THE USE OF OESTROUS COWS FOR THE PRE-COLLECTION PREPARATION OF MATURE BULLS STANDING AT AN ARTIFICIAL BREEDING CENTRE

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INTRODUCTION

Since 1949, a marked expansion in the commercial operation of artificial breeding has taken place in New Zealand. This expansion represents an increase from 1,400 inseminated cows in 1949, to 556,000 inseminated cows in 1961. (New Zealand Dairy Prod. and Marketing Board Ann. Rept.(1962)). The principle objective of the Artificial Breeding Service is to offer farmers the use of top sires from each of the main dairy breeds in New Zealand. These sires are selected on the basis of progeny test records. The rating which each proven sire receives is calculated from the butterfat production records of a sire's daughters. The butterfat records of each daughter are corrected for age differences and compared with the age-corrected average production of the herd-mates. Bulls selected for use as A.B. sires are placed at one of the two Artificial Breeding Centres which provide a Dominion-wide coverage.

Because of the seasonal nature of dairy farming in New Zealand, the bulk of the demand for semen occurs during the spring mating period, and since chilled semen is the principle form of service offered, the bulls experience a peak working period of eight to twelve weeks at this time of the year. The objective in development at the Centres has been to obtain maximum coverage from top sires compatible with satisfactory conception rates. In 1961, the 49-day non-return rate to first inseminations with chilled semen was 63.3% (New Zealand Dairy Production and Marketing Board Ann. Rept. (1962)).

Three approaches have been adopted to obtain the maximum coverage from top sires and to this end work has been undertaken in an

endeavour to:

(i) increase the harvest of sperm from each bull;

(ii) extend the harvested sperm to as great a degree as possible.

and (iii) improve storage techniques by the use of improved diluents.

The current procedure involves the collection of two services from each bull once every three days. Prior to each collection, each bull is prepared by teasing in the presence of a cow restrained within a small collection bail. Minimum quality requirements comprising an initial motility estimate and a live-dead sperm count must be satisfied before a service is diluted to a minimum concentration of 12.5×10^6 sperm per millilitre. The dosage rate is $\frac{1}{2}$ ml. of diluted semen per cow which is equivalent to 6.25×10^6 sperm per insemination.

The trial reported in this thesis was conducted at the Awahuri Artificial Breeding Centre. Its principle objective was to determine whether or not the use of oestrous cows as teasers could increase the number of sperm harvested from mature bulls under otherwise routine management conditions. Other effects upon the quantity and quality of ejaculates obtained and upon sexual response were also investigated. Comparisons were made with the semen characteristics of the collections taken when the oestrous teasers were used as their own non-oestrous controls.

Some reports, summarised in the Review of Literature, mentioned that sexual interest can frequently be re-vitalised in sexually slothful bulls by using an oestrous cow as a teaser. Other reports considered that the presence of an oestrous cow in the immediate environment increased the excitability of all bulls. However, no trials in

which the bulls were subjected to controlled pre-collection preparation in the presence of an oestrous cow have been found in the literature.

In the current experiment 22 mature bulls were selected from the sires standing at the Centre. While variable factors were restricted as much as possible, experimental techniques bore a close relationship to the normal routine practised at the Centre during the spring mating season.

Part 1.

REVIEW OF LITERATURE

The review of literature has been presented in 3 major sections:-

(i) The effects of oestrous cows on bull behaviour.

(ii) Major considerations relating to the management of the bulls and the selection of teaser cows.

and (iii) The laboratory techniques used in measuring semen quality and quantity and the measurement of a bull's sexual response.

Chapter I

The Effects of Oestrous Cows on Bull Behaviour

The reproductive system of the cow undergoes a rhythmical change called the oestrous cycle and the highlight of this cycle is the period of oestrus at which time the female is receptive to the male. Besides being receptive, an oestrous cow may also excite bulls in the immediate environment. James (1952) and Hart et. al. (1946) reported that when an oestrous cow was used as a teaser, interest could be re-vitalised in sexually slothful bulls. The latter workers suggested that because interest was aroused as soon as the oestrous cow was led into the barn "there was an odour from the female rather than her behaviour that attracted and psychically stimulated the male under conditions obtaining in an artificial breeding chute". These comments were limited to aged bulls which had suffered a loss of libido.

Several other papers have noted a generalised effect in all bulls. A recommendation of the Milk Marketing Board for the preparation of a sire prior to service was the parading of an oestrous cow in front of the bulls,

with the resultant creation of what was termed "a pandemonium of ecstasy" (Brit. Milk Marketing Board Rpt. (1954)). Cordts (1953) reported that the presence of an oestrous cow increased the excitability of bulls tethered in stalls. Neither report mentioned the use of the oestrous cow as a teaser, nor whether her presence influenced the service quality or quantity.

Almquist and Hale (1956) used reaction time as an index of sexual activity and stated that smearing the rump of a teaser with oestrous mucus or wormwood oil did not influence a bull's responsiveness. They defined reaction time as the interval between a bull's being introduced to a teaser animal and the collection of a service, no restraint being applied to the bull. When several ejaculates were collected, the response was measured in terms of the number of ejaculates per unit time. The report did not record how much mucus was applied nor whether it was re-applied prior to introducing each bull. No data was presented in this report.

James (1952) was prompted to make specific investigations into the effects of using oestrous teasers when he noticed a marked increase in the semen volume of 2 bulls which had an oestrous cow substituted for a non-oestrous teaser. Four pairs of monozygotic[3] twin bulls were used in the investigation. Eight services were collected from each bull on each of 2 collection days. A reversal design was used, one twin being collected over the oestrous teaser for the first 4 services, and over a non-oestrous teaser for the second 4 services. The twin mate received the opposite treatment. James considered that "the results did not suggest that there was any stimulating effect in collecting semen using an oestral cow as a decoy". An examination of the data showed a 12.2% increase in sperm output and

an 11.5% increase in the total semen volume for the first 4 services, when the collections were made over an oestrous teaser. 4 of the bulls showed a marked increase, 2 a decrease and the other 2 bulls showed little variation. The use of oestrous teasers did not greatly increase the total sperm output nor the semen volume of the second 4 ejaculates.

James also examined the possibility that tactile sensations produced in bulls after being allowed to serve an oestrous cow could influence the sperm content and semen volume from subsequent collections. One twin of each pair was alternately allowed to serve an oestrous teaser and an artificial vagina. The services collected with the artificial vagina were then compared with the complementary ejaculates collected from the twin mate which was collected over a non-oestrous teaser. Although only one oestrous cow was used, no differences in semen volume or sperm content were apparent.

Prabhu et. al. (1954) used oestrous buffalo cows as teasers with Indian water buffalo bulls in an attempt to reduce the between service variation in sperm output. Oestrous and non-oestrous teasers were alternated at intervals of a few minutes, 24-48 hours and 7 days, but no effect on semen quality or reaction time could be attributed to the physiological state of the teasers. With the varied collection intervals and apparently irregular teasing procedure used by these workers, real differences would have been difficult to detect.

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Summary

Several observations cited from the literature indicated that the presence of an oestrous cow appeared to excite most bulls, but the trials

in which oestrous cows were used as teasers or in which oestrous mucus was smeared on the rump of a teaser cow, showed no significant effect on either the quality or the quantity of semen collected or upon the sexual precocity of the bulls. None of the references cited used standardised teasing procedures in the presence of an oestrous cow as a pre-collection routine. This could well have masked what may have been real differences in the "stimulus value" of the teaser cows.

CHAPTER II

Major Considerations Relating to the Management of the Bulls and the Selection of the Teaser Cows.

