to support the idea of the SDO being unstable in young bulls.

Influence of growth rate, scrotal circumference and physical size on sexual behaviour

Hentges (1973) reported that excessive fatness following an extended period of concentrate feeding impaired libido in young bulls. Ologun et al. (1981) found a significant negative correlation between SC and ADG in the feedlot. Makarechian, Mahone & Maynard (1983), however, reported no consistent relationship between libido and pre- and post-weaning gains in bulls.

In the present study growth rate did not affect SC significantly nor consistently. Average daily gain over winter was positively correlated with SC while ADGS and ADGF were negatively correlated. The performance of some bulls was not consistent over all three phases of feeding. This was obviously the result of environmental factors. In the dual purpose bulls differences in ADGF could predict 12,1 per cent of the variation in SC. Further investigation under carefully controlled conditions is indicated. Paterson (personal communication) has suggested that research at a more basic level is called for since an attempt is being made to relate one complex hormonal system (libido) to another (growth) by measuring libido and growth per sé; a relationship confused by environmental influences.

Some of the larger framed Simmentaler bulls appeared to experience difficulty serving some of the smaller heifers. Shoulder height was identified as being one of the more important variates influencing SC in dual purpose bulls. The importance of using teaser females of adequate size is stressed.

Scrotal circumference accounted for 16,4 per cent of the variation in SC in the British beef breeds. A belief popularly held is that scrotal circumference is positively related to libido, testicular size being associated with ability to produce testosterone (Wildeus, Entwistle & Holroyd, 1984). However, testosterone levels per sé do not determine differences in libido in the bull (Blockey & Galloway, 1978; Chenoweth et al., 1979). The relative important of scrotal circumference as a factor in determining difference in SC among British beef bulls was thus confusing. The fact that Hereford bulls had smaller testicles than the other British beef breeds and at the same time had the lowest SC would appear to explain the discrepancy.

CHAPTER 3

FACTORS INFLUENCING SEXUAL BEHAVIOUR OF YOUNG <u>BOS INDICUS</u> BULLS UNDER PEN AND PASTURE MATING CONDITIONS

INTRODUCTION

Although <u>B. indicus</u> breeds have been used widely in cross-breeding programs some of these breeds have a record of sub-fertility relative to that in <u>B. taurus</u> breeds (Seebeck, 1973). Thus Wells (1986) maintained that Afrikaner cow-herds seldom achieve conception rates above 65% in a 90-day breeding season and van Niekerk, Lishman & Lesch (1985) obtained between 5 and 10% fewer calves from Afrikaner cows than from Simmentaler cows even when the breeds were compared under an environment to which Afrikaners are said to be well adapted. Similarly, Brahman cross genotypes developed in Australia display a reproductive performance which is in some cases lower than <u>B. taurus</u> breeds (Seebeck, 1973) and a male contribution to lowered fertility has been suggested. The latter might be explained by the finding that <u>B. indicus</u> bulls have less sperm per gram of testicular tissue and a lower sperm production potential than <u>B. taurus</u> bulls (Entwistle, Winantea & Holroyd, 1980). Since bull libido is also a factor influencing fertility (Blockey, 1978) <u>B. indicus</u> bulls are at a further disadvantage because they display marked sexual sluggishness in pen libido tests (Chenoweth, 1981; confirmed in Chapter 2). There is perhaps a need to modify accepted pen tests since Chenoweth (1981) suggested that <u>B. indicus</u> bulls prefer to mount cows which are in full oestrus, and Price (1985) speculated that the use of unrestrained oestrous females is preferred when conducting pen libido tests with <u>B. indicus</u> bulls. Furthermore, in the tests conducted in Chapter 2 the <u>B. indicus</u> bulls often appeared to be more aware of the observer and/or aggressive threats made by other bulls than of the oestrous females.

Commercial producers believe that *B. indicus* bulls are more likely to engage in sexual behaviour during the hours of darkness. Such a belief, while apparently unsubstantiated and based largely on casual observation, cannot be ignored particularly when the reproductive characteristics of *B. indicus* females are considered. Oestrous behaviour is less intense (Dobson & Kamonpatona, 1986), of shorter duration (Baker, 1969) and more silent ovulations are noted (van der Westhuysen, 1972) than in *B. taurus* cows.

In the light of the differences between *B. taurus* and *B. indicus* females it may be speculated that in the ontogeny of the latter breed little emphasis has been placed on fertility in the male thereby leading to sub-optimal fertility.

The aim of the present study was to investigate whether *B. indicus* bulls mate more frequently at night than during the day, to determine the effect of the presence or absence of an observer on performance during the pen test (Blockey, 1981c) and to determine the extent to which the pen test is able to predict sexual behaviour under more natural conditions in *B. indicus* bulls.

PROCEDURE

Animals

The trial was conducted using 18 heterosexually inexperienced Afrikaner stud bulls with a mean age of 601,7 ± 9,3 days. Bulls were raised primarily on natural grazing (Northern Tall Grassveld, Acocks, 1975) during summer and maize residues with limited maize silage during winter. Their mean mass and scrotal circumference were 351,0 ± 7,4 kg and 31,2 ± 0,4 cm respectively.

Night versus Day

The effect of night or day on sexual behaviour was evaluated over eight serving capacity tests (Blockey, 1981c) in a cross-over design. Bulls were subdivided into six groups of three, blocked for body mass with three groups being allocated to each treatment (night or day). Bulls were tested under conditions of either night or day for four serving capacity tests following which treatments were switched for a further four tests. Each group of three bulls was exposed to two restrained, ovariectomized, oestrous-induced (Lunstra, 1980) cows for 30 minutes following a minimum of 10 minutes sexual stimulation. Cows were mildly sedated[11] and were replaced by fresh cows once they refused to remain immobile during mating. The frequency of mounts (without ejaculation) and completed services was recorded by means of a tape recorder.

For the purpose of night tests the bulls were marked with luminous paint numbers. The position of bulls relative to one another and the teaser females was ascertained at the start of a test using a flashlight. Thereafter, use of the flashlight was kept to a minimum to avoid disturbing the bulls excessively. Tests (both night and day) were conducted with the observer located in a hide near the pen.

Observer versus no observer

The effect of the presence or absence of an observer was evaluated over four serving capacity tests, these tests followed the initial series of eight tests. Bulls were reallocated to six groups in accordance with their mass and serving capacity (mean for the first eight tests). Treatments were switched alternatively in a cross-over design. Test procedure was the same as for the first eight tests. During the "no observer" tests personnel hid in a hut on one side of the pen. In addition to a record of sexual behaviour, agonistic interactions were also noted. The following agonistic activities were recorded, disrupted mounts, encounters won or lost and encounters with no out-

[11] Rompun, Bayer

come. Encounters usually took the form of physical contact but could take the form of threats with no physical interaction.

Natural mating

Following termination of the pen tests a subsample of eight bulls was selected for evaluation under conditions of natural mating. Bulls were selected on the basis of their mean serving capacity over the 12 pen tests. The four "high" bulls achieved a mean serving capacity of 2,0 ± 0,5 services while the four "low" bulls achieved 0,3 ± 0,1 services.

A group of 72 females (heifers and dry cows) was used during this phase of the trial. Oestrus was induced by administering a single injection of prostaglandin[12]. The trial began 48 hours after injection and was terminated 72 hours later. The females were kept in a single group separate from the bulls with oestrus detection being carried out between 04h30 and 18h30 daily. The recording of sexual behaviour amongst the bulls was carried out during two periods each day. The first period began at 05h00 with the allocation of oestrous females and ended at 10h00. The second period began at 16h00 (fresh oestrous females allocated) and ended at 19h00. Once allocated, a female remained with a particular bull until conclusion of the trial.

In order to facilitate observation, this phase of the trial was spread over two weeks with four bulls being evaluated in each week. Thus, the females to be synchronised were subdivided into two groups of 36 each. Bulls were evaluated as single-sires in four adjacent paddocks. The number of mounts (without ejaculation) and services were recorded for each bull.

Within a given observation period oestrous females were allocated to bulls on a random basis. Oestrous females were not always available in sufficient numbers to allow simultaneous allotment to bulls at the start of an observation period. Furthermore, bulls were not necessarily exposed to a fresh oestrous female in every observation period. This led to some bulls being mated to more females than others and the mating pressure varied between four and seven cows over three days. With bulls not being exposed to a fresh oestrous female in every period a bull's previous activity could have influenced his performance at any time. Previous activity was quantified as follows:-

1 = bull exposed to no female on heat or showed no sexual behaviour.

2 = bull exposed to previously mated female at the end of her oestrus.

3 = bull exposed to a fresh female at the start of her oestrus.

A bull's previous activity was included as a variate in the analysis of data.

Oestrous females showed varying degrees of receptiveness (in spite of being prepared to stand for other females). Cow receptivity was quantified as follows:-

1 = female at the end of her heat, previously mated, willing to stand.

2 = female at the start of her heat, partially receptive, stood when clasped or female at the end of her heat, not previously mated, but partially receptive.

3 = female freshly on heat willing to stand.

Cow receptivity was also included as a variate in the analysis of data.

Following completion of the pasture mating, females were joined with Sussex bulls which were fitted with chinball harnesses and were checked daily for signs of oestrus and paint marks. Conception rates were evaluated by means of returns to service and confirmed following calving on the basis of date of calving and breed characteristics.

Statistical analysis

The night/daylight and observer/no observer evaluations were analysed by means of analysis of variance for a cross-over design (Genstat V Mark 4,03 C, 1980. Lawes Agricultural Trust, Rothamsted Experimental Station). The effect of agonistic behaviour on serving capacity was analysed by means of correlation techniques (Rayner, 1967). Data from the evaluation of sexual behaviour under natural mating conditions were subjected to multiple regression analysis (Genstat V Mark 4,04B 1984). Services per 100 minutes (Y), cow receptivity (X_1) bulls previous activity (X_2), duration of exposure (X_3) and serving capacity (X_4, expressed in terms of 0 = high and 1 = low serving capacity) were included as variables in the model.

The qualitative nature of the variates X_1 and X_2 necessitated the creation of dummy variates which served to establish whether or not the relationship between Y and X_3 and X_4 changed for the various combinations of X_1 and X_2 (**Table** 9). There were nine possible combinations of X_1 and X_2, two of which were excluded from analysis (no observations were recorded for these two combinations).

Table 9 The seven combinations of variates X_1 (cow receptivity) and X_2 (bulls previous activity) used in the multiple regression analysis

	Variate	
Combination	X_1	X_2
1	1	3
2	2	1
3	2	2
4	2	3
5	3	1
6	3	2
7	3	3

RESULTS

Neither time of day (night versus daytime) nor the presence of an observer had any measurable effect on the serving capacity of the bulls (**Table 10**) and data were pooled for further analysis.

Table 10 Treatment means for mounting (without ejaculation) and serving behaviour recorded during the night/ daytime and observer/no observer comparisons[1]

	Night versus Day		Observer versus No observer	
Sexual Behaviour	Night	Day	Observer	No observer
Mounts	12,360	11,510	12,560	11,580
Services	0,694	0,708	0,917	0,861

[1] All differences between means non-significant

The bulls did not serve consistently from one test to another (**Figure 16**). On average only 43,05 ± 1,69% of the bulls served in any test. Seven bulls (38,9%) served in less than four tests, 27,8% (n=5) served in between five to eight tests and 33,3% (n=6) served in nine to twelve tests. Only one bull served in all the tests, while two bulls failed to serve in any of the tests.

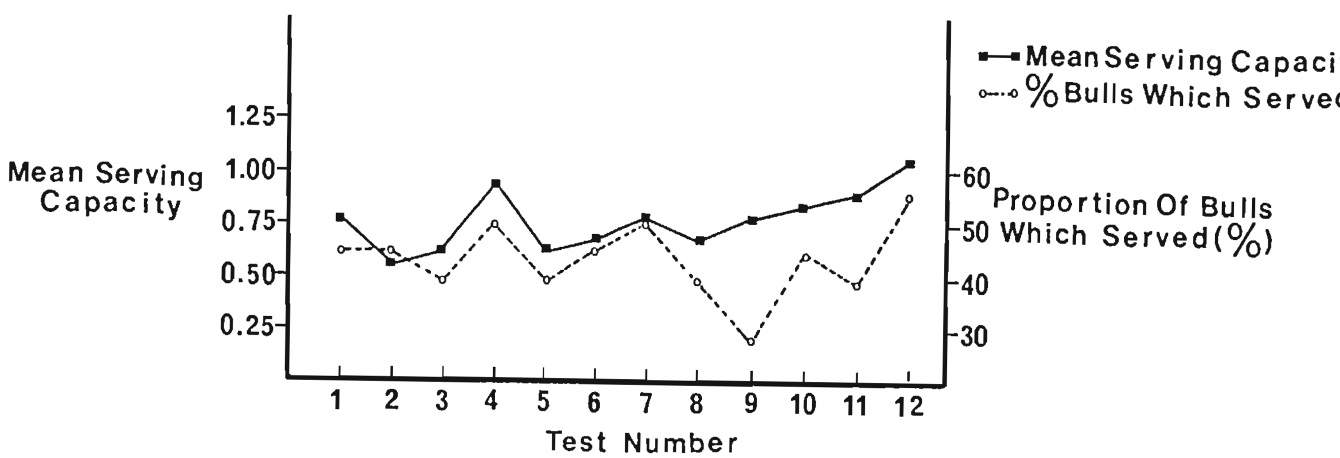

Figure 16 The mean serving capacity and proportion of bulls which served over the 12 serving capacity tests

Despite some marked fluctuations, serving capacity increased significantly (r=0,59; P < 0,05) with increasing number of exposures to oestrous females. Fluctuations in serving capacity tended to be associated with changes in the proportion of bulls mating (**Figure 16**). A notable exception was in test nine where the smallest proportion (27,8%) of bulls served. This test was the first of the last four (observer versus no observer) tests and bulls had been allocated to new groups. Re-allocation appears to have stimulated agonistic behaviour and 35,8% of all such interactions recorded during the last four tests occurred during test nine. This undoubtedly led to the reduction in the proportion of bulls serving. However, mean serving capacity for test nine was not similarly depressed because one bull doubled his serving capacity (two services in test eight versus four in test nine) while another bull increased from 0 to 2 services.

The number of interactions won, expressed as a proportion of the number of interactions in any given test (percentage wins), had a low positive effect on serving capacity ($r=0,19$; $P<0,10$). The number of mounts (without ejaculation) completed by a given bull was positively associated with percentage wins ($r=0,28$; $P<0,02$). The number of occasions on which mounting behaviour was disrupted had a negative effect on serving capacity ($r=-0,28$, $P<0,02$). In a number of groups a clear dominance order emerged. In most cases two of the bulls effectively gained access to the two cows, thereby excluding the third bull. Furthermore, bulls tended to concentrate on individual cows.

Under pasture mating conditions the bulls with high serving capacity displayed a higher level of serving behaviour ($P<0,05$) and a lower, non-significantly different, level of mounting behaviour than low serving capacity bulls (**Figure 17**). Thus high serving capacity bulls showed a superior but non-significantly different, degree of serving efficiency (Lane, Kiracofe, Craig & Schalles, 1983) when compared with low serving capacity bulls (**Figure 17**). The higher level of mounting behaviour and poorer serving efficiency of the low serving capacity bulls may be largely attributed to two bulls, which displayed poor sexual technique. At times, these two bulls displayed high levels of mounting behaviour (between 21 and 25 mounts/100 minutes), but ejaculated infrequently.

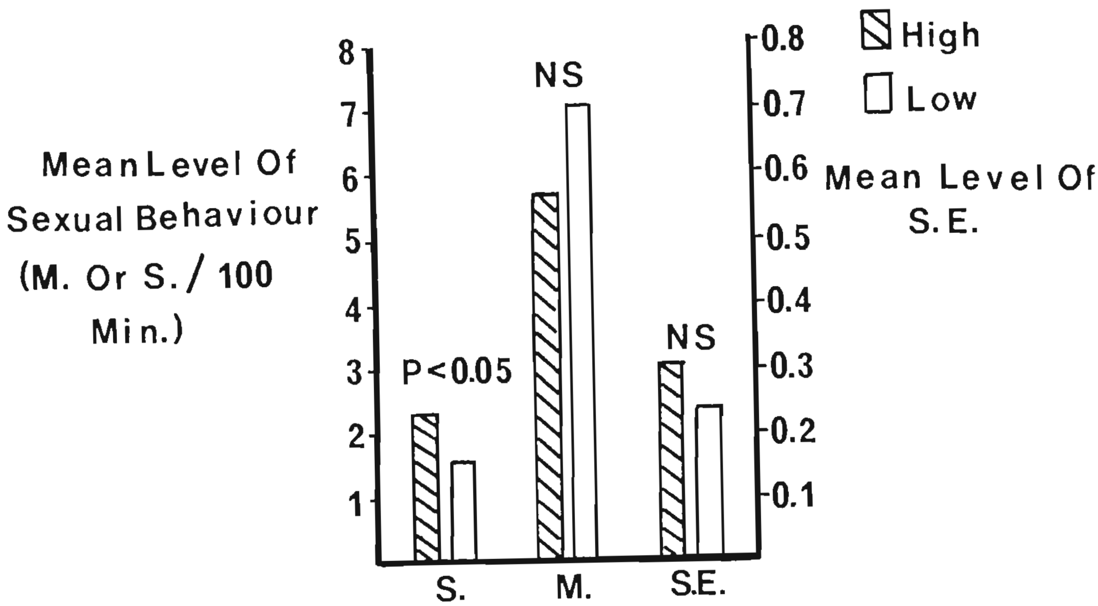

Figure 17 Mean serving (S) and mounting (M) response per 100 minutes and level of serving efficiency (SE) for high and low serving capacity bulls under pasture mating conditions

As each pen test was completed the results were combined with those from previous tests and a mean score was calculated. The relationship between means calculated in this way and the mean number of services completed per 100 minutes of pasture mating was tested. Following seven tests a significant association was obtained (Table 11).

Table 11 The relationship between mean number of services/100 minutes of pasture mating (y), and mean serving capacity (x) with increasing number of serving capacity tests[1]

	PEN TEST NUMBER											
	1	2	3	4	5	6	7	8	9	10	11	12
r	0,450 [a]	0,592 [a]	0,637 [b]	0,665 [b]	0,630 [b]	0,693 [b]	0,718 [c]	0,718 [c]	0,737 [c]	0,721 [c]	0,712 [c]	0,677 [b]
a	1,747	1,547	1,522	1,492	1,521	1,464	1,465	1,474	1,470	1,491	1,513	1,521
b	0,176	0,393	0,431	0,430	0,416	0,495	0,497	0,477	0,479	0,463	0,445	0,403
r^2	20,250	35,046	40,577	44,222	39,690	48,025	51,552	50,694	54,317	51,984	50,694	45,833

1 Mean serving capacity for individual bulls (n=8) derived on the basis of scores obtained in preceding tests

a Not significant

b P < 0,10

P < 0,05

The multiple regression analysis accounted for 66,9% ($P < 0,01$) of the variation in serving activity under pasture mating conditions. Duration of exposure (X_3) had a significant negative effect on services/100 minutes (Partial regression co-efficient = -0,486; $P < 0,05$). The bulls showed their highest rate of work during the first hour in which they were exposed to a fresh oestrous female. Consequently, bulls with a shorter duration of exposure (females were not necessarily allocated to all bulls at the start of a given observation period) achieved more services per unit time. Serving capacity (X_4), coded 0 for high and 1 for low serving capacity, also contributed significantly to the regression equation (Partial regression co-efficient = -83,3; $P < 0,001$). Arising from the analysis were seven equations, one for each possible combination of X_1 and X_2, expressed in terms of Y and X_3 and X_4 (**Table 12**). The significance of these regression equations was measured relative to the seventh combination of X_1 and X_2 (**Table 9**). It is evident (**Table 12**) that combination 5 represented the most ideal and combination 1 the least favourable mating situation.