The sections of this chapter review the pertinent literature related to the preparation of the trial design and involve a consideration of various aspects of bull management relevant to the investigation and the selection of cows as teasers.

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2.1 Frequency of Collection

Many trials have been reported in which bulls have been collected at different frequencies. The results of these trials will be considered in 3 sections.

(a) Sperm output:- Earlier workers recommended low frequencies of 1 or 2 collections per week from mature bulls. Patrick et. al. (1949) studied an ejaculation pattern of 1 every fourth day, 2 every eighth day or three every twelfth day, and found no significant treatment differences in volume, % motile sperm, methylene blue reduction time or conception rates in the 6 bulls studied. Mercier et al (1949) found a higher percentage of ejaculates were satisfactory for use in artificial insemination when bulls were ejaculated once every 6 days instead of twice every twelfth day or 3 times every eighteenth day. Neither group of workers restrained the bulls prior to service.

The following table summarises results obtained in later trials.

Reference	No. of Bulls	Restraint	Frequencies	% increase with greater frequency
Bratton & Foote (1954) (a)	16	None	1 x 8 vs 1 x 4	63 M.S.
	14	None	1 x 8 vs 2 x 8*	60 M.S.
Bratton & Foote (1954) (b)	14	Prolonged	1 x 8 vs 2 x 8*	60 M.S.
Almquist & Hale (1956)	15	Short	1 x 7 vs 2 x 7	67 M.S.
	10	Varied	2 x 7 vs 6 x 7	112 M.Sa
Hafs et.al. (1959)	10	Varied	1 x 7 vs 7 x 7	90 T.S.
Hale & Almquist (1960)	2	Short vs None	6 x 7 vs 70 x 7	17 T.S.

1 x 8 indicates 1 collection every 8 days

* indicates 2 collections every eighth day

M.S. - % increase in motile sperm output

T.S. - % increase in total sperm output

Table 1. The Effect of Frequency of Collection on The Sperm Output of Mature Bulls.

Comparison between the results obtained is difficult because of the different methods adopted in sexual preparation. Bratton and Foote (1954) (b) applied 20 minutes restraint before the first collections were made and after a 10 minutes rest, a further 10 minutes restraint before second collections were made. In contrast to this prolonged preparation, Almquist and Hale (1956) restrained the bulls in their first trial for only 4 - 5 minutes prior to the collection of first services.

Hale and Almquist (1960) presented a table intending to show the effect of increased collection frequencies on sperm output. The data as presented was of questionable value because of the few bulls used at all

collection frequencies and the confounding of different collection frequencies with different methods of sexual preparation.

The other results included in Table 1 support the conclusion of Hafs et. al. (1959) who reported that increasing frequencies of collection produce diminishing increments in the sperm output as each service has a reduced sperm content.

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(b) Effect on libido:- Higher frequencies of collection have been reported to reduce libido. At a frequency on 2 collections on each of 5 days per week Almquist and Hale (1956) reported that during the 24-week trial period one bull showed a 47% decline in sperm count and another an 18% reduction in semen volume. These workers concluded that such reductions indicated that certain bulls could not be ejaculated so frequently for extended periods. With only 2 collections per week, only 3 of the 10 bulls used required a new stimulus, whereas with 6 collections per week, 7 bulls required new stimuli. Although the motile sperm output per week was increased by 112% by making 6 rather than 2 collections per week, more frequent changes in the teasing routine were required to maintain the bulls' sexual activity.

Hafs et. al. (1959) reported a similar decline in libido. Bulls collected once a week required an average of 9.2 minutes of active sexual stimulation and 2.9 false mounts compared with bulls collected daily which required an average of 16 minutes sexual stimulation and 3.7 false mounts. While daily collection led to a decline in libido, these workers found that a weekly change in the collection routine maintained the required teasing time within workable limits.

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(c) Recommended frequencies:- In a review publication, Melrose (1962) concluded that "although individual bulls vary in their reactions to frequent semen collections, the available evidence indicates that, with adequate stimulation, the average mature bull could be expected to ejaculate at least 4 times per week". Salisbury and Van Demark (1961) suggested a frequency of 2 - 3 ejaculates every 2 - 3 days as they considered that daily ejaculation of bulls over an extended period of time did not impair spermatogenesis. This contention was confirmed by Amann and Almquist (1962) who reported that even with intensive pre-collection preparation prior to each of 6 collections made each week from each of 12 mature bulls, the weekly sperm output represented only 42% of the weekly testicular sperm production.

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2.2 Sexual Preparation

Hale and Almquist (1960) considered that "the function of sexual preparation was to provide high quality semen containing the greatest possible number of sperm per ejaculate". Quantitative data shown in the accompanying table summarises the reports of several investigations.

Reference	No. of Bulls	Weeks of Preparation	No. of Ejac/ Week	Preparation	% Increase* 1st Ejac	2nd Ejac.
Collins et.al (1951)	22	5	1	None vs 2-3 mins restraint + 1 false mount	36	
Branton et.al (1952)	9	6	2	None vs 1 false mount	42	
				None vs 2 false mounts	41	
Crombach (1958)	6	4	4	None vs 1 false mount	129	
	2	4	4	None vs 10 mins restraint	147	
	5**	13	4	None vs 5 mins restraint + 1 false mount	112	109
Almquist et.al (1958)	6	3-6	2	None vs 1 false mount	72	29
	3	12	6	None vs 2 mins restraint + 3 false mounts		45
				1 false mount vs 2 mins restraint + 3 false mounts	44	
Hafs et al. (1962)	11	10	1	None vs 5 mins restraint	99	
	14	10	1	10 mins restraint vs 10 mins restraint + 3 false mounts	31	

* % Increase with Second Method

** Identical twins used as controls.

Table 2:- The Effect of Sexual Preparation on The Sperm Output of Bulls.

All the investigations showed that sexual preparation produced a valuable increase in sperm output. The techniques used in sexual preparation were:-

(a) Allowing the bulls to have a number of false mounts

(b) Restraining the bulls for varying periods of time,

and (c) A combination of the other two methods.

The most comprehensive trial was conducted by Hafs et. al. (1962) In their trial, none, 1, 2 and 3 false mounts were combined with none, 5 or 10 minutes restraint. The results showed that progressively increasing the intensity of sexual preparation produced decreasing increments in sperm output. The concentration of sperm in the semen was not significantly affected by the number of false mounts but was affected by the duration of preparation. From this work it appeared that a restraint period of at least 5 or 10 minutes was more essential than allowing false mounts.

The only work noted in which sexual preparation did not augment total sperm output was reported in a trial conducted by James (1952). Five pairs of monozygous twins were used. Ten consecutive services were collected from each bull on 2 collection days. On each day one twin was restrained prior to the collection of each service and the twin mate was not restrained. The results showed marked variations in response both between and within pairs. Preparation was possibly carried to the extreme as 3 false mounts were allowed prior to the collection of each of the 10 services. Although neither the first nor the second services showed any response, James also mentioned that a bull was prepared and collected in front of his [redacted] twin mate who was not prepared. This in itself could have acted as a form of sexual preparation.

The effectiveness of sexual preparation does not appear to decline with its continued application. Sexual preparation continued to be effective even at a collection frequency of 6 ejaculates per week over a period of 3 months (Almquist et. al (1958)) or after 4 months of receiving the same preparation prior to each service (Crombach et. al (1956)). No detrimental psychological effects resulting from any of the 12 methods of sexual preparation imposed were reported by Hafs et. al (1962).