Table 12 The multiple regression equations for each of the seven combinations of X_1 and X_2 expressed in terms of Y and X_3 and X_4[1]

Combination	Regression Equation	Level of significance
1	$Y = (369,3 - 160,2) - 0,486\ X_3 - 83,3\ X_4$	***
2	$Y = (369,3 + 47,8) - 0,486\ X_3 - 83,3\ X_4$	NS
3	$Y = (369,3 - 148,2) - 0,486\ X_3 - 83,3\ X_4$	**
4	$Y = (369,3 - 116,4) - 0,486\ X_3 - 83,3\ X_4$	**
5	$Y = (369,3 + 92,9) - 0,486\ X_3 - 83,3\ X_4$	*
6	$Y = (369,3 - 0,2) - 0,486\ X_3 - 83,3\ X_4$	NS
7	$Y = 369,3 - 0,486\ X_3 - 83,3\ X_4$	-

[1] *** denotes $P<0,001$; ** denotes $P<0,01$; * denotes $P<0,05$; NS denotes not significant. In each case significance was measured relative to combination 7

The high serving capacity bulls achieved a mean conception rate of 67,1 ± 5,3%, while the low serving capacity bulls impregnated 56,3 ± 16,0% of their cows (Table 13).

Table 13 The conception rates achieved by low and high serving capacity bulls during the pasture mating period of evaluation

	SERVING CAPACITY							
	LOW				HIGH			
Bull ID	12	19	18	13	2	4	11	3
Conception rate (%)	57,1	42,9	100,0	25,0	71,4	57,1	80,0	60,0
Number of cows conceiving	4	3	4	1	5	4	4	3
Number of cows mated	7	7	4	4	7	7	5	5
Mean conception[1] rate (%) ± S.E.	56,3 ± 16,0				67,1 ± 5,3			

[1] Means not significantly different

The 100% conception achieved by bull No. 18 was surprising since this bull showed poor mating technique which was associated with a low level of serving behaviour.

DISCUSSION

Nocturnal sexual behaviour

There was no evidence to support the popularly held belief that <u>B. indicus</u> bulls mate with greater frequency at night than during daylight hours. Should more <u>B. indicus</u> cows conceive as a result of nocturnal sexual behaviour, it is probably the result of cows coming into oestrus at night, rather than enhanced bull activity, a possibility suggested by Wells (1986). Furthermore, the low incidence of homosexual and heterosexual behaviour associated with oestrus in Afrikaner cows (Wells, 1986) could readily lead the casual observer to believe that sexual behaviour occurs exclusively at night.

Effect of an observer

The absence of any detrimental effect of the presence of an observer (if such an effect exists) suggests that bulls used in this study were accustomed to the presence of people. In the period leading up to the pen tests bulls were mustered daily for the purposes of feeding. At the termination of their pen tests certain bulls showed a reluctance to leave the test area, despite attempts to remove them.

Nature of the stimulus offered by restrained oestrous females

Bulls which performed inconsistently or did not serve at all during the pen tests did not necessarily continue to perform poorly under pasture mating conditions. This is indicated by the magnitude of the constant term (a) in the regression equations for the relationship between pasture and pen mating (**Table 11**). Thus a bull with a pen test score of zero is capable of attaining a score of approximately 1,5 serves /100 minutes under pasture conditions. Clearly then, the teaser females used in this study did not represent an ideal stimulus for bulls. Garcia, McDonnell, Kenny & Osborne (1986) have recently demonstrated that in restrained cows the stage of oestrus, relative to ovulation, had bearing on the behavioural response elicited in Santa Gertrudis bulls. Cows which were at the pre-ovulatory stage of oestrus offered a better stimulus than post-ovulatory cows. This is contrary to the finding of Blockey (1981c) who worked with B. taurus bulls and to those in Chapter 2, where both B. taurus and B. indicus bulls were tested. In both cases adequately restrained non-oestrous cows elicited the same behavioural response, in bulls, as oestrous cows. However, no attempt was made to differentiate between ovulatory status and cows were utilised as teasers when they displayed "standing" oestrous. While the serving behaviour of the _B. indicus_ bulls in Chapter 2 appeared to be unhindered by the use of non-oestrous teasers, the low level of serving behaviour recorded for these bulls and the high proportion (55,6%) of bulls which did not serve may have limited the value of the oestrous/non-oestrous comparison. Garcia _et al_. (1986) speculated, as did Chenoweth (1981), that _B. indicus_ bulls are more discriminating with respect to stage of oestrus than are _B. taurus_ bulls.

Adaption to pen test conditions

The significant improvement in serving capacity, with the progression of tests, can be attributed to a few bulls which showed a gradual improvement with increasing number of exposures and to some bulls which began serving, although erratically, at a relatively later stage. The improvement in serving capacity suggests some form of adaption to the test situation as well as improved copulatory technique. This improvement in serving behaviour occurred more gradually and was not as dramatic as the change observed in Chapter 2 which may indicate breed-type differences in rate of learning/adaptation.

The detrimental effects of agonistic behaviour

Clearly, agonistic behaviour exerted a detrimental effect on the success of sexual behaviour. Blockey (1979) studied _B. taurus_ bulls and found that agonistic behaviour did not influence serving capacity in two year-old bulls since such bulls did not have a stable social dominance order. The results obtained in Chapter 2 largely confirmed these findings.

It is possible therefore that the bulls in the present study could have been forced to establish a social structure during the period of feeding just prior to the serving capacity tests when trough space and/or silage may have been limiting. A social dominance order develops more slowly in young animals which are kept in intact peer groups where resources are not limited (Craig, 1986). However, where feed was limiting, social rank influenced weight gain in 15-19½ month-old Hereford bulls (Blockey & Lade, 1974). A further consideration could be that *B. indicus* bulls with their higher threshold for sexual behaviour (Houpt & Wolski, 1982) are more readily put off by agonistic behaviour than are *B. taurus* breeds suggesting that *B. indicus* bulls would be tested more effectively in a single-sire situation.

The relationship between pen and pasture mating behaviour

The significant association between serving capacity and pasture mating score was obtained in spite of the inconsistent performance of bulls in the pen tests and the depressive effect of agonistic behaviour. Following pasture mating, the bulls remained within either the high or low serving capacity groups with the exception of one animal which moved into the high scoring category displacing the lowest rank bull within this group. This bull did not serve in any but the last pen test. Chenoweth (1975) referred to bulls, particularly *B. indicus* bulls, which show temporary psychogenic or central nervous inhibition of libido and commence copulatory behaviour unpredictably at a later stage.

Serving capacity and conception rate

The 10,8%, though non-significant, advantage in conception rate recorded for the high serving capacity bulls was unexpected in view of the relatively small (0,78 services/ 100 minutes), although significant, advantage maintained by the high serving capacity bulls during pasture mating and the small numbers of bulls and cows evaluated. The mating load of between four and seven oestrous females over three days is similar to that in Chapter 4, where bulls, mated as single-sires, covered between 35 and 40 cows over 21 days, and should therefore be considered realistic. While the beneficial effect of high serving capacity on conception rates has been clearly demonstrated by a number of workers (Blockey, 1978; Lunstra, 1980; Birkner, Garcia, Vincent, Alberio & Butler, 1984), others have reported little or no effect (Makarechian & Farid, 1985; Makarechian, Farid & Berg, 1985; results Chapter 4). The aforementioned trials were carried out using B. taurus bulls and only limited information on the relationship in B. indicus bulls exists. Christensen, Seifert & Post (1982) could find no positive relationship, whereas Smith, Morris, Amoss, Parish, Williams & Wiltbank (1981) and Post, Christensen & Seifert (1987) concluded that mating behaviour assessed under field rather than pen conditions provided a useful estimate of bull performance. In the present study, bulls were selected on the basis of a large number of pen tests (most other studies used one or two such tests). This meant that successive tests resulted in an improved mean estimate of serving capacity (**Table 11**). These data suggest that a minimum of seven tests is needed for B. indicus bulls, which is hardly practical for routine testing of serving capacity.

Use of unrestrained teaser females

The inconsistent serving behaviour of B. indicus bulls during pen tests suggests that unrestrained oestrous cows are to be preferred. The problem which presents itself in such a test is the difficulty of selecting cows which are properly receptive to the bull. Degree of cow receptivity clearly influenced bull behaviour (**Table 12**). Thus large numbers of intact females are necessary to evaluate sexual behaviour under pasture conditions since in the present study females did not always come on heat in sufficent numbers to ensure that each bull received a fresh oestrous female at the start of, or during, every observation period.

Since this criterion could not be met in the present trial an attempt was made to allow for "bull's previous activity" and "duration of exposure" by the inclusion of dummy variates in the multiple regression analysis (**Table 12**). Continuous observation represents another alternative but is clearly costly in terms of manpower needs.

The findings of Garcia et al. (1986) regarding the differences between B. taurus and B. indicus bulls further complicated the conduct of sexual behaviour tests. Clearly the specific characteristics which make oestrous females attractive to B. indicus bulls need to be identified before pen tests can be used effectively to measure serving capacity in these breeds.

CHAPTER 4

THE RELATIONSHIP BETWEEN BULL LIBIDO AND FERTILITY

INTRODUCTION

Under conditions of natural mating the bull must ensure that every cow capable of producing a calf does so. In order to fertilize an acceptable number of females the bull must produce sufficient semen of good quality, possess a high level of sex-drive to seek out receptive females which are then mated with a high degree of expertise. According to Blockey (1980) farmers mate more bulls per 100 females than is necessary to ensure good herd fertility. Thus by identifying high libido bulls the producer can effectively reduce bull costs per calf and obtain more calves earlier in the season (and therefore improved weaning masses) as a result of higher first oestrus conception rates (Blockey, 1978).

The ability of the SC test (bulls tested in groups) to predict veld mating performance and therefore conception rate (CR) has been demonstrated by Blockey (1978) and Lunstra (1980). Although Christensen, Seifert and Post (1982) queried these findings.

Application of the SC test to small groups of mixed age bulls poses a problem in that age is related to position in the social hierarchy, which is in turn related to sexual behaviour (Blockey, 1981c). Reports on the predictive ability of the libido score test (bulls tested individually) are limited. Sullins, Tomky, Farin, Chenoweth and Pexton (1979) found that high libido bulls (average libido score of 9,7 on a scale of 0-10, see Chapter 2) served a greater proportion of females available and achieved better CR's than medium libido bulls (average score 7,8). Makarechian, Farid and Berg (1983) could not confirm these results. In a trial using Santa Gertrudis bulls (n=40) mated as single sires to 100 heifers for short periods. Smith, Morris, Amoss, Parish, Williams and Wiltbank (1981) found that libido score (number mated/number in oestrous x 100) was positively correlated with CR (r=0,44).

In the light of the highly significant relationship between number of services completed in the libido score test and total SC (Chapter 2) it seems feasible to use the individual test to predict veld mating performance expressed in terms of CR.

The aim of this study was therefore to link the number of services completed in 10 minutes to CR when bulls are mated as single sires to relatively large groups of cows for a limited period.

PROCEDURE

The trial was repeated over two breeding seasons, 1983/84 and 1984/85. During September 1983, 16 bulls (Sussex and Sussex-type including one Aberdeen Angus) were given three 10 minute libido tests (Chenoweth, 1981). Tests were spaced at two day intervals. A given bull's libido score was determined by taking the average of his last two tests. Twelve bulls had previous heterosexual experience. Four two-year old bulls had not. Six bulls were selected for the trial. Three bulls were classified as having high libido (three or more services) and three were classified as medium/low libido (one service). The bulls were examined for breeding soundness in the month before the start of mating. One ejaculate was evaluated for motility and percentage abnormal sperm (Roberts, 1971). All bulls had scrotal circumference greater than 35 cm.

The cow herd (188 Sussex-type cows and 42 Simmentaler-type heifers was rectally palpated to determine the status of ovarian activity. At the same time cows were scored to determine their condition (Van Niekerk & Louw, 1982). The allocation of cows to the six groups was made in such a way as to balance the groups for age, calving date, condition score (CS) and ovarian activity. For two weeks before the start of mating, the cows were observed twice daily for oestrous activity. Mating began on 14/12/83 and continued until 17/1/84. The incidence of heat cycles and of mating activity were determined by means of daily visual observations, heat mount detectors (HMD)[13] and chinball markings from harnesses[14] fitted to the bulls. Following termination of the trial cows were placed with clean-up bulls. These bulls were fitted with harnesses containing ink of a different colour to that used previously. Daily heat spotting continued until 31/1/84. Thereafter daily observations were made for chinball markings until the 7/2/84. A rectal pregnancy diagnosis was made four months after termination of the breeding season. The age of the foetus was matched with the heat spotting data. Successful conceptions were determined on the basis of heat spotting, pregnancy diagnosis and the duration of the gestation period.

In 1984 bulls (n=8) were given four 10 minute libido tests. Tests were spread out over the month of November. A bull's libido score was determined by taking the average of his best two scores. Four bulls were used in the trial. Two bulls had high libido (more than six services) and two were classified as having medium libido (three services). The cow herd (n=218) was divided into four groups, balanced for calving date and CS. Mating began on 14/12/84 and continued until the 17/1/85. Cyclic and mating activity was recorded by observation and chinball markings. After

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[13] Kamar Incorporated, Colorado.

[14] Taurus AI Co Op

17/1/85 cows were subdivided into five groups as follows:

a) Cows expected to return to service. There were two groups, cows which should have cycled between 12/1/85 and 20/1/85 and those expected between 21/1/85 and 31/1/85. An Aberdeen Angus bull fitted with a chinball harness was switched between the two groups.

b) Cows which never cycled (n=35). This group was placed with a Sussex bull (chinball contained a different colour ink to that used in the preceding month). Only one of these cows, which were in poor condition, cycled during the next two weeks.

c) Two groups of cows, determined to be pregnant on the basis of heat spotting data. Bulls were not placed with these cows.

The cows were observed daily for chinball markings. The clean-up period was terminated on 31/1/85. Successful conceptions were determined as in the previous year.

In both years cows were rotationally grazed on veld (Tall Sandy Grassveld, Bioclimatic Group 8). Veld utilization was carefully monitored and groups were most often grazed in camps adjacent to one another in order to minimize any differences in veld quality and quantity.

For the purposes of statistical analysis only the first 21 days of the breeding season were included. During this time mating pressure was most intense. The number of second oestrous conceptions included were thus limited. Cows which cycled after 21 days or did not cycle at all were excluded. The 21 days was further subdivided into seven three-day intervals. In this way a bull's conception rate could be evaluated over a number of episodes of mating activity. Furthermore changes in fertility over time could be evaluated. Conception rate (CR) was determined for each interval. A split-plot analysis was used and the seven time intervals were treated as sub-plots in the design. The relationship between CR for each 3-day interval and the number of cows cycling, lactating, with an interval from calving to cycling (CALCYC) of $\leqslant$ 75 days and with CALCYC $\leqslant$ 80 days was examined using linear regression analysis for both years. In 1984/85 the relationship between CR and the number of cows with CS $\leqslant$ 2,0 and with CS $\leqslant$ 2,5 was also investigated.

RESULTS

The distribution of the bulls (1983/84) among the various libido categories is presented in **Table 14**

Table 14 The distribution of bulls among three libido categories in 1983/84

	Libido		
	Low	Medium	High
Services completed	< 1	1-2	$\geqslant$ 3
No. of bulls	2	11	3

One of the low libido bulls was found to be incapable of serving despite the fact that he mounted frequently. His inability to serve could possibly have been due to arthritic hips and joints since he seemed to be unable to manoeuvre his hindlegs to obtain intromission and complete service. Another bull's libido (medium) was influenced by impaired vision.

In 1984/85 the average number of services a bull completed, increased considerably with three services being the minimum score recorded. Data from 1984/85 are presented in Table 15

Table 15 The number of bulls in each of two libido categories in 1984/85

	Libido	
	Medium	High
Services completed	3 - 4,5	> 5
No. of bulls	5	3

In both seasons bulls had less than 10% abnormalities with motility scores of 3 or more. The average scrotal circumference for bulls mated in 1983/84 was 38,2 cm. In 1984/85 it was 36,1 cm.

The average CS (± S.E.) for cows which cycled during the first three weeks of the season was 2,53 (± 0,51) and 2,55 (± 0,46) in 1983/84 and 1984/85 respectively. The average CALCYC (± S.E.) for years one and two were 87,9 (± 17,5) and 84,6 (± 13,0) days respectively.

In 1983/84 the number of cows cycling in any three-day interval and the number with CALCYC $\leqslant$ 75 days significantly depressed CR ($P < 0,05$). During 1984/85 the number of cows lactating and number with CALCYC $\leqslant$ 75 days had a significant negative effect on CR ($P < 0,05$) while number cycling, number with CS $\leqslant$ 2,5 and the number with CALCYC $\leqslant$ 80 days tended to depress CR ($P < 0,10$). Those variates which had a significant effect on CR were included as covariates in the split-plot analyses.

Table 16 The mean conception rates adjusted for covariates for two libido score treatments over seven 3-day time intervals during two breeding seasons, 1983/84 and 1984/85[1]

			Time interval							
Year	Libido score		1	2	3	4	5	6	7	Treatment means
1983/84	More than 3 services (n=3)	Mean conception[2] rate (%)	79,3^{c}	87,2^{c}	64,5^{c}	96,3^{c}	96,6^{c}	90,9^{c}	70,5^{c}	83,6
	1 service (n=3)	Mean conception Rate (%)	74,4^{c}	79,9^{c}	89,6^{c}	87,4^{c}	61,6^{d}	82,9^{c}	88,8^{c}	80,7
		Mean conception rate (%) for each time interval	76,9	83,5	77,1	91,8	79,1	86,9	79,7	
1984/85	More than 6 services (n=2)	Mean conception rate (%)	55,8	69,5	71,6	63,2	98,9	98,9	60,9	77,0
	3 services (n=2)	Mean conception Rate (%)	64,2	68,6	48,5	59,7	99,0	84,4	82,9	72,3
		Mean conception[3] rate (%) for each time interval	60,0^{a}	69,0^{a}	70,0^{a}	61,5^{a}	99,0^{b}	90,6^{b}	71,9^{a}	

1 Unless otherwise indicated means are not significantly different.

2 LSD for score x times interval =31,3% ($P<0,05$), means within a row are NS, and means within columns with different superscripts are significantly different.

3 LSD for effect of time = 20,8% ($P<0,05$) and 29,3% ($P<0,01$), means within the same row without common superscripts are significantly different.

The effect of bull libido on CR was non-significant in both years (**Table 16**). In the first year the time x score interaction was significant indicating that the performance of high and medium/low bulls differed over time. The differences were not consistently in favour of any one group of bulls.

Time had a significant influence on CR during the 1984/85 season ($P < 0{,}05$). Conception rate increased over time. The increase was not, however, consistent (**Table 16**). The F-value for the interaction (score x time) although less than $P_{0,05}$ was greater then $P_{0,10}$. Again neither group of bulls maintained a steady advantage over the other.

With the relatively small numbers of cows cycling in most time intervals, failure to conceive by only one or two cows greatly influenced CR. For this reason the $\log_e$ (CR) transformation was applied to the data. The outcome of the analysis was not altered in any way. Transformation did, however, smooth out the increase in CR over time (1984/85).

Table 17 The change in mean CALCYC (days) over time in 1983/84 and 1984/85

	Time Interval						
Year	1	2	3	4	5	6	7
1983/84[1]	79,0^{a}	80,2^{a}	87,1^{abc}	85,8^{ab}	94,6^{bc}	91,5^{bc}	98,4^{bc}
1984/85[2]	74,6^{a}	78,5^{ab}	78,9^{ab}	83,2^{bc}	88,3^{cd}	90,9^{d}	90,5^{d}

[1] Means without common superscripts are significantly different (1,2 vs 5,7 ($P < 0{,}01$), the rest are significant at 5%)

[2] Means without common superscripts are significantly different, $P < 0{,}05$

The increase in CR over time during 1984/85 (**Table 16**) was accompanied by a simultaneous increase in CALCYC (**Table 17**). Although CALCYC increased over time during the first season CR did not increase over time.

A greater proportion of cows did not cycle during the second season (from the start of breeding to the start of clean-up) when compared with the first (16,1 versus 5,2%). Furthermore 10,6% cycled after the first 21 days in year two as opposed to 4,4% in 1983/84. Cows which did not cycle at all were generally in poor condition.

DISCUSSION

Change in libido score between years

Bulls increased their libido score considerably from year one to year two, particularly bulls with no heterosexual experience prior to their first test. This would seem to suggest that learning of sexual behaviour extends beyond the first few exposures to oestrous females and into the first season of herd mating. All bulls used during the first season either displayed a constant level of behaviour in all three tests or improved from test one to test two and remained constant in test three. Two of the three high libido bulls used in 1983/84 were sexually experienced while the remaining bull had limited experience. Two of the medium/low bulls were virgins while the third had limited experience. Thus the lack of experience of the medium/low libido bulls might have explained the non-significant effect of libido score on CR in year one. However, such a conclusion presents an anomaly when the data from both years are considered since all the bulls but one in 1984/85 were experienced (this bull achieved the highest score).

Mating pressure

The insignificant effect of a bull's libido score on CR in 1983/84 could have been largely due to insufficient numbers of cows' cycling over the first 21 days. In 1984/85 an attempt was made to highlight differences in CR due to libido by placing bulls under greater mating pressure (the number of bulls used was decreased). The attempt did not succeed because of the larger proportion of cows which failed to cycle or cycled after 21 days. These cows were generally in poorer condition than those which cycled within 21 days.

On the basis of the results from this study it appears as if the generally accepted BFR of 1:25 is conservative and that bulls of average libido are capable of covering at least 40 cows over a standard 90-day breeding season. Therefore it appears as if high libido bulls are capable of covering more than 40 cows over a limited period of time. Neville *et al*. (1979) found no difference in CR between bulls mated to either 25 or 40 cows for 90 days and Rup, Ball, Shoop and Chenoweth (1977) reported that most bulls achieved good reproductive efficiency when mated to 40 or 60 heifers for 21 days.

Increase in CR over time in 1984/85

During the second season the number of cows lactating had a significant influence on CR. This was not the case in 1983/84. The interval from calving to cycling (CALCYC) increased over time in both years and a longer post-partum interval would have improved the chances of conception in lactating females. This effect was probably masked by the greater proportion of dry cows and heifers in 1983/84, 33,9 versus 20,6% in 1984/85 (there were no heifers in this year).

A second factor which could have influenced CR in 1984/85 was limited veld availability. Rainfall at the peak of the growing season (December/January) was 40,4% below average. The shortage of grazing became noticeable during early January.

Graham (1982) demonstrated that poor nutrition can prolong the post-partum interval. No doubt this effect is more marked in cows of suboptimal condition. Van Niekerk (1982) considered a CS of 3,0 at the start of mating to be ideal whereas cows in this study had an average CS of less than 3,0.

In order to minimize the effect of grazing on CR, cows were grazed on veld which had been spared for winter grazing. Grazing on rested veld lasted from early to mid-January.

The need for libido testing

Results from the present study indicate that bulls of high libido have no advantage over those of medium libdio in terms of CR when mated at BFR's of 1:35 and 1:40 for 21 days even though the high libido bulls in 1984/85 were more than 100% superior to their medium libido counterparts as regards number of services completed in 10 minutes.

In the present trial, bulls with obviously inadequate libido were not identified (remembering that number of bulls were limited). Furthermore, of the phase D_2 bulls tested in 1983 (Chapter 2) and 1984 (not included) only a small percentage of bulls did not serve (zebu bulls excluded). Thus it would appear that only a limited number of bulls with questionable libido and/or mating ability are going to be encountered in general beef farming practice. However, this does not mean that the occurrence of low libido bulls can be ignored particularly when one considers the potential for increased weaning weights, reduced bull costs and the more effective dissemination of superior genetic material to greater numbers of offspring through the use of high libido bulls. Whether or not the cost of testing, balanced against the benefits of using high libido bulls, is justified can only be determined by further investigations using larger groups of males and females.

Bull libido as a component of fertility, cannot be ignored. The need to accurately predict male fertility using semen quality, testicular size and sex-drive is pressing and accentuates the need for a suitable test for libido.

CHAPTER 5

HORMONAL CONTROL OF SEXUAL BEHAVIOUR IN THE RAM

INTRODUCTION

The maintenance of a high level of sexual activity in breeding sires could be of considerable economic value. Within the last decade research into the control of sexual behaviour in the ram has demonstrated that the simultaneous administration of dihydrotestosterone (DHT) and oestradiol (E_2) in wethers induces the complete pattern of male sexual behaviour (D'Occhio & Brooks, 1976; Mattner, 1976). The manner in which these testosterone metabolites exert their control on behaviour is unclear.

The administration of E_2 alone elicits lower levels of behaviour than those seen with testosterone therapy (D'Occhio & Brooks, 1976, 1980; Mattner, 1976). Only after the administration of DHT in conjunction with E_2 does the full pattern of sexual behaviour arise. It appears as if DHT potentiates the action of E_2 (D'Occhio & Brooks, 1980). The administration of DHT alone does not give rise to sexual behaviour (D'Occhio & Brooks, 1976; Mattner, 1976; Parrot, 1978).

Parrot (1975) demonstrated that DHT was responsible for sensitization of the penis in rats. The implication therefore is that DHT facilitates the action of E_2 through its action on the genitals. Parrot (1978), observing that E_2 was not as effective as T in eliciting courtship behaviour, suggested that part of DHT's action is in the central control of courtship. Further support for this idea comes from the work of Thieulant and Pelletier (1979) and Pelletier and Caraty (1981) who characterized cystolic receptors for DHT and E_2 in the pituitary and hypothalamus of the ram.

Thus it would appear as if DHT exerts its influence on sexual behaviour both centrally (by facilitating the action of E_2) and peripherally (Parrot & Baldwin, 1984).

The technique of using active or passive immunization against specific steroid hormones has been used to create deficiencies of hormones at their sites of action and to disrupt their feedback mechanisms (Nieschlag, Usadel, Kley, Schwedes, Schöffling & Krüskemper, 1974; Wickings & Nieschlag, 1978; Thompson & Honey, 1984; Walker, Thompson, Godke & Honey, 1984).

The aim of the present investigation was to negate the action of E_2 in T implanted wethers by means of active immunization. In this way it was hoped to highlight possible central action of DHT on sexual behaviour.

PROCEDURE

Animals and treatments

The trial was repeated over three years (1983, 1984 and 1987). In the first year a group of thirty 18 month old S.A. Mutton Merino wethers with average mass (± S.E.) of 38,8 ± 4,5 kg was used. In 1984, 31 Merino wethers were used. The group consisted of eight 24 month old and twenty-three 18 month old wethers of average mass (± S.E.) 52,7 ± 6,1 and 40,0 ± 4,1 kg respectively. In the last year, 44 wethers were utilized. There were 29 Merino wethers from two age groups, 43 (n=14) and 31 (n=15) months of age, and 15 Dohne Merinos 31 months of age. The mean mass (± S.E.) of the wethers immediately prior to the assessment of sexual behaviour was 40,26 ± 0,77 kg. There were no differences in mass between breeds and age groups.

The wethers were castrated at approximately four weeks of age, using elastrator rings in 1983 and surgical removal in 1984 and 1987.

There were six treatments, namely:

a) Control (C);
b) Immunization against E_2, implantation with testosterone propionate (TP + Anti E_2);
c) Implantation with TP;
d) Implantation with DHT;
e) Oestradiol dipropionate (ODP) implantation;
f) DHT plus ODP implantation (DHT + ODP).

An additional treatment was applied in 1987. The treatment involved immunizing wethers against both oestrone (E_1) and E_2 followed by implantation with TP (TP + Anti $E_{1,2}$). The intention being to negate the action of both these hormones.

In each year treatments were balanced for bodymass with five animals being allocated per treatment. In 1983 DHT wethers were given an additional 2,5 cm long oestrone (E_1) implant in their last test in order to determine its effectiveness with regard to mounting. Oestrone has been shown to induce mounting in wethers (D'Occhio & Brooks, 1980). The antiserum raised in the TP + Anti E_2 wethers was specific for E_2 therefore any residual mounting behaviour in these wethers could have been due to E_1.

Immunization

The method used was according to Hurn and Chantler (1980). In each year the TP + Anti E_2 wethers (10 in 1983 and 1984 and nine in 1987) were immunized with 2,5 mg β-oestradiol-6-O-carboxymethyloximine bovine serum albumin conjugate[15]. The conjugate was dissolved in physiological saline and mixed with two parts complete Freunds adjuvant. The mixture was injected subcutaneously at four sterile sites on the inside of the hindleg. The wethers which displayed the highest levels of binding (five in 1983, seven in 1984 and seven in 1987) received booster injections.

[15] Sigma Chemical Company

In 1983 wethers received one booster injection six weeks after immunization while in 1984 and 1987 they received two booster injections. In 1984 the two injections were spaced at four week intervals while in 1987 the first booster injection was given five weeks after primary immunization with the second booster being given 30 weeks after the first (only five wethers received the second booster in both years). By using two booster injections in 1984 and 1987, it was hoped to produce superior antibody to that obtained in 1983. In 1984 the second booster was given only in saline, which according to Butcher (pers. comm., 1983) minimizes the chances of abscesses at the injection sites. However, the use of saline gave rise to a rapid drop in antibody titre following peak production and so it was decided to retain the saline / complete Freunds adjuvant mixture in 1987.

The immunization procedure for the TP + Anti $E_{1,2}$ treatment in 1987 was the same as for the TP + Anti E_2 with the exception that wethers were immunized with 1,25 mg of β-oestradiol-6-0-carboxymethyloxime bovine serum albumin and 1,25 mg Oestrone-3-carboxymethyloxime bovine serum albumin.[16]

Implant manufacture

The implants were manufactured using silastic tubing (3,35 mm internal diameter x 4,65 mm external diameter). The desired length of tubing was sealed at one end using elastomer.[17] Crystalline hormone was forced into the tube from the opposite end until it was tightly packed. The open end was then sealed.

For the first two years the implant lengths required to produce levels of hormone within the physiological range were determined on the basis of results from a number of

[16] Dr J. Cutting, Citrus and Subtropical Research Institute, Nelspruit.

[17] Dow Corning Corp, Michigan.

investigations (Schanbacher, 1980; D'Occhio, Schanbacher & Kinder, 1982, 1983).

The lengths of implants used were as follows:

a) TP, 5 x 30 cm;
b) DHT, 1 x 30 cm plus 1 x 5 cm;
c) ODP, 1 x 5 cm.

The lengths of implant used in 1983 and 1984 gave rise to supraphysiological levels of hormone and so a preliminary investigation was carried out in 1987 to determine the relationship between implant length and plasma hormone levels. The lengths of implant subsequently used were as follows:

a) TP, 1 x 22 cm for non-immunized and 1 x 11 cm for immunized wethers;
b) DHT, 1 x 10 cm;
c) ODP, 1 x 1,5 cm.

In 1984 immunized wethers had testosterone levels more than double that of non-immunized wethers. For this reason, in 1987, the immunized wethers were implanted with an implant half the length of that implanted in non-immunized wethers.

Determination of the relationship between implant length and plasma hormone levels

Ten Merino wethers were utilized in this investigation. The wethers represented two age groups, 32 and 21 months of age. Their mean mass was 42,53 ± 0,49 kg. The wethers were treated with a given hormone implant, bled seven days later, the implant removed and replaced with an implant of the next hormone to be evaluated. Hormones were evaluated in the following order: ODP, TP and DHT. The range in implant sizes were 13 to 36 mm, 176 to 629 mm and 27 to 226 mm for the three hormones respectively. The plasma samples were frozen at -20°C and subsequently analysed for the appropriate hormone using RIA techniques.

Method of implantation

Implants were placed subcutaneously just posterior to the shoulder. The site was shaved and sterilized. Local anaesthetic[18] was used. A cut 1 cm long was made through the skin. A stainless steel trochar and cannula (**Figure 18**) of appropriate length was inserted subcutaneously. The trochar was removed and the implant inserted into the cannula. Then the cannula was removed leaving the implant behind. Where more than one implant was used they were placed in such a way that they radiated downward starting parallel with the backbone of the wether.

Teaser ewes

A simulated oestrus was achieved in ovariectomized ewes by using progesterone sponges, placed intravaginally, for seven days with an intramuscular injection of ECP (1,4 mg) at sponge withdrawal on the morning before a given test. Ewes were detected on heat by means of intact rams fitted with raddle blocks. During the course of testing, ewes which became non-receptive were removed and replaced by fresh ones.

Test procedure

The wethers were given four tests in 1983, two in 1984 and three in 1987. In 1983 and 1984 the tests were preceded by a non-oestrus test which served the purpose of acclimatization. In the last year the three tests were preceded by an oestrus test prior to implantation. This test served a dual purpose, to identify wethers which mounted without any hormone treatment and as a means of acclimatization. Tests were of 10 minutes duration and one wether was placed with two ewes. The test area was similar to that used in Chapter 1. Tests were spaced at least five days apart.

[18] Lignocaine, Centaur

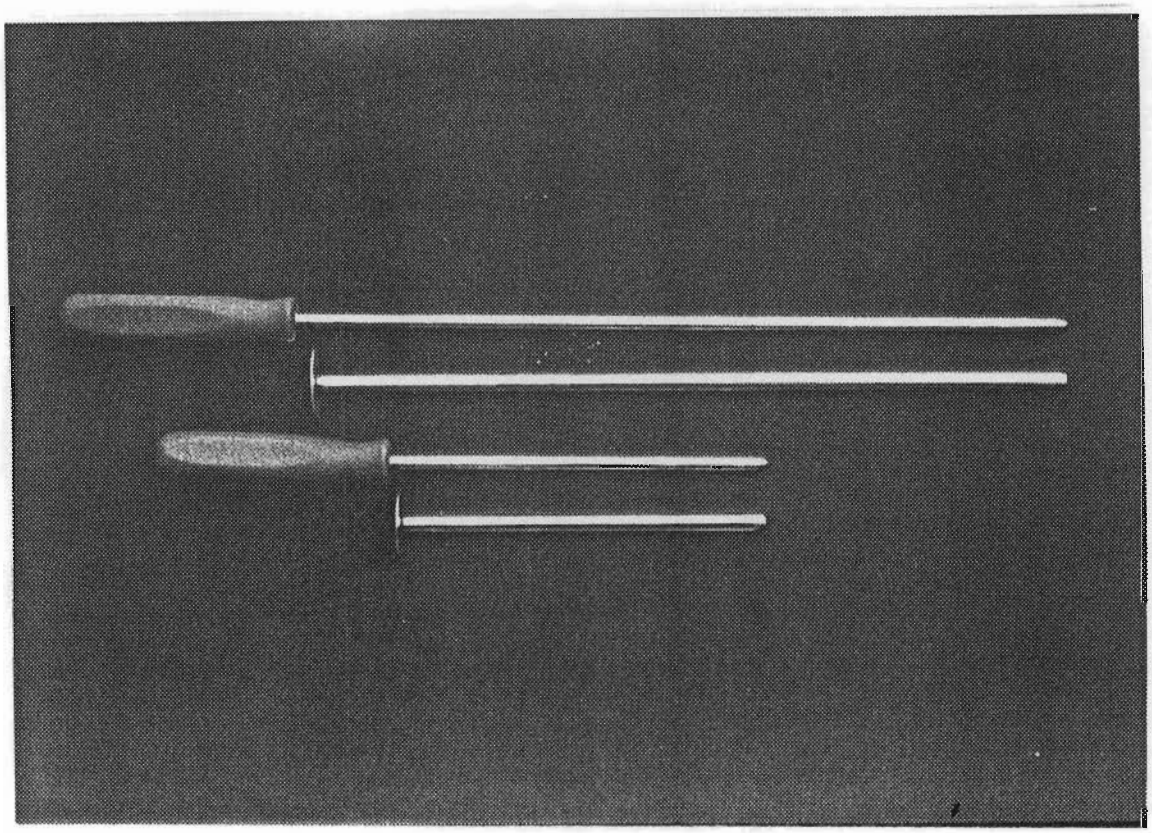

Figure 18 The stainless steel trochars and cannulas used for the insertion of implants

The frequency of the following behavioural traits was recorded:

a) Anogenital sniffing;
b) Staring at the ewe's head;
c) Tongue flicking;
d) Foreleg kicks and nudging;
e) Flehmen;
f) Intention to mount;
g) Attempted mount;
h) Mounting;
i) Mounting with thrusting/seeking movements;
j) Service or serving action.

The determination of antibody titres

Blood (± 10mℓ) from the jugular vein was collected using a sterile syringe, transferred to a test tube, incubated at 37,2°C for 30 minutes to hasten clot formation and left at 4°C overnight to promote clot contraction. The serum was transferred to plastic test tubes in 3mℓ aliquots using a Pasteur pipette, "snap frozen" and stored at -20°C. Just prior to assay, serum was thawed by placing it briefly in a waterbath at 37,2°C. Antibody binding was determined using serum diluted with 0,1% gelatin in phosphate buffered saline (PBS). One hundred µℓ of 0,5% bovine serum albumin in PBS were added to each assay tube together with 100µℓ of the appropriate antibody dilution. Each dilution was assayed in duplicate. Oestradiol -^{3}H (E_2 -^{3}H) was added to each tube (± 10 000 cpm). The tubes were vortexed briefly and incubated for five minutes at 37,2°C, vortexed once more and incubated in ice for a further 40 minutes. Free and bound proteins were then separated using 0,8 mℓ of cold charcoal-dextran suspension (250 mg Norit A charcoal and 1 gm Dextran T-70 in 100 mℓ of distilled H_2O). Tubes were vortexed and allowed to stand in ice for 10 minutes. Thereafter they were centrifuged at 2 600 rpm and 0°C for 10 minutes. The supernatant was subsequently decanted into a scintillation vial containing 12,5 mℓ of scintillant.

The percentage tracer bound was determined following correction for non-specific binding.

In 1983 binding was determined at 14 and 35 days after first immunization and at 16, 38 and 51 days after the booster injection. The dilutions 1:50, 1:100, 1:500 and 1:1 000 were used for all determinations except the last two. For these, dilutions of 1:10, 1:25, 1:50 and 1:100 were used. Days 38 and 51 coincided with one day before test two and one day after test three respectively. In 1984 binding was determined at day 17 after immunization, day 21 after the first booster and at days 11 and 18 after the second booster. The dilutions 1:50, 1:100. 1:500 and 1:1 000 were used throughout.