While sexual preparation produces a significant effect upon the sperm content of services collected from a group of bulls, individual bulls may show a marked variation in their response. Almquist and Hale (1956) noticed that amongst the 22 bulls they used, the greatest number of tests showed that between 3 and 5 services per hour could be collected from bulls which were not sexually prepared nor restrained, but the bulls showed considerable individual variation. This was substantiated in a later report by the same authors when they showed that whereas sexual preparation increased the sperm content of the services from one bull by 52%, another bull showed a negligible response, (Hale and Almquist (1960)). Bonadonna (1956) stressed that "in order to achieve a rational exploitation of sires, it is essential to carefully watch the individual behaviour of each subject, to carefully avoid bringing about acquired reflexes that are inhibitory and to encourage the creation and the maintenance of positive or favourable reflexes".

2.3 Interval Between Services

The interval between services refers to the interval between the collection of the first service and second or subsequent services on

any one collection day. Apart from the trial reported in the First Annual Report of the N.Z. Dairy Production and Marketing Board (1962), no other work has been noted which specifically examined this point. In the particular trial referred to, collection at a 10 minute interval as against a 60 minute interval produced a highly significant difference in the sperm content of the second service in favour of the shorter period. The trial involved 30 mature Jersey bulls, 3 collections being made at each interval from each bull. The report concluded that "there would appear to be a very real benefit in collecting second or third services as soon as possible after the preceding service."

Crombach et. al (1956) carried out a series of tests to specifically determine the effect of sexual preparation on the second ejaculate. They found that a better quality service was obtained if the second service was collected after an additional five minutes restraint than if the second service was collected immediately after the first had been taken. While no substantiating data were presented, Bonadonna (1956) recommended a 10-15 minute interval between collections.

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2.4 Seasonal and Climatic Variations in Semen Characteristics

Most investigations recorded in the literature were conducted to determine seasonal variation in fertility and not sperm output. Anderson (1942) reviewed the earlier work and all references quoted showed that there were significant monthly and seasonal differences in semen quality. Erb et al. (1942) recorded highly significant monthly and seasonal differences in motility, volume, sperm concentration and total sperm output. These latter workers only used 4 bulls, 2 of which were immature. Because

of this and because the authors also noted that there were marked differences between successive weeks in the same bull, these results should be interpreted with caution.

Anderson's (1945) study indicated that under climatic conditions experienced at the experimental station in Kenya where his trial was conducted, semen was poorest during the months of maximum temperature and humidity. Although there appeared to be a basic seasonal rhythm in the quality of bull semen, Anderson's results showed considerable variation in seasonal effects upon bulls and between years by the same bull.

Johnston et. al (1953) found that under sub-tropical conditions, bulls produced greatest concentrations of spermatozoa in summer and winter, although several bulls showed marked decreases during the late summer and fall periods. Figures for sperm output were not mentioned. Casaday et. al (1953) found that under climate chamber conditions, spermatogenesis in the young dairy bull was impaired by continuous exposure to temperatures exceeding 85°F.

Steif (1954) noted seasonal variation in the number of abnormal sperm, semen volume, and sperm concentration, the greatest quantity of best quality semen being collected in the spring. Schmidt (1954) studied the effect of day length and temperature on bulls and found positive correlations between each of these two factors, sperm concentration and sperm numbers. The influence of day length was probably confounded by seasonal temperature variations, particularly since the occurrence of photoperiodicity in bulls has not been demonstrated.

Hafs et. al (1955) used the semen production records of 68 bulls from 5 dairy breeds in 4 studs in the state of Michigan, which experienced

severe winters, to estimate the bull ejaculate, breed, season and interaction variance components of 5 criteria used to measure semen production. All studs showed significant seasonal variation in semen volume. While the 2 smaller studs showed significant seasonal variation in sperm output, the 2 larger studs did not show this variation. These workers concluded that although significant seasonal differences were noted "actual seasonal differences were considered to be too small to be of much practical value in the design of experiments".

Bonadonna (1956) suggested that "the variations in seminal activity were not always clearly seasonal in South European countries having a mild climate. The climatic conditions which were likely to affect animals were long rainy periods, or damp cold and sudden and deep depressions associated with thunderstorms, particularly in summer." Although James (1954) did not specifically investigate seasonal variation in semen quantity and quality, perusal of other data from collections taken through the whole year suggested that there were no seasonal trends in the sexual performance of bulls in New Zealand.

While seasonal variation in semen properties has been reported by some workers, the variations appear to be associated with temperature extremes. In warm-temperate or Mediterranean climates, this variation has not been noticed.

2.5 Semen Collection

Melrose (1962) considered that the use of the artificial vagina appeared to be universal as a method for the routine collection of semen. Perry (1960) noted that most males would readily respond to this method of

collection provided that proper precautions were taken in the preparation of the equipment. In this connection, the peculiar temperature requirements of each male should be studied to avoid the development of what Bonadonna (1956) has termed inhibitory reflexes. The temperature range suggested by Bonadonna was 43–45°C but Perry provided an even wider range of 40.5–46°C. Comparative figures quoted by Salisbury and Van Demark (1961) were 42–44°C.

Modifications to the standard rubber-lined vagina have been developed by several workers. Melrose (1962) mentioned that "the use of a roughened type of liner has now been more widely adopted; although there is little published data on this, the generally accepted view is that such a liner ensures a more constant service behaviour in the bull". Since no references were quoted by Melrose and no mention of such work has been found in the literature, this "generally accepted view" could not be substantiated. Millar (1958) developed an elaborate modification to prevent temperature shock to the raw semen, but Melrose considered that it had not been extensively used, probably because of the time required for assembling and dismantling the modified equipment.

Bonadonna (1956) suggested that some operators made poor collections because they underestimated the importance of applying the artificial vagina to the erect penis of the bull at the right time. Faulty application encouraged the onset of inhibitory reflexes. The collection should be made on the ascending extra-vaginal stage and never on the descending stage. Bonadonna mentioned no optimum time during the ascending stage since individual bulls vary and a knowledge of each bull's behaviour becomes necessary.

Contrary to the implications of Bonadonna's review, James (1954) found that the skill of the operator making a collection did not appear to influence its quality or quantity. Only 4 pairs of identical twins were involved in the trial and all the bulls were well used to collections being taken with an artificial vagina. James rightly pointed out that these results were only applicable to bulls well used to the collection techniques.

2.6 Teaser Cow Considerations

The pertinent points relevant to the design of the current trial have been reviewed under 3 sub-sections covering the manifestations, the duration and the control of oestrus. Salisbury and Van Demark (1961) considered that the teaser cow should be an animal that will stand quietly or "she should be well restrained within a breeding rack". Their impression was that although some movement by the teaser cow encouraged some bulls, excessive restlessness caused other bulls to refuse to mount and collecting the ejaculate became difficult for the operator.

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(a) Manifestations of oestrous:- Rottensten and Touchberry (1957) scored the degree of expression of heat symptoms in 554 first-calf heifers. Scores varied from 1 to 4 for symptoms varying from a vague expression of oestrus to oestrus being more evident than normal. While these workers did not state the frequency distribution, the calculated repeatability of single ratings among unrelated animals was only 0.29. This low estimate could possibly be due to the highly subjective nature of the rating given and the fact that the observations were only made at milkings. In comparison to these workers, Olsson (1957) found that 98% of his sample of over

200,000 cows showed distinct symptoms of oestrus.

Salisbury and Van Demark (1961) have described oestrus as the period of desire which is characterised by the psychic manifestations of heat. With the onset of oestrus a cow may become restless and start bellowing but will not stand whilst another animal, be it a cow or a bull, attempts to mount. It is this duration during which a cow will stand when mounted that Hansel (1959) has defined as the period of oestrus. He suggested that "other manifestations of oestrus such as a flow of clear mucus from the vulva, swollen lips of the vulva, restlessness, bellowing and attempts to mount other females were too variable to be used alone as criteria for detecting oestrus".