In 1987 binding was determined at 35 days following the first immunization, at 48 days after the first booster injection and at 12 and 14 days after the second booster injection. The dilutions used were the same as for 1984. In 1987 antibody titres were also determined using oestrone-$^3H(E_1$ -$^3H)$.

The determination of serum hormone levels

a) <u>Oestradiol</u>

Serum levels of E_2 were determined using radioimmunoassay (RIA), the method and antiserum as described by Butcher, Collins and Fugo (1974).

Serum E_2 levels were determined for the TP + Anti E_2, TP + Anti $E_{1,2}$ (1987), TP, DHT + ODP and ODP wethers. In each year two blood samples were collected. These were obtained one day prior to test two and one day after test three in 1983, one day before tests one and two in 1984 and one day before tests two and three in 1987.

b) Testosterone and dihydrotestosterone

In 1983, an RIA kit[19] was used for the following determinations:

(i) The total adrogen levels for all wethers prior to implantation;

(ii) The total androgen levels for the TP and TP+Anti E_2 wethers for the two sample dates;

(iii) The DHT levels for the DHT and DHT + ODP wethers for the two sample dates.

In 1984, the same determinations were carried out, with the exception that total androgen levels were determined using testosterone antiserum[20] developed in a rabbit (RIA procedure the same as for E_2) while DHT levels were determined using a kit. The rabbit antiserum cross reacted 26,7% with DHT. In 1987, the testosterone antiserum was obtained from another source[21] and cross reacted 69,0% with DHT (Pruitt, Corah, Stevenson & Kiracofe, 1986). Consequently the same antiserum was used for DHT determinations. Separation of the DHT and testosterone fractions was achieved by column chromatography (Abraham, Buster, Lucas, Corrales & Teller, 1972). The DHT fraction was subsequently determined using the procedure of Butcher et al. (1974). High reagent blanks in the DHT assay were accounted for by subjecting the DHT standard curve to column chromatography.

Antiserum cross reactivity

The extent to which the antiserum developed in the TP + Anti E_2 and TP + Anti $E_{1,2}$ (1987) wethers cross reacted with

[19] Amersham, England.

[20] Dr J. Morgenthal, Dept. of Human and Animal Physiology, University of Stellenbosch.

[21] Prof. G.D. Niswender, College of Veterinary Medicine and Biomedical Sciences, Dept. of Physiology, Colorado State University.

E_1 was determined for each animal. Two standard curves, one for E_1 and one for E_2 were run simultaneously for each antiserum. The percentage cross reaction was determined by taking the value for E_1 at 50% binding and expressing it as a percentage of the value for E_2 at 50% binding.

Statistical analysis

Linear regression analysis was used to establish the relationship between implant length and plasma hormone level.

The data on traits of sexual behaviour were analysed by means of split-plot analysis. Successive observations on the same animal were treated as sub-plots in the design. Differences between specific treatments were investigated using orthogonal comparisons.

RESULTS

Relationship between implant length and plasma hormone level

The relationship between implant length (x, in mm) and plasma hormone level (y expressed as ng or pg/mℓ) was highly significant ($P < 0,01$) for all three hormones (**Figure 19**). The r-values for DHT, TP and ODP were 0,878, 0,842 and 0,883 respectively.

Sexual behaviour

The data from the three years will be presented independently. In each year all forms of mounting, with the exception of intention to mount, were included as one variate. Data from the last test in 1983 were excluded (DHT wethers received E_1 implant).

a) <u>1983</u>

The various aspects of sexual behaviour (with the exception of serve) were displayed by all the treatments (**Table 18**).

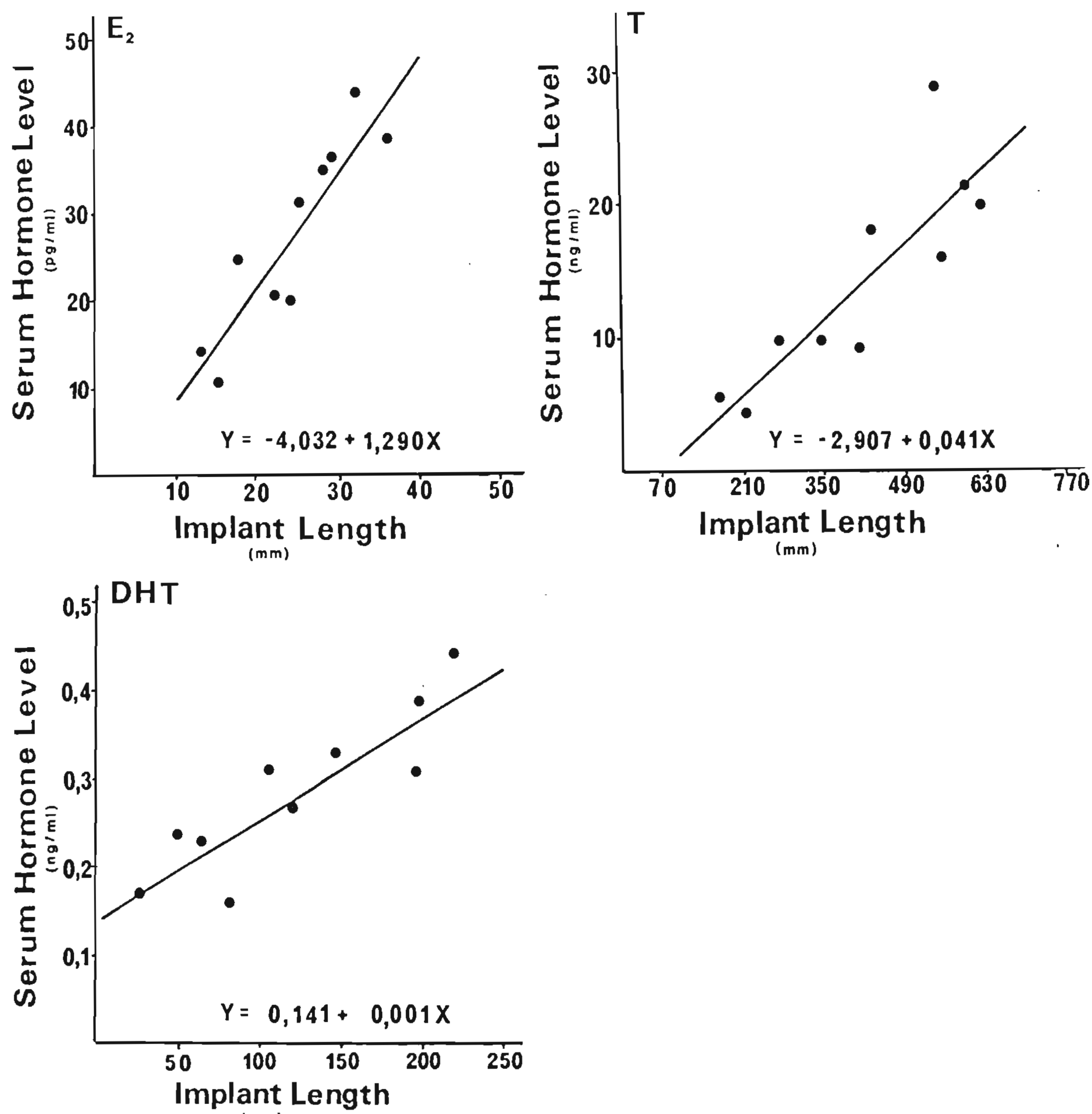

Figure 19 The relationship between implant length and plasma hormone levels for oestradiol (E_2), testosterone (T) and dihydrotestosterone (DHT)

The services seen were without erection, intromission or seminal discharge but were accompanied by a refractory period. Such behaviour was described by Parrot and Baldwin (1984) as "pseudoejaculation".

The incidence of sniffing, staring, tongue flicks and nudging increased over time ($P < 0,01$). There was a tendency for time to influence the level of mounting behaviour. The increase in mounting was almost significant at the 5% level. The incidence of staring showed a downward trend over time.

The hormone x time interaction was significant for tongue flicks, nudging and mounting ($P < 0,05$). The TP, TP + Anti E_2 and ODP wethers showed an increase in the incidence of tongue flicks from t_1 to t_2 (**Table 19**). The increase was followed by a decrease from t_2 to t_3. The changes were most dramatic for the ODP wethers.

The highest level of mounting behaviour was displayed by the TP and DHT + ODP wethers (**Table 18**). Mounting behaviour was depressed, though non-significantly, in the TP + Anti E_2 wethers but occurred at a higher level than in the ODP wethers. The C and DHT wethers displayed the lowest levels of mounting. Mounting behaviour was not shown by all C and DHT wethers. Those which mounted did so consistently.

Among the most notable treatment differences were the high levels of tongue flicks and nudging shown by the TP and DHT + ODP wethers (particularly the TP, **Table 18**).

The incidence of sniffing, flehmen, intention to mount and serve were not significantly influenced by treatment. The C and DHT wethers displayed the lowest levels of activity for these traits (**Table 18**). For staring, differences were significant, the C and DHT wethers were inferior to the remaining four treatments.

The changes in the incidence of nudging, over time (**Table**

Table 18 The mean levels of sexual activity for eight behavioural traits measured for the six treatments over three tests in 1983[1]

	HORMONE TREATMENT							
	1	2	3	4	5	6	L.S.D. VALUES	
TRAIT	CONTROL	TP+ANTI E_2	TP	ODP	DHT	DHT + ODP	5%	1%
Sniffing	6,27	15,20	13,47	19,20	8,47	10,53	NS	NS
Staring	$0,73^{a}$	$5,87^{ab}$	$5,87^{ab}$	$5,87^{ab}$	$1,33^{ab}$	$6,27^{b}$	3,90	5,32
Tongue flicks	$0,20^{a}$	$26,10^{b}$	$52,30^{c}$	$25,90^{b}$	$0,90^{a}$	$35,00^{bc}$	18,05	24,62
Nudging	$0,73^{a}$	$11,27^{a}$	$39,13^{b}$	$11,20^{a}$	$0,13^{a}$	$21,13^{ac}$	16,18	22,06
Flehmen	0,533	1,133	0,867	0,867	0,133	1,000	NS	NS
Intention to mount	0,07	1,27	1,27	1,00	0,53	1,60	NS	NS
Mount	$0,20^{a}$	$11,47^{ab}$	$18,80^{b}$	$7,00^{a}$	$2,20^{a}$	$18,13^{b}$	8,28	11,29
Serve	0,000	0,000	0,200	0,067	0,000	0,267	NS	NS

[1] Means in the same row without common superscripts are significantly different

Table 19 Changes in the mean levels of tongue flicks for the six treatments over time for 1983[1,2]

TREATMENT	TEST NUMBER 1	2	3
Control	0,4[a]	0,2[a]	0,0[a]
TP + Anti E_2	21,4[ab]	40,0[a]	16,8[b]
TP	40,6[a]	62,8[bc]	53,6[ac]
ODP	5,8[a]	58,4[b]	13,4[a]
DHT	0,6[a]	1,2[a]	1,0[a]
DHT + ODP	36,6[a]	38,8[a]	29,6[a]

1 Means in the same row without the same superscripts are significantly different

2 The L.S.D.'s for $P<0,05$ and $P<0,01$ are 23,9 and 31,9 respectively for different and 20,1 and 26,8 respectively for the same levels of hormone

Table 20 Changes in the mean levels of nudging for the six treatments over time for 1983[1,2]

TREATMENT	TEST NUMBER 1	2	3
Control	1,20[a]	1,00[a]	0,00[a]
TP + Anti E_2	9,00[a]	15,80[a]	9,00[a]
TP	30,80[a]	46,40[b]	40,20[b]
ODP	3,80[a]	22,80[b]	7,00[a]
DHT	0,40[a]	0,00[a]	0,00[a]
DHT + ODP	26,00[a]	24,00[a]	13,40[b]

1 Means within the same row without the same superscripts are significantly different

2 The L.S.D.'s for $P<0,05$ and $P<0,01$ are 17,7 and 23,5 respectively for different and 10,1 and 13,5 respectively for the same levels of hormone

20) shown by the TP and ODP wethers were similar to those shown for tongue flicks (Table 19). The incidence of nudging displayed by the DHT + ODP wethers remained static in t_1 and t_2, a significant decrease occurred in t_3 ($P < 0,05$).

The incidence of mounting (Table 21) displayed by the ODP wethers increased dramatically from t_1 to t_2 and dropped to an intermediate level in t_3. The DHT + ODP wethers showed a consistent significant decrease in mounting over time.

Table 21 **Changes in the mean levels of mounting for the six treatments over time for 1983** [1 2]

TREATMENT	TEST NUMBER 1	2	3
Control	0,00 [a]	0,00 [a]	0,60 [a]
TP + Anti E_2	9,60 [a]	14,00 [a]	10,80 [a]
TP	16,00 [a]	22,80 [a]	17,60 [a]
ODP	2,00 [a]	13,40 [b]	5,60 [ab]
DHT	1,60 [a]	2,40 [a]	2,60 [a]
DHT + ODP	27,20 [a]	18,40 [b]	8,80 [c]

1 Means within the same row without the same superscripts are significantly different.

2 The L.S.D.'s for $P < 0,05$ and $P < 0,01$ are 10,5 and 14,0 respectively for different and 8,3 and 11,1 respectively for the same levels of hormone.

The following orthogonal comparisons were included in the analysis:

a) TP versus TP + Anti E_2.

b) ODP and DHT + ODP versus DHT and C.

c) DHT and DHT + ODP versus ODP and C.

d) DHT + ODP and C versus ODP and DHT.

The combination of ODP and DHT + ODP was superior to DHT and C for sniffing, flehmen, intention to mount (all $P<0,05$), staring, tongue flicks, nudges and mounts (all $P<0,01$). Immunization against E_2 significantly reduced the level of tongue flicks and nudging in TP + Anti E_2 wethers ($P<0,01$). There was also a tendency for mounting to be reduced by immunization ($P<0,10$). The comparison of DHT and DHT + ODP versus ODP and C was significantly different for mounting ($P<0,05$). The simultaneous administration of DHT and ODP was therefore superior to ODP with respect to the restoration of mounting in wethers.

The results obtained from the orthogonal comparisons confirm some of the observations made earlier with respect to the data presented in **Table 18**.

The orthogonal comparisons which included ODP showed significant changes over time for most variates ($P<0,05$). This no doubt was largely due to the dramatic increase in activity shown by the ODP wethers from t_1 to t_2 followed by the decrease in t_3.

There appears to be no obvious reasons for the changes in the incidence of the various traits of sexual behaviour seen over time.

Four of the DHT + E_1 wethers mounted. Only one of these wethers had not mounted previously. In another, mounting increased to a higher level than that previously observed.

b) 1984

No wethers displayed "pseudoejaculation" in 1984 (**Table 22**). The differences due to treatment were not as dramatic as those observed in 1983, however, the overall trends are similar.

Table 22 The mean levels of sexual activity for seven behavioural traits measured for six treatments over two tests in 1984[1]

	HORMONE TREATMENT							
	1	2	3	4	5	6	L.S.D. VALUES	
TRAIT	CONTROL	TP+ANTI E_2	TP	ODP	DHT	DHT+ODP	5%	1%
Sniffing	$6,10^{a}$	$8,40^{a}$	$6,00^{a}$	$9,50^{a}$	$4,00^{a}$	$9,40^{a}$	NS	NS
Staring	$0,80^{a}$	$4,40^{a}$	$2,90^{a}$	$2,70^{a}$	$0,90^{a}$	$2,40^{a}$	NS	NS
Tongue flicks	$0,00^{a}$	$9,30^{b}$	$9,90^{b}$	$12,80^{b}$	$0,00^{a}$	$8,80^{b}$	8,08	11,24
Nudging	$0,50^{a}$	$8,50^{b}$	$11,70^{b}$	$10,00^{b}$	$0,70^{a}$	$7,40^{ab}$	7,62	10,39
Flehmen	$0,10^{a}$	$0,90^{bc}$	$0,60^{ab}$	$1,50^{c}$	$0,20^{a}$	$1,40^{c}$	0,70	1,08
Intention to mount	$0,50^{a}$	$1,80^{a}$	$0,90^{a}$	$0,90^{a}$	$0,00^{a}$	$0,80^{a}$	NS	NS
Mount	$2,60^{a}$	$9,50^{bc}$	$11,00^{c}$	$4,10^{ab}$	$2,10^{a}$	$9,70^{bc}$	6,23	8,50

[1] Means within the same row without common superscripts are significantly different

The TP wethers displayed the highest levels of mounting behaviour (**Table 22**). The DHT + ODP and TP + Anti E_2 wethers showed similar levels of mounting (marginally lower than TP). Again the level of mounting shown by the ODP wethers were suboptimal when compared to the TP wethers. The C and DHT wethers showed the lowest levels of mounting.

The suppression of mounting caused by immunity to E_2 was not as great as in 1983. This was largely due to the fact that one TP wether displayed no sexual behaviour in either test. In t_1 this wether showed aggression toward the ewe and jumped against the sides of the pen in an attempt to escape (its behaviour was similar to the "fear" response described in Chapter 1). When placed in a pen with its fellow TP wethers it courted them in preference to the ewe. Another TP wether showed a low level of mounting in t_1 this was followed by a dramatic increase in t_2. The orthogonal comparison (mounting) for TP versus TP + Anti E_2 interacted significantly over time ($P < 0{,}05$). The TP + Anti E_2 wethers mounted at a higher level than the TP wethers in t_1. The situation was reversed in t_2.

The levels of tongue flicks and nudging were similar for the TP and ODP wethers (**Table 22**). This was in contrast to 1983 where the TP wethers displayed levels at least twice those shown by the ODP wethers. In 1983, the DHT + ODP wethers ranked second to the TP wethers, with respect to nudging and tongue flicks, however, in 1984 they displayed levels slightly lower than the TP + Anti E_2 wethers which were in turn less active than the TP and ODP wethers. These differences were not significant. The C and DHT wethers showed the lowest levels of tongue flicks and nudging.

The ODP and DHT + ODP wethers showed the highest levels of the flehmen response (**Table 22**). The TP + Anti E_2 and TP wethers showed flehmen at a level intermediate between the ODP and DHT + ODP and the C and DHT wethers.

Differences in the incidence of sniffing, staring and intention to mount were not significant (Table 22).

The only variate to show a significant increase over time was tongue flicks ($P < 0,05$). The incidence of nudging, intention to mount and mounting showed a slight tendency to increase from t_1 to t_2 (the increase for mounting was significant at the 10% level). Thus the increase in the incidence of tongue flicks and nudging which are aspects of courtship behaviour was associated with increased mounting behaviour.

In the orthogonal comparison ODP and DHT + ODP versus DHT and C, the combination containing ODP was superior to DHT and C for sniffing and mounting ($P < 0,05$) and for tongue flicks, nudging and flehmen ($P < 0,01$). The same result was obtained in 1983.

c) <u>1987</u>

Treatment differences were significantly different for all traits of sexual behaviour (**Table 23**) with exception of sniffing ($P < 0,08$). The TP wethers showed the highest levels of behaviour for staring, tongue flicks, nudging, intention to mount and mounting. The DHT + ODP wethers were ranked second to the TP treated group in respect of these traits. Treatment differences between the TP and DHT + ODP wethers were significantly different (**Table 23**) for tongue flicks ($P < 0,01$) and mounting behaviour ($P < 0,01$). For flehmen behaviour however, the DHT + ODP wethers showed the highest level of response ($P < 0,01$) which was associated with the highest, though non-significantly different, level of sniffing behaviour. Wethers treated with TP displayed significantly superior levels of staring ($P < 0,05$), tongue flicks ($P < 0,01$), nudging ($P < 0,01$), intention to mount ($P < 0,10$) and mounting behaviour ($P < 0,01$) than the ODP treated group (**Table 23**). The DHT + ODP wethers were superior

Table 23 The means levels of sexual activity for seven behavioural traits measured for the seven treatments over three tests in 1987[1]

	HORMONE TREATMENT									
	1	2	3	4	5	6	7	L.S.D. VALUES		
TRAIT	CONTROL	TP+ANTI E_1	TP+ANTI $E_{1,2}$	TP	ODP	DHT	DHT+ODP	10%	5%	1%
Sniffing	2,87[a]	6,33[a]	2,33[a]	4,93[a]	5,80[a]	2,47[a]	7,73[a]	NS	NS	NS
Staring	1,53[a]	1,40[a]	0,33[a]	14,67[b]	10,20[c]	1,20[a]	12,67[bc]	3,10	3,74	5,06
Tongue Flicks	3,30[a]	2,30[a]	1,70[a]	57,30[b]	30,70[c]	3,90[a]	38,90[d]	8,00	9,67	13,10
Nudging	2,60[a]	2,27[a]	2,40[a]	36,67[b]	18,93[c]	2,53[a]	30,47[bd]	7,43	8,96	12,15
Flehmen	0,00[a]	0,333[a]	0,067[a]	0,533[a]	0,267[a]	0,000[a]	1,800[b]	0,620	0,748	1,013
Intention to mount	0,00[a]	0,33[ac]	0,13[a]	1,67[b]	0,93[cd]	0,00[a]	1,07[bd]	0,72	0,87	1,18
Mount	0,67[a]	1,33[a]	0,60[a]	21,20[b]	8,13[c]	0,67[c]	9,53[c]	4,35	5,24	7,11

[1] Means within the same row without common superscripts are significantly different

to the ODP wethers in three aspects of behaviour namely, tongue flicks ($P < 0,10$), nudging ($P < 0,05$) and flehmen ($P < 0,01$).

Immunization against E_2 (TP + Anti E_2) was most successful in 1987 when compared to the previous two years. Sexual behaviour was significantly depressed to levels similar to those recorded for C and DHT wethers. Immunization against $E_{1,2}$ (TP + Anti $E_{1,2}$) appeared to be more successful than immunization against E_2 alone. Differences between these two treatments were not reflected statistically even though many were of the order of 100% and more.

Two variates changed significantly over the three tests. The level of sniffing was highest in t_1 (**Table 24**), declined significantly from t_1 to t_2 ($P < 0,01$) and remained low for t_3 ($P < 0,05$). The difference between t_2 and t_3 was not significant. Mounting behaviour increased significantly from t_1 to t_2 ($P < 0,01$) then declined significantly in t_3 ($P < 0,01$). The level of mounting in t_1 and t_3 were similar and not significantly different (**Table 24**).

Table 24 Changes in the mean levels of seven traits of sexual behaviour over time for 1987 [1]

	TEST NUMBER			L.S.D. VALUES	
TRAIT	**1**	**2**	**3**	**5%**	**1%**
Sniffing	$5,94^{a}$	$3,89^{b}$	$4,09^{b}$	1,45	1,94
Staring	$6,77^{a}$	$5,86^{a}$	$5,37^{a}$	NS	
Tongue flicks	$19,10^{a}$	$20,90^{a}$	$19,30^{a}$	NS	
Nudging	$13,00^{a}$	$14,09^{a}$	$14,00^{a}$	NS	
Flehmen	$0,371^{a}$	$0,400^{a}$	$0,514^{a}$	NS	
Intention to mount	$0,57^{a}$	$0,63^{a}$	$0,57^{a}$	NS	
Mount	$4,63^{a}$	$8,03^{b}$	$5,40^{a}$	1,91	2,54

[1] Means without common superscripts are significantly different

The dramatic change in mounting behaviour over the three tests (**Tables 24** and 25) could most likely be attributed to a similar pattern of change in environmental temperature rather than any effect peculiar to hormone treatments. The average temperatures (recorded half-hourly) for t_1, t_2 and t_3 were 18,6, 14,4 and 16,9°C respectively.

The only hormone x time interaction to attain significance ($P < 0{,}05$) was that for mounting behaviour (**Table 25**). All treatments, with the exception of the ODP and TP + Anti $E_{1,2}$ wethers, showed their highest level of mounting behaviour in t_2. The ODP wethers showed a gradual decrease in mounting behaviour over the three tests. The TP + Anti $E_{1,2}$ wethers increased their mounting behaviour from t_1 to t_2 and it remained static in t_3. Changes in mounting behaviour, over time, were non-significant for the C, DHT, ODP, TP + Anti E_2 and TP + Anti $E_{1,2}$ wethers (**Table 25**).

Table 25 Changes in the mean levels of mounting for the seven treatments over time for 1987[1, 2]

	TEST NUMBER		
TREATMENT	**1**	**2**	**3**
Control	0,40 [a]	1,00 [a]	0,60 [a]
TP + Anti E_2	1,20 [a]	2,60 [a]	0,20 [a]
TP + Anti $E_{1,2}$	0,20 [a]	0,80 [a]	0,80 [a]
TP	14,40 [a]	27,40 [b]	21,80 [c]
ODP	9,80 [a]	8,00 [a]	6,60 [a]
DHT	0,00 [a]	1,80 [a]	0,20 [a]
DHT + ODP	6,40 [a]	14,60 [b]	7,60 [a]

1 Means within the same row without commcn superscripts are significantly different.

2 The L.S.D.'s for $P < 0{,}05$ and $P < 0{,}01$ are 6,67 and 8,98 respectively for different and 5,05 and 6,73 respectively for the same hormone treatments.

In t_1 , the TP wethers showed the highest level of mounting (**Table 25**) and the only treatment which did not mount at a significantly lower level than the TP wethers was the ODP group. The TP treated wethers maintained the highest, significantly different, level of mounting in t_2 and t_3 when compared to all treatments (**Table 25**). The ODP wethers mounted at a higher, non-significantly different, level than the DHT + ODP wethers, in the first test (**Table 25**). The situation was reversed in t_2 with the DHT + ODP wethers showing a higher incidence of mounting when compared to the ODP wethers. The difference between these two treatments approached significance at the 5% level. The situation remained the same in t_3 however, the difference was non-significant (**Table 25**).

In the first test the immunized wethers (both TP + Anti E_2 and TP + Anti $E_{1,2}$) showed a lower, though non-significantly different, level of mounting when compared to the DHT + ODP wethers. In t_2 the difference between these treatments was significant ($P<0,01$, **Table 25**), while the difference between the immunized and ODP wethers which was significant in t_1 ($P<0,05$), was non-significant in t_2. In t_3 the ODP treated wethers displayed a level of mounting which was almost signficantly different from the C, DHT, TP + Anti E_2 and TP + Anti $E_{1,2}$ treated wethers (**Table 25**).

The orthogonal comparisons carried out in this last year were the same as in the previous two years however, the TP versus TP + Anti E_2 comparison was changed to include the TP + Anti $E_{1,2}$ treatment. Thus, the TP wethers were evaluated against the average of the two immunized treatments.

The comparisons of TP versus immunized wethers and DHT + ODP and ODP versus DHT and C were both significant ($P< 0,01$) for staring, tongue flicks, nudging, intention to mount and mounting behaviour. The DHT + ODP and ODP combination of treatments was also significantly different to the DHT and C treatments for sniffing and flehmen ($P<0,01$). The superiority of the DHT + ODP treatment in terms of flehmen behaviour was further demonstrated by the fact that both the DHT + ODP and DHT versus ODP and C and DHT + ODP and C versus ODP and DHT comparisons were significantly different for this trait ($P < 0{,}01$). The significant relationships reported for the orthogonal comparisons coincide with and reinforce the results presented for main effects (**Table 23**).

The hormone x time interaction was significant for sniffing behaviour for the DHT + ODP and ODP versus DHT and C comparison ($P < 0{,}05$). The interaction for mounting behaviour was significant for the TP versus TP + Immunization comparison ($P < 0{,}01$).

The significant interaction for sniffing behaviour may be attributed to the fact that the wethers treated with DHT + ODP displayed their highest level of sniffing in t_3, a similar slightly lower level in t_1 and the lowest level in t_2. The ODP wethers showed a similar but less dramatic trend. These changes contrast with those observed for sniffing across all treatments (**Table 24**).

Both the TP wethers and the average of the immunized treatments follow a change in mounting behaviour similar to that seen for all treatments (**Table 25**) however, the magnitude of responses for TP was much greater than for the TP implanted immunized wethers. This no doubt was the cause of the significant interaction for the TP versus TP + Immunization comparison.

Antibody production

High levels of binding were produced in all years. During the early stages of immunization binding tended to drop rather sharply as the serum became more dilute. Following boosting, the curves flattened with high levels of binding being maintained for all dilutions.

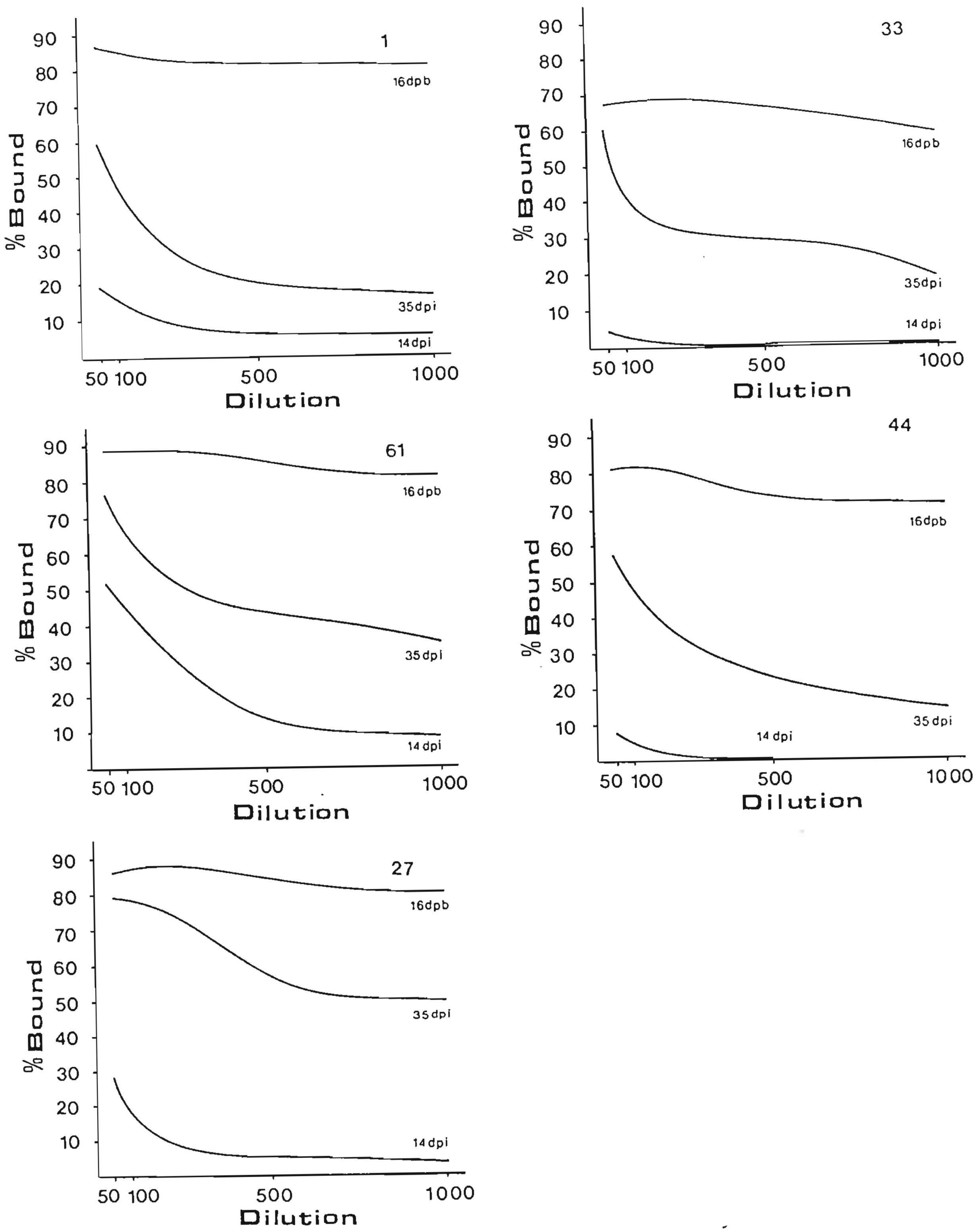

Figure 20 The % oestradiol-^{3}H tracer bound by antibody in serial dilutions of serum obtained from TP + Anti E_2 wethers in 1983. The five wethers (namely 1,61,27,33 and 44) were bled at 14 and 35 days post immunization (dpi) and at 16 days post boosting (dpb).

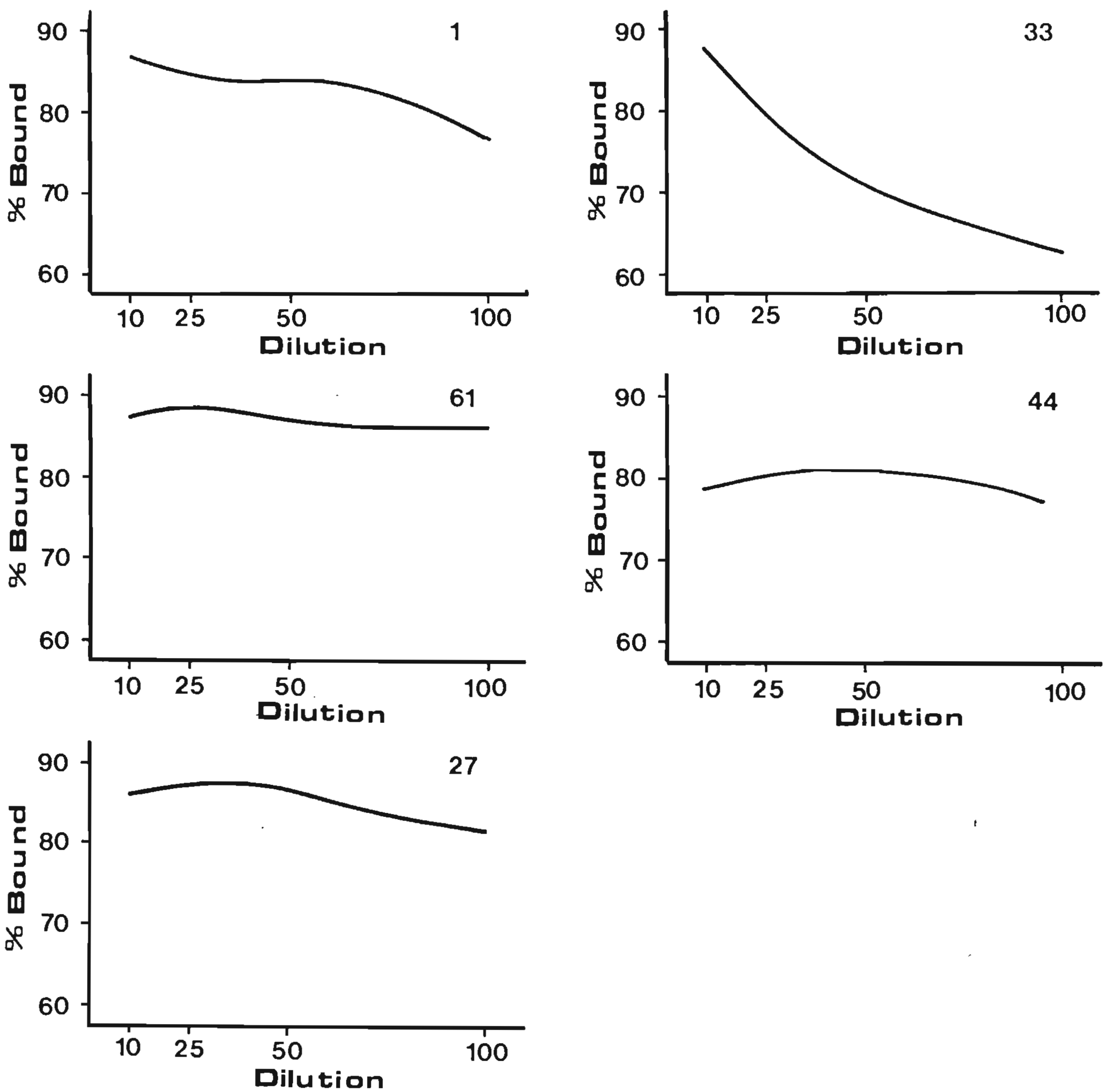

Figure 21 The average % oestradiol^{-3}H tracer bound, at 38 and 51 days post boosting, by antibody in serial dilutions of serum obtained from the five TP + Anti E_2 wethers (namely 1,61,27,33 and 44) in 1983

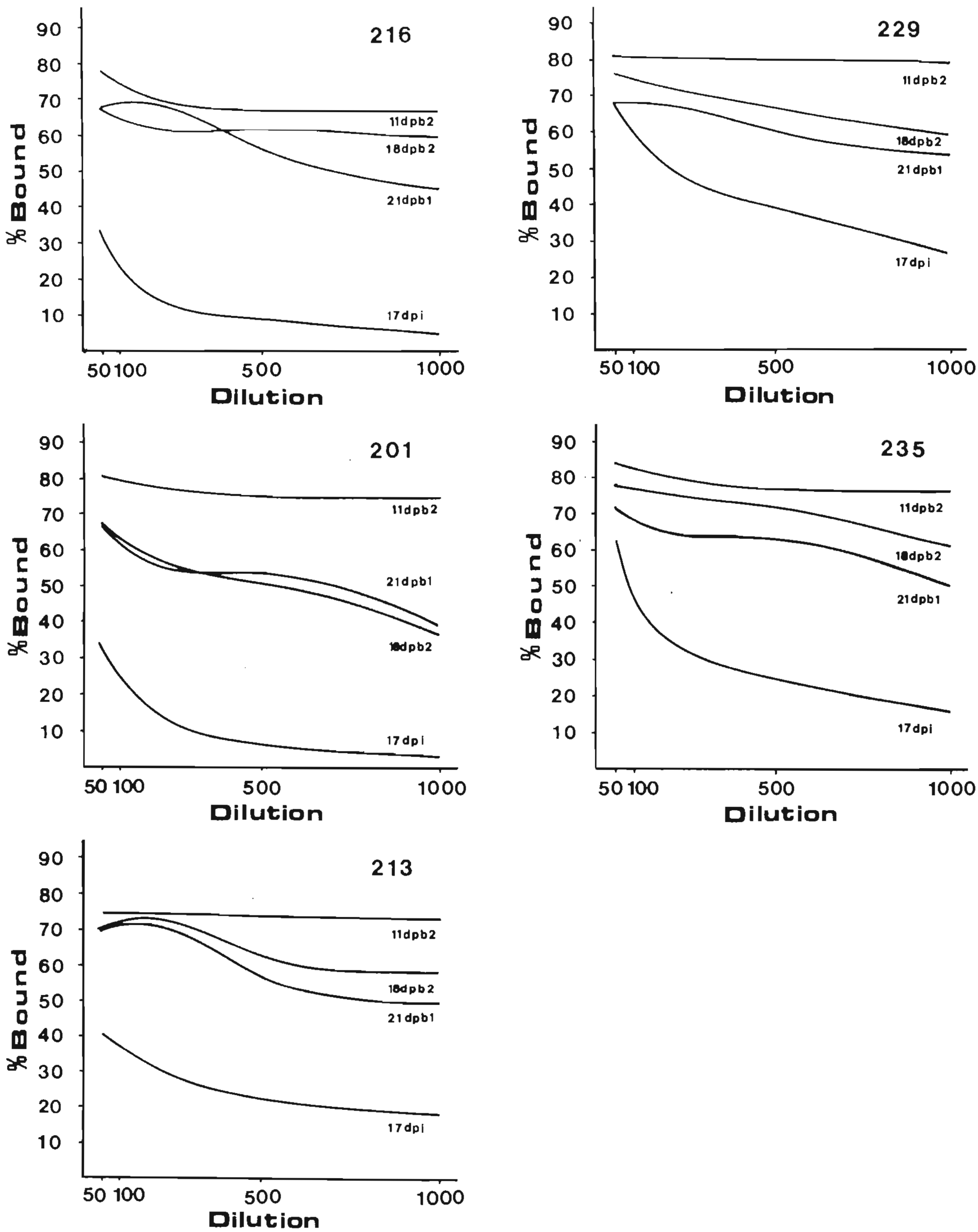

Figure 22 The % oestradiol^{-3}H tracer bound by antibody in serial dilutions of serum obtained from the TP + Anti E_2 wethers in 1984. The five wethers (namely 216,201, 213,229 and 235) were bled at 17 days post immunization (dpi), 21 days post booster 1 (dpb1) and 11 and 18 days post booster 2 (dpb2).