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(b) The Duration of Oestrus: - Unlike some domestic animals, the cow has a comparatively short heat period. Hammond (1927) found that the duration of oestrus in the 11 heifers and 4 cows studied varied from 6 to 30 hours with a mean of about 17 hours. Whilst Hammond's results were obtained from a small population, the average length of the heat period was greatest in the warm summer months and least in the winter. Trimberger (1948) studied a considerably larger population and could not detect this seasonal variation. Other observations reported by Trimberger were that the duration of oestrous averaged 17.8 hours in cows (range 2.5 to 38 hours) and 15.3 hours in heifers. Cows and heifers appeared to come into heat throughout the day and night with a fairly equal distribution, but animals that first showed oestrus in the afternoon stayed in heat 2 to 4 hours longer than those that showed it in the evening.

After a comprehensive review of the relevant literature Hansel

(1959) concluded that the range in the length of oestrus reported in the papers reviewed varied from 6 to 30 hours in most studies, with a standard deviation of approximately 4 hours.

From a very limited number of tests conducted by Hammond (1927) the effect of service by a bull appeared to shorten the duration of oestrus, although the psychological effect of the close proximity of a bull did not appear to have any influence. Marion et. al (1950) in a more extensive experiment, found that although sterile copulation hastened ovulation, it did not reduce the duration of oestrus.

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(c) The Artificial Control of Oestrus:- Because cattle experience a comparatively short oestrous period, any suitable artificial measure of control would be an asset in designing a trial in which oestrous teasers were required. Many of the studies illustrating the effects of the injection of gonadotrophins on ovarian function in the bovine have been carried out for the purpose of inducing "super-ovulation" and few record the effects of such treatments on oestrous behaviour. Rowson (1951) used P.M.S. to induce multiple ovulation. Although no comment was made on the manifestations of the subsequent visible heat periods experienced, many cows did not exhibit visible oestrus. Hansel (1959) has mentioned that other workers encountered similar problems.

Daily injections of large doses of progesterone from mid-cycle onwards prevent oestrus and ovulation occurring at the normal time, but Hansel concluded from a review of the results reported that oestrus and ovulation occurred from 4 to 7 days after the cessation of treatment. This was supported by Trimberger and Hansel (1955) who found that although

progesterone injections altered the time of oestrus, such treatment had no advantage in predicting oestrus on a particular day. Another disadvantage was that during the experimental period, of the 30 cows used, only 4 had oestrous periods of normal length, 4 had silent heats and 7 had a marked disturbance of either the length of oestrus or ovulation time.

Oestrogens have been extensively used in lactation studies. Folly and Malpress (1944) and Hammond Jnr. and Day (1944) conducted extensive studies. Both pairs of workers reported the occurrence of irregular heat periods varying in intensity and the development of nymphomania, which occasionally terminated in broken pelvises.

Perhaps the simplest method for altering the bovine oestrous cycle involves the manual removal of the corpus luteum through the rectal wall. Hammond and Bhattacharya (1944) used the technique in a trial and noted that the interval between removal of the corpus luteum and the following heat varied with the breed of the cow and the age of the expressed corpus luteum. About 10% showed oestrus within two days, but the majority came on heat on the third or fourth day. The average time was 4.2 days.

Hammond (1927) used only 2 cows which had corpora lutea expressed 7 days after the previous heat period, and both showed oestrus 2 days later, but the length of oestrus was only half that of the previous oestrus. Roberts (1956) summarised the results of a number of similar studies and concluded that observable oestrus occurred within 2 to 7 days in 50-80% of the cows in which a corpus luteum had been expressed. Although they did not quote the variation, Salisbury and Van Demark (1961) presented a table from work by Jakobsen and Teige (1956) who showed that expression of the corpus luteum induced oestrus in 90% of the 2,000 cows studied.

Although hormonal therapy and expression of the corpus luteum alter the bovine oestrous cycle, none of the methods improved to a great degree the predictability of the onset of oestrus. Moreover the oestrous period subsequent to treatment may be abnormal, either in its length or in its manifestations. Because of these limitations, the artificial control of oestrus was not attempted in the experiment reported in this thesis.

2.7 Conclusions

After a consideration of the reports reviewed in this chapter the following points were thought relevant to any trial aimed at determining the effect of using oestrous cows as teasers:-

(a) Most mature bulls will withstand a collection frequency of 2 services every third day but because varying frequencies influence the total sperm output and the sperm content of an ejaculate, the selected frequency should not be varied.

(b) The intensity of pre-collection sexual preparation has a significant effect upon the sperm content of a service and therefore, when the physiological conditions of a teaser are being compared, the preparation routine should be standardised.

(c) Because some bulls show a varied response to sexual preparation the selection of bulls which will respond to standardised teasing routine is warranted.

(d) A suitable interval between the collection of first services from each bull on any one collection day and the commencement of sexual preparation for the second service is 10 minutes.

(e) It would be most unlikely that bulls selected for the trial

would exhibit marked seasonal variation in sperm output or semen output.

(f) The efficiency of the collection team would deserve special consideration since faults in the collection technique and variations in teasing could modify the responsiveness of a bull.

(g) The most reliable test of the oestrous condition in a cow is standing whilst being mounted by another cow.

(h) Because the duration of oestrus is relatively short and variable, the period of time in which a cow is used as an oestrous teaser should be limited,

and (i) Methods for the artificial variation of the oestrous cycle were not considered suitable because no method greatly improved the low predictability of oestrus and because of the tendency to give rise to different manifestations of oestrus.

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CHAPTER III

Laboratory Techniques Used In Measuring Semen Quantity and Quality and The Measurement of Sexual Response.

The sections of this chapter only briefly review pertinent literature which was consulted when developing the quality and quantity tests used during the current trial.

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3.1 Concentration of Sperm Cells

Sperm concentration was first estimated by the counting of a diluted sample of semen mounted on a haemocytometer slide (Walton 1927). Where large numbers of samples have to be counted, this method has proved tedious. Current methods used include the measurement of the opacity of a spermatozoal suspension by standard opacity tubes as described by Kyaw (1944), or by an absorptiometer, the opacity of the suspension being proportional to the spermatozoal concentration. The standardisation of the absorptiometer from haemocytometer counts has been described by Salisbury et al (1943). These workers found that the repeatability of absorptiometer estimates of concentration was almost as high as for repeated haemocytometer counts. Salisbury and Van Demark (1961) quoted standard errors of $\pm$ 5% to $\pm$ 8% of the mean value.

The use of a standard "Zeiss" colorimeter was described by Cox and Melrose (1953), who also indicated the desirability of performing occasional check calibrations and advised the regular use of a standard opacity tube to detect any errors due to light variations. The "Hilger Biochem" absorptiometer was used by Bishop et al (1954), who emphasised the need

for allowing the spermatozoal suspension to stand for a few minutes so that any flow movement in the suspension could cease before measuring its opacity.

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3.2 Assessment of Spermatozoan Motility

"Currently used methods for the assessment of sperm motility are primarily visual, and the results are usually expressed in comparative rather than absolute terms. No means is readily available for characterising the distribution of motility of individual sperm cells in a semen sample". (Salisbury and Van Demark (1961)). Because motility is a subjective assessment Bishop and Walton (1960) and Melrose (1962) considered that the results obtained by different workers could not be compared.

The system currently used at the Awahuri Artificial Breeding Centre involves grading services between the extremes of 0 and 3+. A service is not diluted and dispatched unless the initial motility of the undiluted sample microscopically inspected soon after collection is rated 2 or better.

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3.3 Differential Staining of Live and Dead Spermatozoa

This differentiation was first used for spermatozoa by Lasley et al (1942) who, using eosin and opal blue stain in an isotonic phosphate buffer, showed that dead spermatozoa, which stained with eosin, could be readily distinguished from the living unstained spermatozoa against the background stained with opal blue. Numerous variations in stains and backgrounds in buffers of different pH have been developed, their effectiveness and use being summarised by Melrose (1962).

The repeatability of estimations made of live-dead ratios has

been studied by various authors. Ortavant et al (1952) found no differences in the percentages of dead sperm in the same semen counted by two operators; also the results by counting 150 sperm were not appreciably different from those obtained when 2,000 spermatozoa were counted. Campbell et al (1953) reporting on the counts made by two operators, found that the variation between different counts on one smear was consistent with random sampling from a binomial distribution. These findings were not confirmed by Campbell et al (1956) who, using bull, boar and ram spermatozoa, found larger variation between counts of the same semen than would be expected if the distribution of the stained spermatozoa was uniform. Variations also occurred between operators. They suggested that these variations were caused by the "clumping" of dead sperm and the variation in the interpretation of partially stained spermatozoa between operators.

The occurrence of half stained forms has also been reported by Dott (1956), Brochart (1953) and Meyer et al (1951). Suggested causes included the pH of the solvent, the degree of maturity of the spermatozoa and the interval between the collection of the service and the preparation of the smear (Melrose (1962)). Campbell et al (1956) emphasised that the time between preparation of the sub-sample and the making of the smears should be kept constant, 5 minutes being considered satisfactory. These workers recommended that at the commencement of a trial, an operator should be positive as to which sperm must be counted as dead. Campbell et al (1956), Mayer et al (1951) and Salisbury and Van Demark (1961) all classified partially stained heads as dead sperm. From their data, Campbell et al (1956) suggested that the maximum accuracy could be obtained by preparing one smear from each of several sub-samples and counting 100

spermatozoa on each smear.

The routine procedure at the Awahuri A.B. Centre is to count 200 spermatozoa on the one slide. If the sample shows less than 60% live sperm, the service is rejected. Similar live-dead estimates were conducted in the current trial, not to determine the effect of using an oestrous teaser on total live sperm output, but to determine if any quality effect as measured by live-dead counts, resulted.

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3.4 pH Measurements of Semen

A series of studies conducted by Anderson (1952) showed that pH assessments were of little value as measures of semen quality. Mann (1954) indicated that measurements of pH change after incubation were of limited value as a measure of the metabolic changes. In the current trial the initial pH and the pH change after 1 hour s incubation at 37°C were measured. Much of the previous work had been conducted on a limited number of bulls over short periods. The current trial provided an opportunity for assembling pH data from a larger number of bulls over an extended period of collecting.

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3.5 Initial Fructose Concentration of Semen

Since Mann (1946) proved that the sugar in semen was D(-) fructose many workers have conducted tests on its concentration and utilisation by sperm. Mann (1948) developed a technique for measuring fructose concentration but the procedure used during the current trial was a modification of Mann's technique as suggested by Bishop et al (1954).

The results obtained in the current trial were of particular

interest in view of work reported by Branton et al (1952). These workers reported that "the initial fructose concentration could be markedly influenced by the level of sexual excitement prior to service and, that as sperm density increased, there was generally an accompanying decrease in the fructose concentration of the semen".

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3.6 Measurement of Sexual Response

This measurement is not a laboratory test but is included here, as like the previous tests, the data would assist in showing the effects produced by the use of an oestrous cow as a teaser. Almquist and Hale (1956) developed "an index of response" termed re-action time. This was the time interval between an unrestrained bull being introduced to the teaser cow and the collection of a service; or the number of ejaculates collected per unit time. Although these workers showed that neither oestrous mucus nor wormwood oil smeared on the rump of a teaser cow reduced re-action time, no work has been reported where an oestrous cow has been used. Because the bulls used in the current trial were subjected to a controlled period of pre-collection preparation, only a subjective assessment of the re-action time was obtained.

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3.7 Summary

Because the primary aim of the trial was to determine treatment effects upon sperm output, the most important measurements were volume and sperm concentration. Since every service dispatched from the Awahuri A.B. Centre must also be subjected to quality tests for motility and % live sperm, these tests were included in the trial.

Other tests were conducted to give the researcher experience in developing his own laboratory tests and to assemble additional data on what have been termed "additional treatment effects".

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PART II

THE EXPERIMENTAL DESIGN

CHAPTER IV

THE DESIGN ADOPTED AND ITS LIMITATIONS

The primary aim of the experiment was to determine whether the treatments adopted for the pre-collection preparation of the bulls would yield any significant difference in the total sperm output per collection day of a group of mature bulls. A preliminary estimate of the variation exhibited in the sperm output of mature bulls was calculated from records of the previous spring mating season. These records showed that the coefficient of variation for the total sperm output for mature bulls under routine collection procedures was of the order of 43%.

Substitution with this figure of 43 into the formula:-

$$p = \frac{\text{Coeff. of Var.} \times t_{0.05}}{\sqrt{N}}$$

(where N = no. of replications; p = % treatment difference)

(Snedecor, (1956))

showed that for 60 replications (i.e. 20 bulls each with 3 oestrous-non-oestrous comparisons) a treatment difference of 16% or more would probably prove statistically significant.

Because of the problems associated with the securing of a regular supply of oestrous cows at an Artificial Breeding Centre, it was considered that the use of oestrous teasers would need to augment sperm output to an appreciable degree to be of any practical value. A design involving 60 replications, which was both within the scope of the material available and capable of measuring a real difference of 16% or more if one existed, was accepted as satisfactory for the collection of the

necessary data. The design outlined in Table 3 was adopted.

Day	Bull Group	Teaser Cow
1	I	Oestrus X if available, otherwise non oestrus Y.*
2	II	" T " " " " " Y.*
3	---	
4	I	Oestrus Z if available, otherwise non oestrus X if X used day 1, otherwise non oestrus Y.*
5	II	Oestrus V if available, otherwise non oestrus T if T used day 2, otherwise non oestrus Y.*
6	---	
7	I	Non oestrus X if X used day 1 and not day 4, otherwise oestrus M if available, otherwise non oestrus Z if Z used day 4, otherwise non oestrus Y.*
8	II	Non oestrus T if T used day 2 and not day 5, otherwise oestrus P if available, otherwise non oestrus V if V used day 5, otherwise non oestrus Y.*
9	---	
10	I	Non oestrus Z if used day 4 but not day 7, otherwise oestrus Q if available, otherwise non oestrus M if M used day 7, otherwise non oestrus Y.*
11	II	Non oestrus V if V used day 5 but not day 8, otherwise oestrus L if available, otherwise non oestrus P if P used day 8, otherwise non oestrus Y.*
12	---	
		etc. until a minimum of 60 bull collection days has been obtained on oestrus cows.

Y* Pregnant Cow which was a trained teaser.

Table 3. Experimental Design Adopted To Determine The Effects of Using An Oestrous Teaser Cow.

22 bulls were selected comprising 14 Jerseys, 4 Friesian and 4 Ayrshire bulls, all of which were mature, had experienced at least one season of normal collecting and had not exhibited abnormal collection behaviour patterns. The bulls of each breed were randomly allocated to one or other of the 2 groups. The routine procedure at the Centre was to collect 2 services from each bull every third day and the same frequency of collection applied throughout the experimental period. Each group was collected on different days.