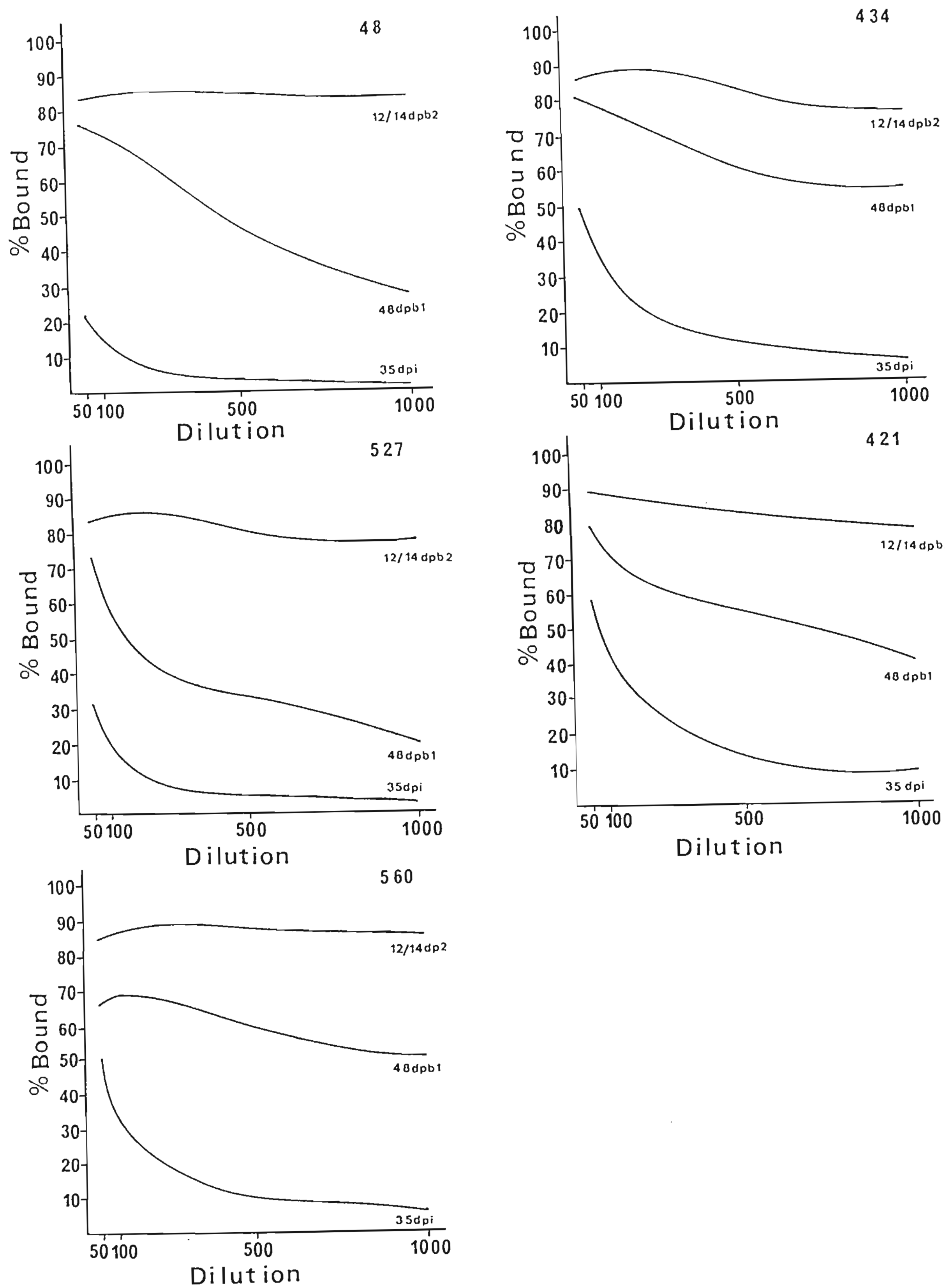

Figure 23 The % oestradiol^{-3}H tracer bound by antibody in serial dilutions of serum obtained from TP + Anti E_2 wethers in 1987. The five wethers (namely 48,527,560, 434 and 421) were bled at 35 days post immunization (dpi), 48 days post booster

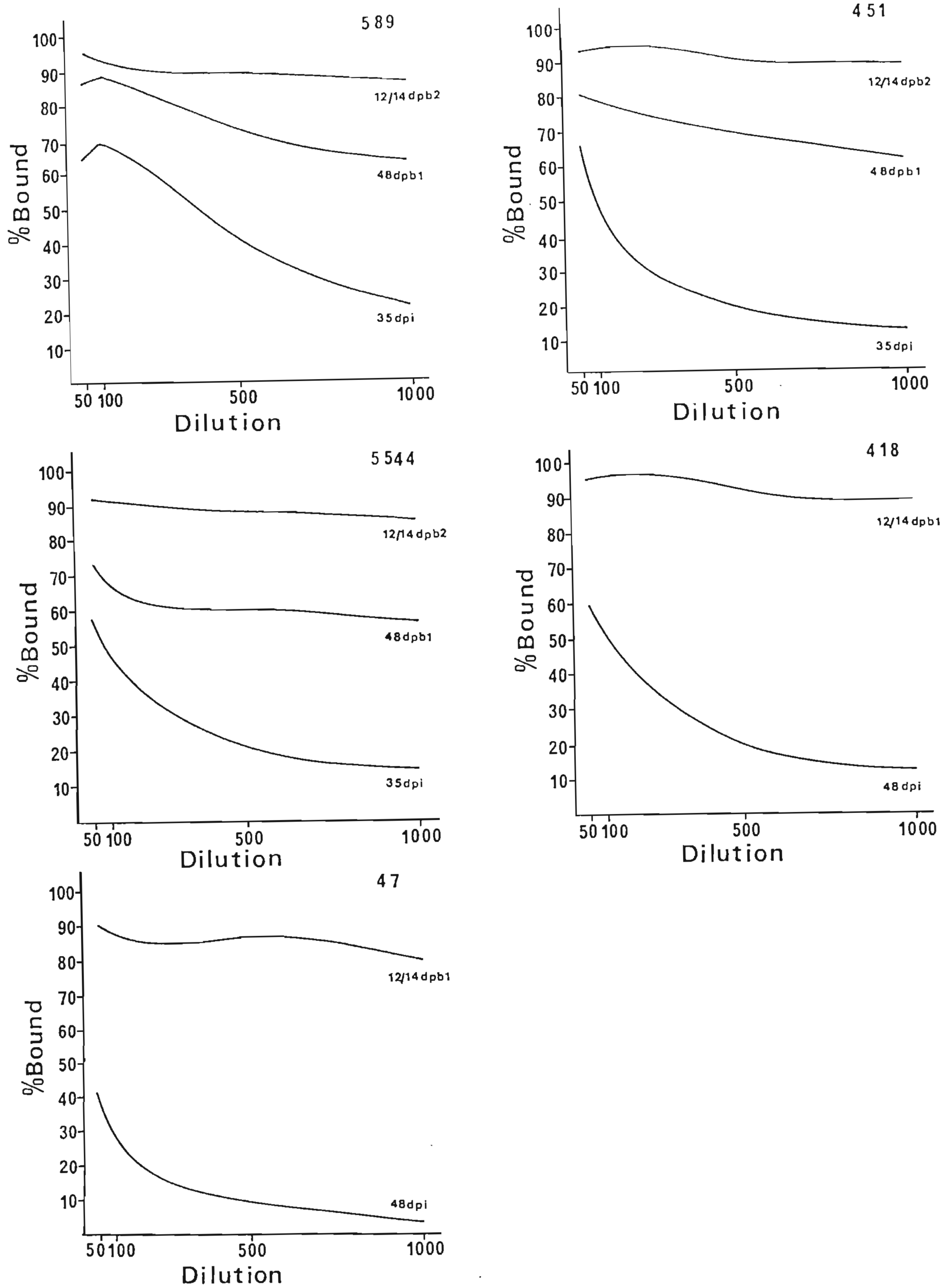

Figure 24 The % oestradiol^{-3}H tracer bound by antibody in serial dilutions of serum obtained from TP + Anti $E_{1,2}$ wethers in 1987. The five wethers (namely 589,5544, 47,451 and 418) were bled at 35 days post immunization (dpi), 48 days post booster one (dpb1) and 12 and 14 days post booster two (dpb2). The average binding for 12 and 14 dpb2 is depicted. Wethers 47 and 418 only received two

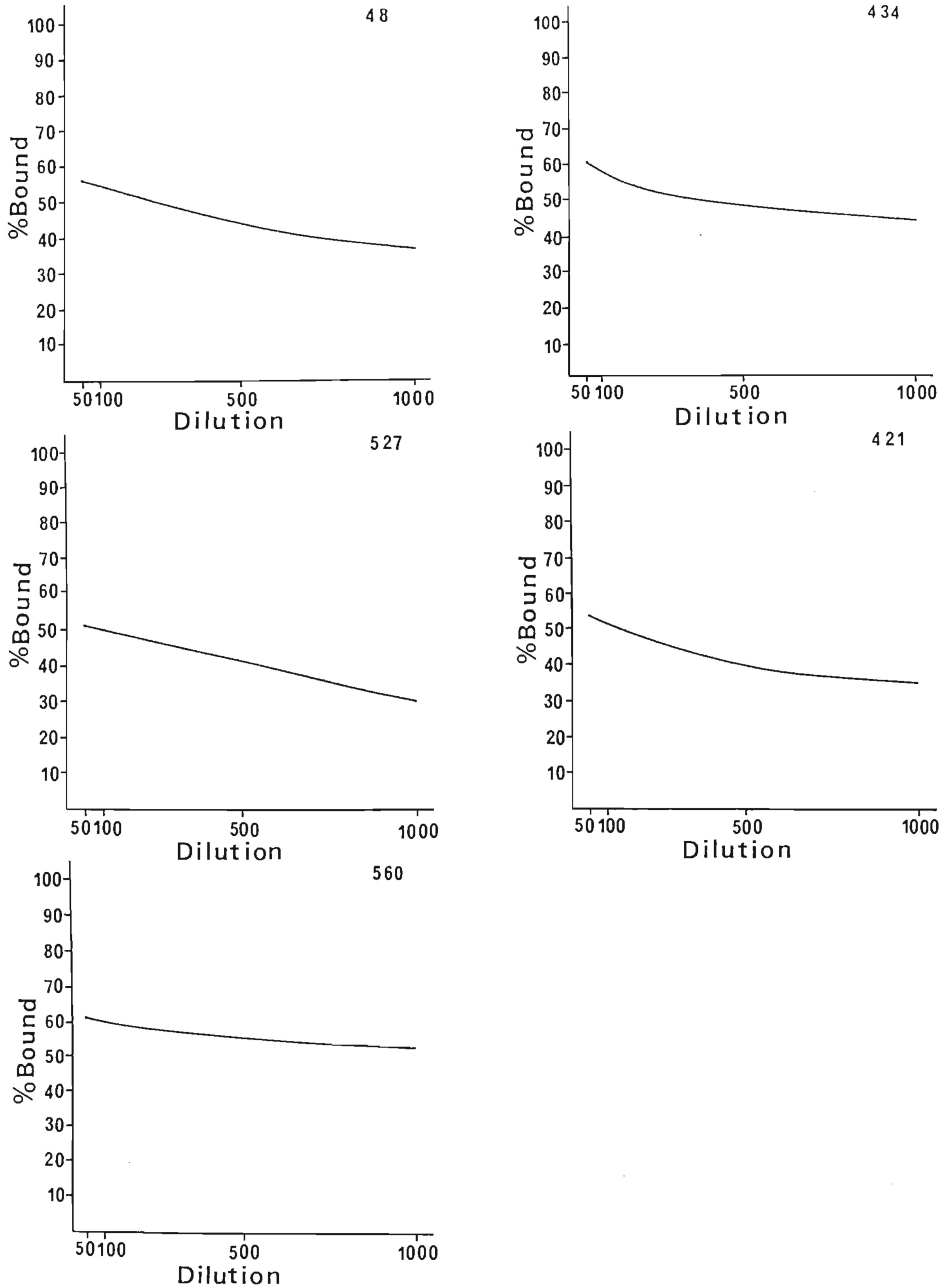

Figure 25 The % oestrone ^{-3}H tracer bound by antibody in serial dilutions of serum obtained from TP + Anti E_2 wethers in 1987. The five wethers (namely 48,527,560, 434 and 421) were bled twice at 12 and 14 days post booster two (dpb2). The

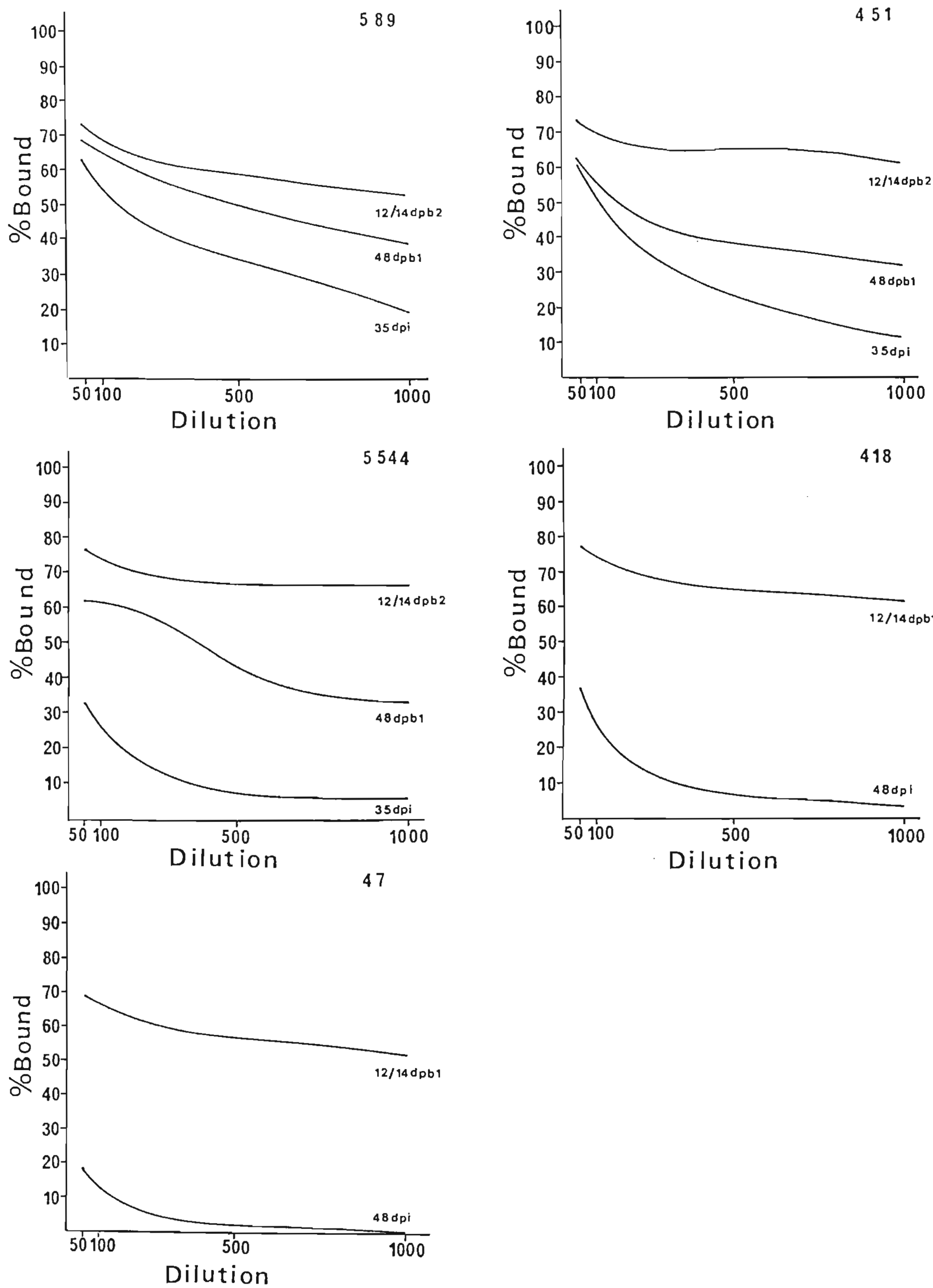

Figure 26 The % oestrone ^{-3}H tracer bound by antibody in serial dilutions of serum obtained from TP + Anti $E_{1,2}$ wethers in 1987. The five wethers (namely 589,5544, 47,451 and 418) were bled at 35 days post immunization (dpi), 48 days post booster one (dpb1) and 12 and 14 days post booster two (dpb2).

In 1983 binding reached a peak close to 16 days post boosting (16dpb, **Figure 20**). Hurn and Chantler (1980) state that peak antibody production is reached 7-10 days after booster injection. At 16dpb the titre curves were generally flat and binding was, with the exception of No. 33, greater than 80% at 1:1 000 dilution.

Although the mean percentage binding at 1:50 dilution was the same at 16 (**Figure 20**), 38 and 51 days (**Figure 21**) after boosting it was apparent that the tail of the curve had dropped considerably by the latter two dates.

In 1984 (**Figure 22**) the average binding at a 1:50 dilution 11 days after the second booster (11dpb2) was similar to that at 16dpb in 1983 (**Figure 20**), 79,1 versus 81,7% respectively. With the exclusion of wether No. 33 in 1983 average binding at 16dpb increased from 81,7 to 85,5%. In 1984 the levels of binding dropped sharply between 11dpb2 and 18dpb2 (**Figure 22**). Binding at 18dpb2 was intermediate between the levels recorded at 21 days after the first booster (21dpb1) and 11dpb2 with the exception of wether No. 201 for which the curve at 18dpb2 was similar to that at 21dpb1.