Prior to the commencement of the experiment, a pre-trial period covering 3 collection days for each group was conducted. The reasons for the pre-trial period were:-

(i) that previous experience at the Centre had shown that the first few services collected after a long rest period were higher in quantity but lower in quality than the services collected under regular working conditions;

(ii) the technicians could become acquainted with the details of the experimental technique;

and (iii) laboratory tests and equipment could be standardised.

The experimental period commenced on the 5th April, 1962 for GROUP I and a day later for GROUP II.

Since it was necessary to maintain each group of bulls on a 3-day roster and, either oestrous cows were not available or non-oestrous cows were not required every collection day, a trained teaser which was 4-5 months pregnant, was used to maintain the 3-day interval between collections. Semen quality and quantity data were still recorded whenever the pregnant cow was used to aid in the calculation of correction factors if any significant decline in semen or sperm output had occurred.

While this experimental design was considered the most suitable, some apparent limitations justify comment.

(a) Between Day Differences.

Since both groups of bulls were collected on different days and oestrous--non-oestrous comparisons for each cow were 3 or 6 days apart, any factors influencing the day-to-day sperm output of bulls could confound any treatment effects. The literature reviewed suggested that only extremes of temperature and high humidity produced any significant variation in sperm output. Furthermore, no previous workers employed a group of control bulls to specifically take account of any day-to-day variation, suggesting that they did not consider this variation in sperm output was significant. Analyses on data abstracted from the records for 12 mature Jersey bulls used by the Centre last spring season indicated that the day-to-day variation in sperm output and semen volume was not extreme. (Appendix V)

To overcome the problem of this variation, all bulls would have had to be collected on the same day and an oestrous and a non-oestrous teaser used together. Since an oestrous cow could not be used as a non-oestrous teaser on the same day, the use of another cow as a non-oestrous teaser would have introduced another undesirable variable. This is apart from the fact that the possible influence of oestrous odours would necessitate making collections over the non-oestrous teaser in completely different surroundings with another collection team.

Other disadvantages through collecting both groups of bulls on the one day were:-

(i) it was doubtful whether each cow could have withstood having

44 services being collected over her each day while she was used as a teaser;

and (ii) it would probably have been beyond the capacity of one collection team to work efficiently throughout such a long collection day.

The preliminary analyses (shown in Appendix V) indicated that any day-to-day variation in sperm output would have been of small magnitude, and to obtain any worthwhile measure of it, a large group of bulls would have needed to be used as a control. These bulls would have had to be collected over a different cow, by a different collection team, in a different collection bay. It was considered that these disadvantages limited the effectiveness of running a control group of bulls.

(b) Two Groups of Bulls.

Apart from the fact that the 2 groups of bulls were collected on different days, each group used different oestrous teasers. The advantages of using 2 groups of bulls instead of one large group were:-

(i) collections could be completed in a short period of time without excessive strain on the collection team or the teaser cow;

(ii) the reduced likelihood that the oestrous condition of the cow would terminate before collections were completed;

and (iii) oestrous cows could be used on 2 days instead of one day out of every 3 days. With the limited number of cows available this would reduce the length of the trial.

Collecting 22 bulls on any one day would have meant using 2 collection teams and having 2 oestrous cows each day. The other alternative was to only use one group of 11 or 12 bulls but this could have prolonged

the duration of the experiment for several months unless more cows had been available for use as teasers.

(c) Treatment Sequence.

Because of the poor predictability for the onset of oestrus in any cow and because the bulls were not varied from a 3-day roster, a cow was always used as an oestrous teaser before being used as her own non-oestrous control. If there had been a decline in sperm output from one collection day to the next, this decline would have inflated any treatment effect. Data recorded from collections taken when the pregnant teaser was used could have been used in calculating correction factors, had a significant decline occurred. The other alternative was to run a control group of bulls each collection day but the limitations of this alternative have been outlined.

(d) Selection of Teaser Animals.

While the criterion for the selection of a cow as a teaser was that she was of a suitable size and not too restless in the collection bail, the 5 cows used as oestrous teasers represent only a small sample of the cow population. Health precautions necessitated the isolation of all cows before their being used as teasers, and cattle from surrounding farms could not be used. Despite the small number of cows used there was nothing to suggest that any showed abnormal manifestations of heat. (Appendix II)

(e) Unequal Interval Between Treatments.

A variation in the experimental design was that 2 oestrous cows could be used as teasers on 2 consecutive collection days with the one group of bulls. This resulted in a 6 day instead of the normal 3 day

interval between using a cow as an oestrous teaser and as her own non-oestrous control. The variation was adopted so that full use could be made of the few cows available as oestrous teasers, thus restricting the duration of the trial period. Salisbury and Van Demark (1961) considered that at 3 dgys a cow would be in metoestrus but at 6 days she would be in dioestrus. No reported work has suggested that a teaser in the metoestrous condition is any more or less effective than a teaser in the dioestrous condition, and such a possibility seems unlikely. Any significant decline in sperm output could still be accounted for by using correction factors irrespective of the interval being 3 or 6 days.

(f) Seasonal Influences.

Conducting the trial during the late autumn need not necessarily imply that results would be only applicable to bulls at that time of the year. Climatic conditions which bulls would experience at the Awahuri Centre are not extreme, and the review of literature has shown that under such conditions there is little reason to suspect seasonal variation in semen output.

Contrasting reports on the seasonal variation in the duration of oestrus have also been noted in the literature. During the experimental period, a cow was only used as an oestrous teaser if she would stand while being mounted by another cow. This meant that she at least excited her herd mates. At any other time of the year a similar criterion for selection could have been used.

CHAPTER V

ASSOCIATED EXPERIMENTAL TECHNIQUES

The previous chapter outlined the design for the trial proper, but detailed techniques were also necessary for supplementary aspects related to stalling, preparing and collecting the bulls of each group.

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5.1 Pre-Collection Preparation.

The review of literature effectively showed that pre-collection sexual preparation produced an increase in the sperm content of an ejaculate. The increment was dependant upon the intensity and duration of preparation. The use of an oestrous cow would alter the teasing environment and where an oestrous--non-oestrous comparison was required, a standardised teasing procedure would be critical. Some flexibility would need to be incorporated to allow for individual variations between bulls, but a standardised teasing routine would not be as prone to variation as a subjective assessment of a bull's readiness by a collector. Standardised teasing procedures were therefore adopted.

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5.2 Stalling and Order of Collection.

Since all the bulls were well accustomed to the teasing and collection procedures, some bulls could have suffered varying degrees of frustration while waiting to be collected. Such an effect could be accentuated by the presence of an oestrous cow. The effects of this frustration would vary between bulls. To confound this possibility, each bull was randomly allocated to a stall prior to the use of a new teaser. The stalling order dictated the collection sequence except when oestrous teasers were used on 2 consecutive collection days for one group of bulls.

In this event, the bulls were randomly selected from their stalls. The same stalling and collection order was adopted for each oestrous--non-oestrous comparison.

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5.3 The Collection Team

Since minor variations in teasing procedure and collection technique would arise between different technicians, the same collection team comprising a collector, a leader and a third technician who prepared the artificial vaginas, handled the bulls throughout the entire trial. All three were experienced. Using such a competent team minimised the chances of collection technique influencing the final result.

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PART III

MATERIAL AND METHODS

CHAPTER VI

THE STOCK USED AND THEIR MANAGEMENT DURING THE TRIAL

This Chapter provides details of the stock used, the manner in which oestrous cows were selected and the collection procedure adopted with each bull.