During 1987 the average binding of E_2 recorded at a 1:50 dilution was 91,4 and 94,0% at 12 and 14dpb2 respectively for the TP + Anti $E_{1,2}$ wethers (**Figure 24**) and 84,9 and 84,7% respectively for the TP + Anti E_2 group (**Figure 23**). The immunization program carried out in this last year appears to have been more effective than in the previous years. With respect to binding of E_1 the $E_{1,2}$ immunized wethers were again superior to the E_2 immunized group. At a 1:50 dilution the TP + Anti $E_{1,2}$ wethers displayed 76,5 and 70,4% binding of E_1 at 12 and 14dpb2 (**Figure 26**) respectively, while the TP + Anti E_2 wethers only achieved 57,0 and 55,5% binding for the two dates respectively (**Figure 25**). The E_2 antibody titre curves were generally flatter than the E_1 curves at 12 and 14dpb2.

For the TP + Anti $E_{1,2}$ wethers suppression of mounting behaviour was complete in three of the five wethers (**Table 26**). For the TP + Anti E_2 wethers suppression was complete in two of the wethers.

Table 26 Number of mounts achieved by individual TP + Anti $E_{1,2}$ and TP + Anti E_2 wethers in each of the three sexual behaviour tests in 1987

		TEST NUMBER		
TREATMENT		1	2	3
TP + Anti $E_{1,2}$	418*	0	3	2
	47*	1	1	2
	589	0	0	0
	5554	0	0	0
	451	0	0	0
TP + Anti E_2	48	0	0	0
	434	5	7	0
	560	0	0	0
	527	1	3	0
	421	0	3	1

* Wethers 418 and 47 only received one booster injection and not two. These wethers served as replacements for some of those originally immunized but which were stolen just prior to the first booster injection.

The two $E_{1,2}$ immunized wethers which did mount had received only a primary immunization and one booster injection (see explanation below **Table 26**) as opposed to the three injections administered to the rest of the wethers. It is thus tempting to speculate that the antibody produced following two injections was inferior to that produced following three. Hurn and Chantler (1980) suggest prolonged immunization may result in a more stable antibody of higher titre.

If wethers are classified as either mounters or non-mounters it is evident (Table 27) that lack of mounting behaviour could to some extent be accounted for by ability to bind E_2 and E_1.

Table 27 The levels of binding of E_2 and E_1 shown by immunized wethers (1987) when classified as either mounters or non-mounters[1]

TRACER	DILUTION ANTIBODY	% BINDING	n MOUNTERS	n NON-MOUNTERS
E_2	1:10 000	> 60%	2(418, 434)	4(48, 560, 589, 5 544)
		< 41%	3(47, 421, 527)	1(451)
E_1	1:1 000	> 51%	2(47,418)	4(451,560, 589, 5 544)
		< 44%	3(421, 434, 527)	1(48)

1 The I.D. No.'s of the wethers are given in parentheses.

Perhaps the explanation for mounting behaviour could lie in the quality of antibody and/or its ability to recognise both E_2 and E_1 well. Based on the cross-reactivity studies conducted, it would appear as if the latter possibility does not account for mounting behaviour or lack of it.

The antisera produced in the first two years appeared not to cross-react significantly with E_1. In 1983 antisera were tested at 23 days after boosting. Two of the wethers (No. 1 and No. 33) produced antiserum which gave rise to reasonable E_2 standard curves implying that their antisera were of acceptable quality. The antisera from the remaining three wethers appeared not to recognise E_2 at levels < 10pg and binding at these levels was equivalent to that at 0pg. At values greater than 10pg binding dropped sharply. The sera from these three wethers was evaluated for cross-reactivity at an earlier date, namely 16dpb. Two of the wethers (No. 27 and No. 44) produced better curves while the remaining wether (No. 61) produced a curve similar to that at 23 days after boosting.

In 1984 the antisera were tested for cross-reactivity at 18dpb2. All the E_2 curves tended to be rather flat. The antiserum from one wether (No. 229) was evaluated at 11dpb2. The slope of the curve indicated that the antibody present was of superior quality to that found at 18dpb2.

The antisera produced in 1987 seemed to be of good quality since the E_2 standard curves dropped sharply from 0 to 500pg. Antisera recognised E_2 more readily than E_1 (regardless of immunization treatment). The mean % crossreaction (±S.E.) for the TP + Anti E_2 and TP + Anti $E_{1,2}$ were 5,65 ± 1,00 and 8,01±1,25% respectively.

Serum hormone levels

a) Testosterone

The values for serum androgen (testosterone plus dihydrotestosterone, **Table 28**) obtained prior to implantation in 1983 (using the RIA kit) were higher than the level of approximately 0,40ng/mℓ expected for wethers (Schanbacher, 1976). The values obtained in 1984 (antiserum donated by Dr. J. Morgenthal) and 1987 (antiserum donated by Dr. G.D. Niswender) were closer to those reported by Schanbacher (1976). However, the immunized wethers (TP + Anti E_2) were an exception, in 1984, with a mean ±S.E. of 2,78 ± 0,094ng/mℓ. This was not the case in 1987 where higher mean values tended to be associated with high values for individual wethers within a given treatment. The fact that the immunized wethers in 1987 did not have abnormally high pre-implant androgen levels may be ascribed to the 30 week interval between the first and second booster injections as opposed to the 4 week interval used in 1984.

In 1983, the assay was split over two consecutive days. The first four treatments were assayed on day one with the remaining two being assayed on the second day (**Table 28**).

Table 28 The mean (±S.E.) testosterone levels (ng/mℓ) for each treatment prior to implantation in 1983, 1984 and 1987

	Treatment	n	1983	1984	1987
1.	Control	5	3,00 ± 0,89	0,21 ± 0,04	0,82 ± 0,72
2.	TP + Anti E_2	5	4,47 ± 1,12	2,78 ± 0,94	0,19 ± 0,07
3.	TP + Anti $E_{1,2}$	5	-	-	0,61 ± 0,47
4	TP	5	4,96 ± 0,90	0,65 ± 0,31	0,20 ± 0,07
5	ODP	5	4,84 ± 1,29	0,41 ± 0,20	1,18 ± 0,64
6.	DHT	5	2,52 ± 0,76	0,34 ± 0,08	0,80 ± 0,39
7	DHT + ODP	5	2,75 ± 0,92	0,27 ± 0,01	1,41 ± 1,21

The values on day one were 3,2 x those obtained on day two, while the pool serum was 1,9 x higher on day one than on day two. The differences were surprising in the light of the good recoveries (> 90%) and similarity of the standard curves obtained in both assays. The values presented for the ODP and DHT + ODP wethers (**Table 28**) have been adjusted for the difference in pool values between the two assays.

Closer examination of the wethers used in 1983 revealed evidence of residual testicular tissue, located subcutaneously, suggesting that castration had been incorrectly carried out. The wethers utilized in 1984 and 1987 showed no evidence of any residual testicular tissue on examination prior to initiation of the investigation in each year. However, of those wethers which initiated some form of sexual behaviour during the pre-implant acclimatization test in 1987 63,6% (seven out of 11) had relatively high pre-implantation androgen levels. Wethers which displayed sexual behaviour prior to hormone treatment did so at a very low level and seldom went beyond courtship behaviour (only two of the 11 showed intention to, or attempted to mount).

The variation between assays using the antiserum donated by Dr. J. Morgenthal was generally small with a C.V. of 9,9% for pool values (1984). The C.V. obtained for pool values in 1987 (antiserum from Dr. G.D. Niswender) was 10,2%.

The level of testosterone measured for immunized wethers following implantation were higher than those measured in the TP wethers (**Table 29**).

The drop in testosterone seen in the TP wethers, of 1983, following implantation suggests that testosterone production by the residual gonadal tissue was being depressed (by negative feedback on LH). At the same time TP released from the implant was also being depressed by gonadal testosterone (low concentration gradient). The levels measured

Table 29 The mean (±S.E.) testosterone levels (ng/mℓ), for two sample dates, in the TP, TP + Anti $_{1,2}$ and TP + Anti E_2 implanted wethers for 1983, 1984 and 1987

TREATMENT	n	DATE	1983 *			1984			1987		
TP + Anti E_2	5	1	8,84	±	1,17	37,46	±	2,48	4,00	±	0,68
	5	2	6,88	±	2,06	43,47	±	3,98	4,13	±	0,97
TP + Anti $E_{1,2}$	5	1		-			-		8,85	±	3,20
	5	2		-			-		7,37	±	1,75
TP	5	1	3,57	±	0,66	18,91	±	0,92	3,38	±	0,45
	5	2	2,54	±	0,48	17,40	±	1,09	2,89	±	0,41

* Samples assayed in the same assay as first four treatments in Table 26

Table 30 The mean (± S.E.) DHT levels (ng/mℓ), for two sample dates, in the DHT and DHT + ODP implanted wethers for 1983, 1984 and 1987

TREATMENT	n	DATE	YEAR 1983	YEAR 1984	YEAR 1987
DHT + ODP	5	1	0,330 ± 0,058	0,413 ± 0,213	0,156 ± 0,041
	5	2	0,372 ± 0,122	0,700 ± 0,190	0,177 ± 0,033
DHT	5	1	0,508 ± 0,080	0,773 ± 0,199	0,146 ± 0,041
	5	2	0,154 ± 0,037	0,775 ± 0,194	0,094 ± 0,016

Table 31 The mean (± S.E.) E levels (pg/mℓ), for two sample dates, in the TP, TP + Anti $E_{1,2}$ TP + Anti E_2, ODP and DHT + ODP wethers for 1983, 1984 and 1987

TREATMENT	DATE	N	1983	N	1984	N	1987
					YEAR		
TP	1	5	16,80 ± 6,36	1	1,83	5	1,51 ± 0,32
	2	5	15,46 ± 3,95	5	1,95 ± 0,28	5	1,48 ± 0,30
TP + Anti E_2	1	5	36,04 ± 6,13	4	13,00 ± 1,88	5	3,57 ± 0,90
	2	5	25,38 ± 6,07	5	6,94 ± 2,08	5	2,55 ± 0,56
TP + Anti $E_{1,2}$	1	5	-		-	5	6,21 ± 1,63
	2	5	-		-	5	4,80 ± 2,22
ODP	1	4*	50,70 ± 6,81	5	26,61 ± 4,35	5	17,06 ± 0,96
	2	4*	31,38 ± 2,86	5	25,20 ± 2,36	5	16,30 ± 1,03
DHT + ODP	1	5	43,45 ± 3,11	5	37,19 ± 2,45	5	16,06 ± 0,49
	2	5	48,36 ± 10,45	5	28,95 ± 1,53	5	17,13 ± 2,68

* One wether displayed levels approximately 3 x those seen in other ODP wethers suggesting that the implant was incorrectly sealed

after implantation may represent a state of equilibrium between negative feedback and the rate of release from the implant.

b) DHT

In the first two years problems were experienced using the RIA kit. Poor DHT recoveries were obtained (< 60%) while in 1984 reconstitution of the freeze-dried charcoal/ dextran mixture was incomplete with resultant poor repeatability between duplicates and poor correspondence between sample dates. Therefore the DHT values from 1983 and 1984 should be regarded with suspicion (**Table 30**). In 1987 a greater degree of success was obtained using column chromatography and a C.V. of 17,3% was obtained for pool values between successive assays.

The DHT levels simulated in 1987 are very close to the levels of 0,14 and 0,16 ng/mℓ reported by Schanbacher (1976) and D'Occhio, Schanbacher & Kinder (1983).

c) Oestradiol

Immunization against E_2 resulted in elevated E levels in the TP + Anti E_2 wethers when compared to the TP wethers (**Table 31**). One TP + Anti E_2 wether in 1984 (first sample date) had an E_2 level of < 1 pg on the standard curve and was not included in the mean. By the second sample date the levels of E_2 for this wether had risen to 4,0 pg/mℓ.

The levels of E_2 recorded for the TP implanted wethers in 1984 and 1987 were generally low (**Table 31**) and in some cases undetectable (1984). These low levels were surprising in the light of the work of Sanford, Simaraks, Palmer and Howland (1982) who reported oestrogen levels for intact rams in the range 8 to 14 pg/mℓ corresponding to testosterone levels of 5 to 17 ng/mℓ. The higher levels of testosterone reported by Sanford <u>et al</u>. (1982) corresponded with the peak of the breeding season and are similar to the levels recorded for the TP wethers (**Table 29**) in 1984. The testosterone levels observed in 1987 (TP wethers, **Table 29**) were considerably lower than those for 1984 and were more consistent with the 4,96ng/mℓ reported by Schanbacher (1976) and the minimum value of Sanford et al. (1982). In spite of the difference between testosterone levels in 1984 and 1987 the E_2 levels recorded for TP wethers were similar (**Table 31**). It is possible that the levels of T which are needed to stimulate E_2 production through increased aromatase activity (Hutchinson & Steimer, 1986) are not very high and that maximum aromatase activity gives rise to low but adequate levels of E_2.

The levels of E obtained for the ODP and DHT + ODP wethers in 1984 (**Table 31**) were considerably higher than those reported by Sanford et al. (1982) while the levels reported for 1987 are just above the maximum reported by these workers.

In 1984 only one oestrogen assay was carried out as a result no between assay C.V. for pool values could be calculated. The C.V. for pool values in 1987 was 9,8%.

DISCUSSION

Role of the various hormones in the control of sexual behaviour

Results from the present research support the findings of previous studies with respect to the differential control of sexual behaviour by the various steroid hormones (Mattner, 1976; D'Occhio & Brooks, 1976; Parrot, 1978; D'Occhio & Brooks, 1980; Parrot & Baldwin, 1984).

The administration of TP stimulated the highest levels of mounting behaviour in each year. Oestradiol dipropionate resulted in lower levels of mounting than did TP. Simultaneous treatment with DHT and ODP gave rise to levels of mounting similar to TP in 1983 and 1984. In 1987 the DHT +

ODP wethers were intermediate between the TP and ODP wethers (non-significantly different from the ODP treatment) for mounting behaviour. Administration of DHT alone did not stimulate mounting beyond the levels seen in the controls. The lack of a strong DHT/ODP synergism in 1987 might be attributed to the low, although realistic, DHT levels when compared to previous years. All the C and two of the DHT wethers mounted at low to negligible levels in 1984. While in 1987, three C and two DHT wethers mounted. Mounting behaviour was generally weak and precopulatory courtship behaviour was not consistently displayed by all wethers. Contrary to these findings most authors report no or negligible mounting behaviour (Parrot, 1978; D'Occhio & Brooks, 1980, 1982).

It has been suggested that CNS tissue has a lower T threshold than the peripheral sex organs (D'Occhio & Brooks, 1982) and levels of T between 0,32 and 0,65 ng/mℓ elicited nudging and mounting behaviour (centrally controlled). Full mating activity (intromission and ejaculatory reflex) was achieved at a level of 1,26 ng/mℓ. On this basis 43,3% of the wethers in 1984 and 34,3% in 1987 should have been capable of mounting prior to implantation. Furthermore, E has a far greater potency than T (microgram versus milligram levels) at the CNS level (Ryan, Naftolin, Reddy, Flores & Petro, 1972). Thus it would appear as if mounting behaviour is possible in a fairly large proportion of wethers without there being overt signs of residual testicular tissue following castration.

Quality of antibodies produced

The procedures carried out in this study to determine the extent of antibody binding are comparable with the technique of equilibrium dialysis currently used by other authors (Thompson & Honey, 1984; Walker et al., 1984). The procedure used for the determination of antibody cross-reactivity was also similar to that used by these workers. Muller (1983) described the technique of competitive RIA, which he considered to be superior to equilibrium dialysis and which facilitates accurate calculation of K, the affinity constant, for a given antiserum. Without K, comparison between various studies is difficult. Notwithstanding the lack of K values, in the present and other studies, the antibodies produced by the TP + Anti E_2 and TP + Anti $E_{1,2}$ wethers appear to be of similar quality to those generated by other workers (Nieschlag et al., 1974; Thompson & Honey, 1984; Walker et al., 1984). However, the nature of the E_2 standard curves obtained for some of the antisera (1984) would suggest that the quality of antibody was not suitable for use in RIA. The antibody produced in1987 appeared to be of superior quality to that in 1984 suggesting that the introduction of a longer interval between the first and second booster injection (30 as opposed to 4 weeks) and the use of Freunds complete adjuvant instead of saline were successful.

Failure to demonstrate the central role of DHT

In spite of the high levels of binding of E_2 stimulated in the immunized wethers in 1983 and 1984 the suppression of E_2 was incomplete.

In the first two years the testosterone levels reported for the TP wethers were at the top of the physiological range (Sanford et al., 1982). The TP + Anti E_2 wethers displayed levels of testosterone that were approximately double those recorded for the TP wethers, giving rise to E_2 levels which overwhelmed the E_2 antibodies present. The supraphysiological testosterone levels in the TP + Anti E_2 wethers probably arose as a result of stimulated testosterone metabolism, caused by the action of the antibodies present, which in turn created a steeper testosterone concentration gradient between the implant and the tissue surrounding it (Kincl & Rudel, 1970). This led to a greater rate of steroid release. Furthermore, testosterone from extragonadal sources such as the adrenal gland was probably much higher (1984). The high levels of testosterone seen in the immunized wethers prior to implantation lends support to this idea. No doubt immunization removed the negative feedback by E_2 on LH (Schanbacher, D'Occhio & Gettys, 1983).

By reducing the length of TP implant administered to the immunized wethers in 1987 it was possible to simulate T and E_2 levels which were within a more realistic range. Immunization against E_2 and E_1 reduced mounting behaviour to levels similar to that seen in the C and DHT wethers. The evidence for E_1 induced mounting behaviour in the DHT wethers (1983) suggests that the high level of sensitivity shown by the antibody for E_1 in 1987 further contributed to the success of immunization in this year.

No behavioural trait/s could be associated exclusively with the TP + Anti E_2 and TP + Anti $E_{1,2}$ wethers which would in turn have highlighted the nature of dihydrotestosterone's central nervous action. Clearly however, DHT (1987) contributed to the action of E_2 in respect of courtship behaviour (specifically tongue flicks and nudging), enhanced the incidence of flehmen and to some extent stimulated mounting behaviour (**Table 23**).

Possible alternate mechanisms for dihydrotestosterones action

Work in the rat (Parrot, 1975) has demonstrated that DHT is responsible for sensitization of the penis. Bonsall, Rees & Michael (1985) reported that DHT is preferentially bound in the male genital tract structures of the monkey. Recently Katz & Price (1986) established that penile neurectomy in young bulls effectively reduced mounting behaviour to levels significantly lower than bulls with surgically deflected penises (intromission prevented, penile sensory system intact) and concluded that sensory stimulation of the penis facilitates the expression of sexual behaviour. In wethers where penile protrusion does not occur during short-term replacement therapy (less than 6-8 weeks duration) stimulation would be via the penile sheath which covers the relatively immobile penis.

The intact male animal shows a sexually quiescent post-ejaculatory refractory period (Katz & Price, 1986). Clearly there must be some form of reward associated with ejaculation. In this regard it is worthwhile to note the performance of the Afrikaner bulls (Chapter 2), which never ejaculated, but displayed the highest level of mounting behaviour of all breeds (B. taurus and B. indicus).

Since courtship behaviour involves the testing of female receptivity it would appear as if an animal motivated by increased penile stimulation (DHT + ODP versus ODP, accepting that E_2 is the primary hormone associated with initiation of mounting) will indulge in higher levels of courtship and as a result mounting behaviour in an attempt to satisfy the urge to copulate.