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6.1 Bulls Selected For the Trial

At the commencement of the pre-trial period, 25 mature bulls standing at the Awahuri A.B. Centre were selected for the experiment. All of the bulls were experienced in collection procedure and none had exhibited abnormal collection behaviour patterns during the previous spring mating season. The bulls of each of the 3 breeds used, were randomly allocated to either one of the 2 groups. Initially GROUP I comprised 8 Jersey, 2 Ayrshire and 2 Friesian bulls and GROUP II comprised 8 Jersey, 2 Ayrshire and 3 Friesian bulls. During the pre-trial period, one Jersey bull was discarded from each group and a Friesian bull was discarded from GROUP II. The criteria used for eliminating these bulls were apparent loss of sexual appetite from that exhibited during the previous spring and/or a marked decline in semen quality since previous services had been collected.

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6.2 Cows From Which Teasers Were Selected

18 cows were available for use as teaser animals. This group comprised

(i) 4 trained teasers (2 Friesian and 2 Jersey) from the Awahuri A.B. Centre;

(ii) 4 trained teasers from the Newstead A.B. Centre;

(iii) 2 untrained Friesian cows from a local farm;

and (iv) 8 untrained cows (6 Jersey and 2 Ayrshire cross) from the Massey College herd of monozygous twins.

All of these cows with the exception of one pregnant teaser from the Awahuri Centre, had exhibited normal cyclical behaviour prior to the trial. During the pre-trial period the untrained cows were tested to determine their suitability as non-oestrous teasers. Because of excessive restlessness 2 of the Massey College cows were discarded.

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6.3 The Collection Procedure

Four factors each deserve separate consideration in defining the collection procedure. They are the stalling and the order of collection of each group of bulls, the pre-collection teasing routine, the team of technicians who handled the bulls and the method of collection.

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(a) The Stalling and Order of Collection

Each group of bulls was collected on different days. On the afternoon prior to a collection day, the bulls of the particular group concerned were taken off their tethering lines and stalled in the bull barn. The bulls were randomly allocated to one of the 11 stalls used. This random stalling order usually dictated the sequence in which the bulls were collected. The bulls were always stalled and collected in the same sequence for each oestrous - non-oestrous comparison. The exception to stalling order dictating the collection sequence was when oestrous teasers were used with one group of bulls on two consecutive collection days.

On the second day the bulls were stalled in readiness for collections being taken over the previous oestrous cow now in the non-oestrous condition but if another oestrous cow was used, the bulls were selected from their stalls at random.

Another exception was that Bull No. 115* was always placed in the same stall but he was always collected in the sequence stipulated by the table. The stalling order and, when necessary, the collection order were determined from tables of random numbers.

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(b) Pre-Collection Teasing Routine

Prior to the collection of the first service each bull was teased for a minimum of 10 minutes from the instant he was led from his stall. Teasing involved allowing the bull to smell and lick the teaser cow, leading the bull away from the cow and then letting him take a false mount at the time which the collector considered was most beneficial to that bull. Slight inflexibility had to be incorporated, as if the bull was not adequately prepared after 10 minutes, teasing was continued. The collector assessed a bull's satisfactory preparation, and when extra teasing time was needed, this was recorded. Towards the end of the trial some bulls did not respond to teasing in which case the bull concerned would be returned to his stall and collected after the remaining bulls had been collected.

After the first service had been collected, each bull was immediately returned to his stall and had a 10 minute rest whilst another bull was being collected. Prior to the collection of a second service, a bull received 7 minutes teasing including a false mount.

The following step-by-step sequence summarises the teasing routine:

* Bull No. 115 always used the same stall because of partial blindness.

Bull 'X' - Taken from his stall; received at least 10 minutes teasing including 1 false mount prior to the 1st service being collected. After collection the bull is returned to his stall.

Bull 'Y' - Receives similar treatment to Bull 'X'.

Bull 'X' - Receives 7 minutes teasing including a false mount prior to the collection of a second service; bull is returned to his stall and collection team wait for 3 minutes before taking Bull 'Y' from his stall.

Bull 'Y' - Receives similar treatment to Bull 'X'

Bulls are collected in pairs (as above) until all bulls have had 12 services collected.

During the pre-trial period it was found that 10 minutes teasing for the second service allowed no flexibility if bulls had to be teased for slightly longer than 10 minutes before collecting the first service. A 7-minute teasing system prior to the second collection was therefore adopted.

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(c) The Collection Team

Throughout the entire pre-trial and trial periods, a team of three experienced technicians handled the bulls. One technician made all the service collections, another teased the bulls and the third technician prepared the artificial vaginas for each bull.

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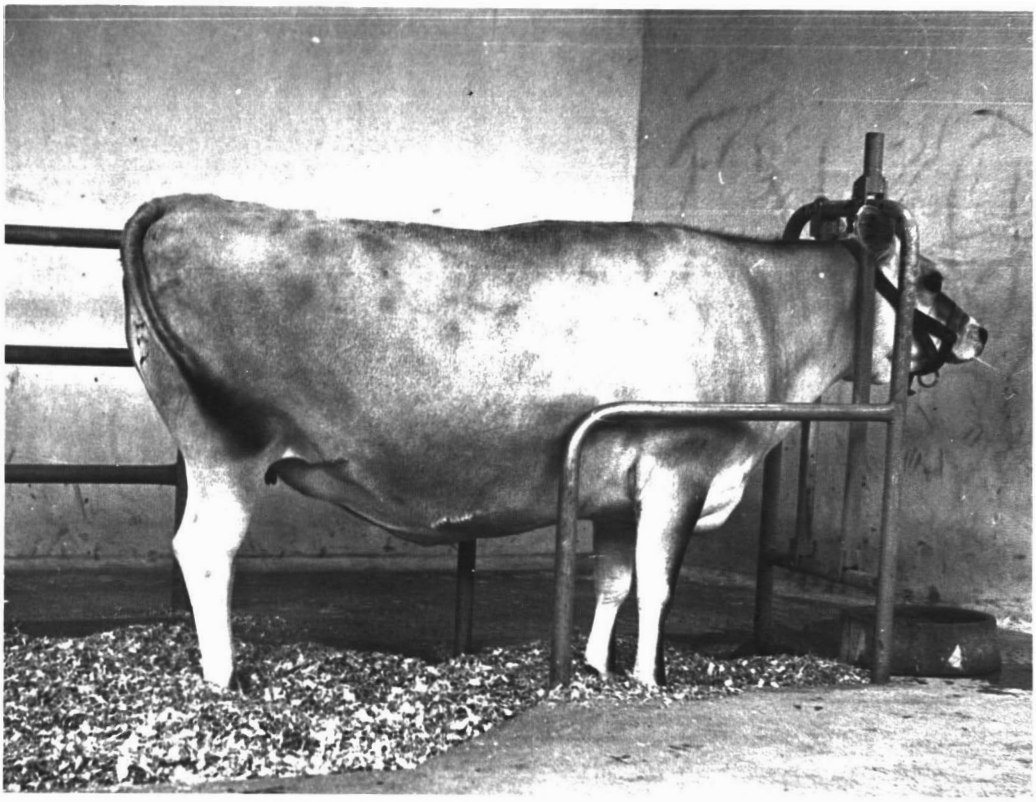
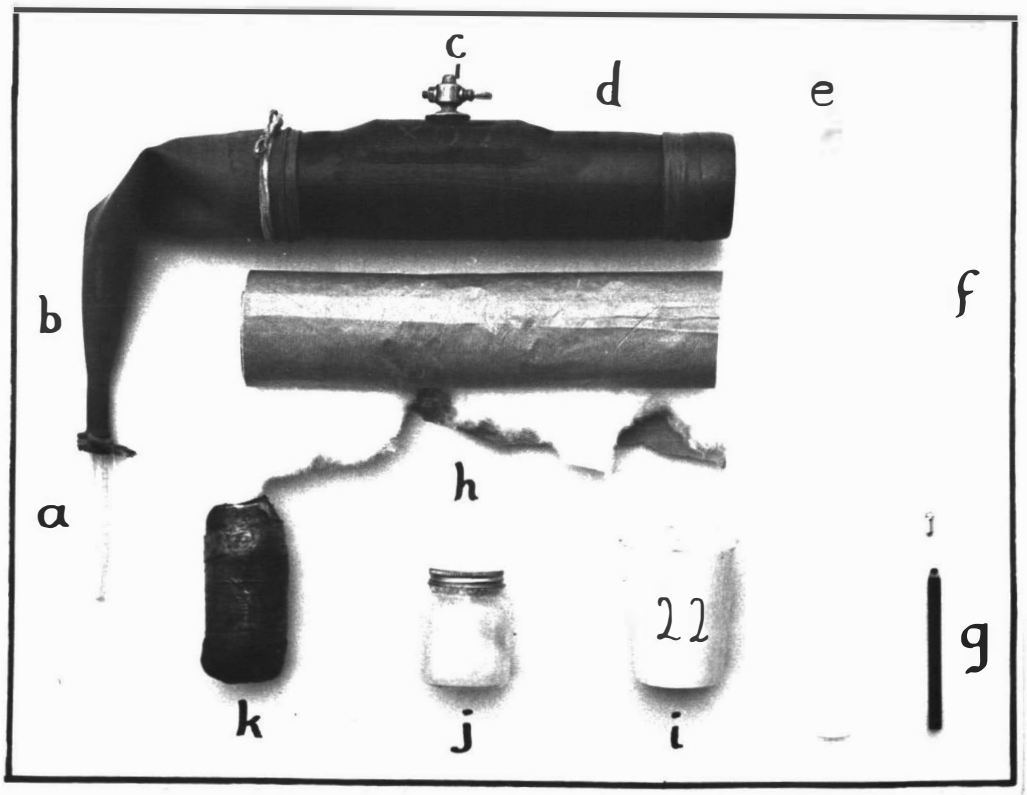
(d) The Method of Collection