The presence of androgen receptors capable of binding both T and DHT, but with higher affinity for DHT, in the pituitary and hypothalamus of the ram (Thieulant & Pelletier, 1979; Pelletier & Caraty, 1981) has been demonstrated. It cannot therefore be categorically stated that DHT has no CNS function. However, Bonsall et al. (1985) point out that T, rather than DHT, is the principal androgen in the brain cell nuclei and state that 5 α-reductase levels may not produce sufficient levels of DHT to compete with T for binding to CNS androgen receptors.

Prospects for further work

The apparent lack of central action by DHT following immunoneutralization of E reported in this study and the failure of a recent study (Parrot & Baldwin, 1984) which made use of implants directly into the appropriate area of the CNS, pose the question as to the direction of future studies.

It has been demonstrated that a low testosterone threshold exists for initiation of mounting behaviour (D'Occhio & Brooks, 1982) in wethers. The relatively high testosterone levels measured in the TP wethers of 1984 and the lower levels reported for 1987 were associated with low but similar levels of E_2 (< 2pg/mℓ) leading to a conclusion that the levels of E_2 in the CNS required to stimulate sexual behaviour may bear little relationship to serum levels, which have been reported to be as high as 13pg/mℓ in intact rams (Sanford et al., 1982).

Parrot & Baldwin (1984) have suggested that the lower levels of mounting generally seen in E_2 treated wethers may in fact be due to E_2 induced hyperprolactineamia (Schanbacher, 1979) which in turn could have impaired the effectiveness of E_2 (Bailey & Herbert, 1982).

It would appear that a new approach is needed in studies of the control of male sexual behaviour. Perhaps work at the receptor level is necessary. There is a clear need to differentiate between the relative importance of T, DHT and E_2 at the central nervous level.

GENERAL DISCUSSION

The high incidence of sexually low-response rams gives cause for concern. In the absence of routine libido tests such animals escape detection and no doubt contribute to poor flock conception rates. Of greater concern is the fact that such behaviour could be inherited. The characteristic behaviour displayed by these rams ensures their ready identification in pen tests. The proportion of low-response rams can be limited by prolonging heterosexual contact after weaning (Le Roux & Barnard, 1974). Whether or not such rams can be identified on the basis of some readily determined endocrinological parameter remains to be clarified and should act as a catalyst for more intensive research into the mechanism controlling behaviour.

There can be no doubt that bulls undergo some form of learning process during their first few exposures to oestrous females. The implication that the majority of bulls undergo a gradual learning process at a similar rate to one another suggests that two or three tests are sufficient for a bull to show his true potential. Clearly, the rate of learning /adaptation in B. indicus bulls appears to be slower than in B. taurus bulls with approximately seven tests being needed before a reliable estimate of SC can be obtained.

Evidence for a negative relationship between growth rate and libido was inconclusive. If such a relationship was present it is likely that it was masked by environmental effects. Sundby and Velle (1983) have demonstrated that bulls with higher plasma testosterone levels (following human chorionic gonadotrophin injection) had significantly higher growth rates from 3- to 11- months of age than those with lower levels. Thus if a negative relationship exists between growth rate and libido, the suggestion is that high testosterone levels are related to poor libido and bull libido has not been found to be related to testosterone levels *per sè* (Blockey & Galloway, 1978). The possibility exists that libido could be mediated by hormones arising from extragonadal sources. For example, Johnson, Welsh and Juniewicz (1982) have demonstrated an antagonistic relationship between plasma cortisol and testosterone, while Galbraith, Dempster and Miller (1978) demonstrated that bulls exhibited both a greater ADG and higher growth hormone and prolactin levels than steers. It would appear as if research into growth rate versus libido should be aimed at clarification of the role of the various sex hormones in respect to their control of libido, at the same time their relationship with the growth regulating hormones should be investigated.

The poor performance by the *B. indicus* bulls (Chapter 2) and the suggestion of an observer/environment effect gave rise to speculation as to the suitability of the SC test for the determination of libido in these breeds. The observer effect, together with the possibility of enhanced nocturnal sexual behaviour were evaluated for Afrikaner bulls in Chapter 3. The fact that bulls showed no preference for nocturnal mating behaviour and were not influenced by the presence of an observer suggested that restrained oestrous females do not present the ideal stimulus to bulls. The ability of the test technician to identify suitably receptive or sexually attractive teaser females using conventional heat spotting techniques is to be questioned indicating a need for research into this area of reproduction physiology. However, the willingness of the Brahman and Afrikaner bulls to mount suggests that intensive selection for performance on such tests could lower their sexual threshold and help them overcome the depressive effects of agonistic interaction and less than perfectly receptive teaser females while at the same time improving the fertility of *B. indicus* breeds.

The absence of a significant relationship between a bull's libido and herd fertility suggests that the 4% bulling rate commonly recommended is wasteful, particularly when one considers that bulls achieved acceptable conception rates (CR), over a limited period of time, when mated at rates of 2,9 and 2,5% in 1983/84 and 1984/85 respectively. Certainly it would appear as if differences due to libido, as measured in the present research, would only become readily apparent at 2% bulls. To recommend heavier work loads for high libido bulls without due consideration being given to semen quality and testicular size would be foolhardy. Thus a holistic approach to research into bull fertility is necessary with suitable techniques being developed for the accurate prediction of each aspect of fertility.

In spite of the non-significant effect of SC on CR the differences of 2,9 and 4,7% (**Table 16**) in the veld mating study (Chapter 4) using high and medium SC bulls and the 10,8% advantage in CR held by high over low SC bulls in the pasture mating study (Chapter 3) makes interesting economic analysis (**Appendix A3**). It would appear that at an 11% advantage in CR utilization of high SC bulls becomes a profitable option.

Results from the first three investigations carried out indicated the urgency for a better understanding of the nature of steroid hormone control of sexual behaviour. The llast investigation suggested that dihydrotestosterone plays little or no role in the central control of sexual behaviour. The effect of dihydrotestosterone appears to be largely via its peripheral sensitisation of the sex organs leading to increased stimulation being received at CNS level which in turn could enhance mounting behaviour. The likelihood that serum hormone levels bear little relationship to the levels active in the CNS suggests that research continue at central hormone receptor level. The relationship between testosterone and its metabolites needs to be clearly established at this level.

Research into the relationship between the various steroid hormones and their role in male reproduction has gathered momentum in recent years. The technique of testosterone response to exogenous GnRH administration for the prediction of SC in bulls has shown some promise but the relationship is somewhat confused in rams. A bull or ram's ability to respond to the visual stimuli associated with courtship measured in terms of LH response could modify rate of testosterone induced aromatase activity which may in turn enhance sexual behaviour. It is the elucidation of such interrelationships which will ultimately lead to the manipulation of the reproductive capacity in genetically superior sires.

SUMMARY

Chapter 1

Thirty-one Ile de France stud rams with no previous heterosexual experience were subjected to three libido tests. Eight rams (25,8%) were classified as sexually low-response. During pen tests such rams displayed a characteristic "fear" response which included odd baaing sounds, agitation, attempts to leave the pen, staring through cracks in the pen and avoiding eye contact with the ewe. The proportion of rams showing normal sexual behaviour did not increase over the three tests. The level of mounting increased significantly, with increasing experience, while the number of services completed showed a non-significant increase. Following flock mating two of the low-response rams showed no evidence of having marked any ewes. These rams showed a preference for homosexual company and displayed sex-like behaviour toward the fellow rams. Five of the original eight low-response rams had a common sire suggesting that sexual behaviour may be influenced genetically.

Chapter 2

A group of young beef bulls (n=54) representing nine breeds were evaluated for libido using five serving capacity (SC) tests (bulls tested in groups of three) followed by one libido score test (bulls tested as individuals). Bos indicus bulls displayed significantly lower levels of libido than did Bos taurus bulls. It was suggested that their poor performance may have been in part due to their inability to adapt to the test environment. Bulls underwent a definite learning process over the five SC tests. The increase in SC was not significant for all breeds. Serving capacity reached a peak after four tests. However, a bull's SC relative to his conspecifics appeared to have stabilised after two or three tests. The use of oestrous teaser females held no advantage over the use of properly restrained non-oestrous

teaser females. Agonistic behaviour did not depress SC but individual exceptions were noted. Growth rate had an inconsistent effect on SC. The number of services completed during the individual test was highly significantly correlated with SC.

Chapter 3

Some of the factors which influence the pen and subsequent pasture mating activity of young (20 months of age) Afrikaner bulls were investigated. The bulls (n=18) were subjected to 12 Blockey-type pen tests. Eight of these bulls, four high and four low serving capacity, were subsequently mated to small groups of oestrous-synchronised females under pasture conditions. During the serving capacity tests bulls showed no preference for nocturnal sexual behaviour and were not hindered by the presence of personnel scoring their activity. Many of the bulls did not serve consistently from one test to another; however, their serving capacity improved with progression of the tests. The inconsistent serving behaviour of the bulls suggests that the restrained females do not present the ideal mating stimulus. Furthermore, agonistic interactions had a depressive effect on sexual behaviour. There was however, a significant relationship between pen and pasture mating behaviour. High serving capacity bulls achieved superior, though non-significantly different, conception rates to low serving capacity bulls. Pasture mating performance was significantly influenced by degree of cow receptiveness, the bull's previous activity and duration of his exposure to an oestrous female within a given observation period. The need to identify the specific oestral characteristics which attract and promote copulatory behaviour in <u>B. indicus</u> bulls clearly exists.

Chapter 4

A trial was conducted over two years in an attempt to relate bull libido (libido score method) to conception rate (CR) following a restricted breeding season (21 days). Six bulls (three high and three medium to low) were evaluated in the first year and four (two high and two medium) in the second. The average bulling percentages were 2,9 and 2,5% in the first and second seasons respectively. Cow groups were balanced for calving date, condition score and lactational status.

There was no significant difference in CR between high and medium libido bulls in both years. The commonly recommended bulling rate of 4% appears to be somewhat conservative. Further work using larger groups of males and females is suggested. By identifying superior bulls a farmer stands to increase weaning mass and reduce bull costs per calf born.

Chapter 5

An investigation was conducted into the hormonal control of sexual behaviour in the ram. The technique of active immunization against specific steroid hormones was used in an attempt to highlight the possible action of dihydrotestosterone (DHT) on mounting behaviour at the level of the central nervous system (CNS). In wethers oestradiol (E_2) therapy causes suboptimal levels of mounting when compared to testosterone (T) therapy. Simultaneous administration of DHT and E_2 results in levels of behaviour equivalent to those seen in T treated wethers. Central aromatization of T gives rise to DHT and E_2. Wethers immunized against E_2 and a combination of oestrone (E_1) and E_2 were implanted with testosterone propionate (TP). Any mounting behaviour seen in treated wethers above that seen in the controls could have been attributed to the central action of DHT.

Immunization reduced mounting behaviour to levels similar to that seen in the control wethers. It would appear as if DHT has no or limited central nervous action. Dihydrotestosterone probably exerts its effect peripherally through sensitisation of the penis leading to enhanced mounting behaviour. Suggestions have been made for further work.

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APPENDICES

APPENDIX A1

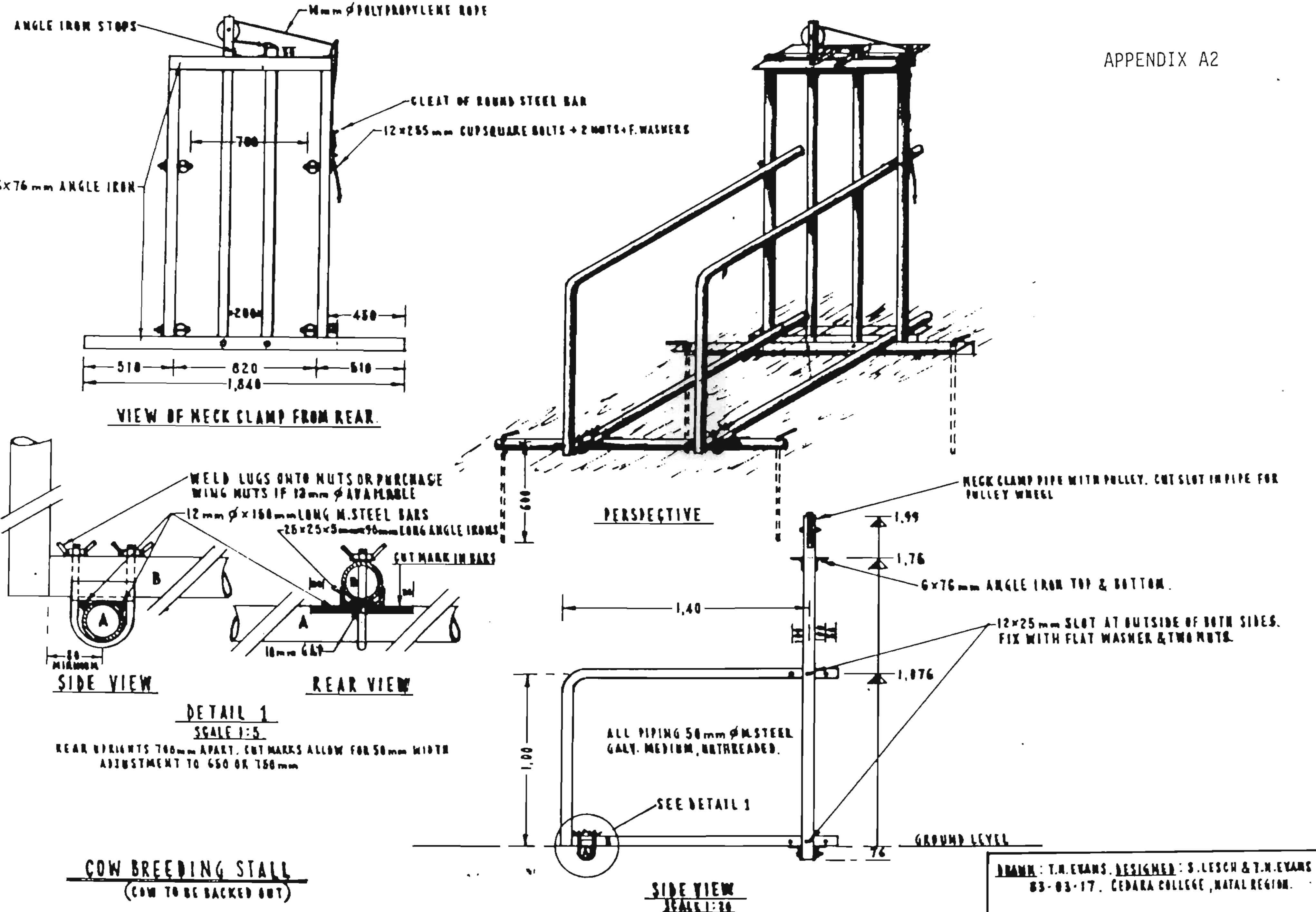
SCHEDULE FOR BULL LIBIDO TESTS

BREED TYPE	TEST NO.	DATE	ORDER OF TESTING[1] 1st	2nd	3rd	4th	5th	6th	7th	HEIFER GROUP (n)
British Beef	1	2/5/83	5	3	6	4	2	1	-	A (5)
	2	5/5/83	1	5	6	3	2	4	-	B (5)
	3	9/5/83	4	1	5	6	3	4	-	C (4)
	4	13/5/83	5	2	3	6	4	1	-	Non-oestrus
	5	16/5/83	6	4	2	1	3	5	-	A (5)
Dual Purpose	1	18/5/83	5	3	4	7	1	6	2	B (5)
	2	21/5/83	6	7	5	4	2	1	3	C (4)
	3	24/5/83	1	5	7	4	2	3	6	Non-oestrus
	4	27/5/83	5	6	1	7	4	3	2	A (5)
	5	30/5/83	4	6	5	2	7	1	3	B (5)
Zebu	1	1/6/83	5	3	7	4	2	6	1	C (4)
	2	[2] 4/6/83	1	2	3	6	7	5	4	Non-oestrus
	3	[2] 7/6/83	2	1	7	5	3	4	6	A (5)
	4	10/6/83	7	2	1	3	4	6	5	B (5)
	5	13/6/83	6	2	3	7	5	4	1	C (4)

[1] Each group of three bulls was allocated a group number. On any given day the first group was preceded by a stimulator group. The order of testing was allocated at random

[2] The Brahman bulls were tested first in order to determine whether earlier testing did not stimulate activity since it is often claimed that these breeds are more active early or late

APPENDIX A2


ANGLE IRON STOPS
16mm ø POLYPROPYLENE ROPE
CLEAT OF ROUND STEEL BAR
12×255mm CUPSQUARE BOLTS + 2 NUTS + F. WASHERS
6×76mm ANGLE IRON
700
200
450
510
820
510
1,840
VIEW OF NECK CLAMP FROM REAR
PERSPECTIVE
600
WELD LUGS ONTO NUTS OR PURCHASE WING NUTS IF 12mm ø AVAILABLE
12mm ø × 160mm LONG M.STEEL BARS
25×25×5mm×90mm LONG ANGLE IRONS
CUT MARK IN BARS
10mm GAP
80 MINIMUM
SIDE VIEW
REAR VIEW
DETAIL 1
SCALE 1:5
REAR UPRIGHTS 700mm APART. CUT MARKS ALLOW FOR 50mm WIDTH ADJUSTMENT TO 650 OR 750mm
NECK CLAMP PIPE WITH PULLEY. CUT SLOT IN PIPE FOR PULLEY WHEEL
1,99
1,76
6×76mm ANGLE IRON TOP & BOTTOM.
1,40
12×25mm SLOT AT OUTSIDE OF BOTH SIDES. FIX WITH FLAT WASHER & TWO NUTS.
1,076
ALL PIPING 50mm ø M.STEEL GALV. MEDIUM, UNTHREADED.
1,00
SEE DETAIL 1
GROUND LEVEL
76
SIDE VIEW
SCALE 1:20
COW BREEDING STALL
(COW TO BE BACKED OUT)
DRAWN: T.N.EVANS. DESIGNED: S.LESCH & T.N.EVANS
83-03-17. CEDARA COLLEGE, NATAL REGION.

APPENDIX A3

THE ECONOMIC ADVANTAGE OF USING HIGH SC BULLS

ASSUMPTIONS

1. Purchase price R4 000

2. Bull to cow ratio 1 : 30

3. Period of stay 3 Breeding seasons or 2,7 years.

4. Advantage in conception rate (CR) over medium or low SC Bulls:

   (a) 3% (b) 5% (c) 11%

5. Feed costs:

Veld grazing	R150	(R50/summer)
Silage	R360	(R120/winter)
Summer lick	R48	(R16/summer)
Winter lick	R162	(R54/winter)
	R720	

6. Slaughter Value:

Carcass mass	400 kg	
Grade C_1/C_3	R4,40 kg.	R1 760

APPENDIX A3

7. Interest costs on purchase price @ 15%/annum

R1 800

8. Cost of SC tests R170/bull

TOTAL cost/bull = R6 691

LESS slaughter value = R4 931

PROFITABILITY

Income from sale of additional weaners :

Advantage in CR	No.extra calves/annum	X 3	Income *
3%	0,9	2,7	R1 928
5%	1,5	4,5	R3 213
11%	3,3	9,9	R7 069

* Weaning mass = 210 kg

Weaner price = R3,40/kg or R714/weaner

C O N C L U S I O N

The increased profitability generated by the use of hich SC bulls although small on a farm scale is nevertheless worthy of consideration since bulls which achieved an 11% advantage in CR paid for themselves.