The equipment used in preparing the artificial vagina and the



TEASER COW RESTRAINED WITHIN COLLECTING BAIL.



COLLECTING APPARATUS.

(a) Sterilised centrifuge tube. (b) Drainer. (c) Pressure regulating tap. (d) Rubber casing and liner. (e) Glass lubricating rod. (f) Thermometer. (g) Marking pencil. (h) Cotton wool. (i) Warmed beaker. (j) White vaseline. (k) Thimble.

type of artificial vagina used are shown in the accompanying photograph. The details of this type of artificial vagina have been described by Perry (1960). Each bull had his own artificial vagina. The temperature of the artificial vagina was checked immediately prior to a collection and adjustments made as required so that the artificial vagina, when presented to the bull, was in the temperature range from 42-45°C. White vaseline was used as a lubricant. The sterilised centrifuge tube was warmed and put into the warm thimble immediately before each collection was made.

When making a collection, the leader would allow the bull to mount and the collector would grasp the penis sheath in his left hand and apply the artificial vagina with his right hand. Any faulty collections were recorded. After the technician had made the collection, the artificial vagina was placed flat on the bench and left for one minute with the collection tube hanging from the drainer. After removal of the insulating thimble the centrifuge tube was placed in a warmed beaker on the outside of which the coded number for that bull on that day and the service number were recorded. The service was then passed through to the laboratory.

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6.4 Daily Selection of The Teaser Cow.

Each morning that an oestrous cow was required, the cows were inspected by the stockman and the Station Superintendent. Any cow showing the characteristic symptoms of oestrus such as a copious discharge of clear mucus, attempted mounting of other cows and in particular standing while being mounted by other cows, was selected as an oestrous teaser. After the completion of a day's collecting, a veterinary surgeon conducted a rectal examination on the oestrous cow as an additional check that the

uterus and ovaries exhibited signs characteristic of the oestrous condition. When none of the cows were displaying oestrous symptoms, the teaser cow was selected in accordance with the stipulations of the experimental design.

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6.5 Stock Management

All the bulls used during the trial were kept on tethering lines allowing restricted movement and grazing. Each bull received ½ bale of meadow hay plus 4-5 lbs of meal per day. When stalled overnight each bull received additional meal.

The cows were grazed as one herd under open paddock conditions. The paddocks used were several hundred yards away from the bull barn and out of the view of the bulls to be collected. The cows occasionally received hay.

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CHAPTER VII

LABORATORY TECHNIQUES USED IN SEMEN ASSESSMENT AND THE MEASUREMENT OF SEXUAL RESPONSE.

The details of each assessment will be outlined and the summary describes the sequence in which the tests were conducted.

7.1 Volume of Semen

The volume of semen ejaculated was read directly from the graduated centrifuge tube in which the service had been collected. Readings were recorded to the nearest 0.1 ml. Froth often capped a service but this was not included when reading the volume. A visual assessment of appearance was also recorded. The assessments ranged from "good" for a thick service (over 1000 x 10^6 sperm/ml.) of good colour, through "moderately good" and "moderate" to "thin" for a low density service (under 300 x 10^6 sperm/ml.)

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7.2 Concentration of Sperm Cells

The sperm concentration of each service was determined using a "Hilger Biochem" absorptiometer which had been calibrated against sperm counts made with a Fuchs Rothenthal cytometer. Details of the calibration and results are recorded in Appendix 3. The detailed procedure was as follows:

(i) 0.1 ml. of semen was removed with a micropipette, the exterior of which was then wiped clean.

(ii) the semen sample was expelled into 9.9 mls. of formalin-saline solution,

(iii) the pipette was rinsed by drawing the mixture up three times

(iv) The diluted semen was inverted at least 10 times to ensure adequate mixing;

(v) 1-2 mls of the mixed sample was used to rinse a curette and the remainder poured into the rinsed curette, the exterior of which was carefully wiped with a soft cloth;

(vi) the curette was placed in the absorptiometer which was standardised against formalin-saline solution prior to each reading;

(vii) the diluted semen sample remained in the absorptiometer for 5 minutes before the reading was recorded from the density scale;

(viii) after recording the result, the curette, micropipette and graduated 10 ml micro-cylinder were rinsed with formalin-saline solution.

If the result showed a concentration of 1000 x 10^6 sperm per ml., or greater, the test was repeated and the mean of the two readings used in the analyses. The light filter used in the absorptiometer was No. 58. The formalin-saline solution was made up of 9 grms of NaCl and 2 ~~grms~~ mls. of 40% formalin per litre.

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7.3 % Live Sperm

All the live-dead counts throughout the trial were made by the one technician who was experienced in the technique. Within 3-4 minutes of a service being collected, a drop of semen was prepared for staining. Details of the procedure were:-

(i) a warm, clean glass rod was dipped into the semen in the centrifuge tube and a drop of semen placed on a warm glass slide;

(ii) 2 drops of warm stain were added to the drop of semen and well mixed with the glass rod;

(iii) the ~~smear~~ sample was left in the warming cupboard for 5 minutes after which the smear was prepared;

(iv) after the smear had dried a total of 200 sperm were counted at 400 x magnification, and all partially stained sperm were counted as dead. Between 5-10 fields were covered in the count;

The stain was prepared as follows. Solution A comprised 17.75 grms. Na_2HPO_4 in 1 litre of distilled water. Solution B contained 17.01 grms KH_2PO_4 per litre. 50 mls of Solution B were added to 950 mls of Solution A together with 6 grms of aqueous eosin yellow and 30 grms of analine blue. This mixture was allowed to stand for 24 hours prior to filtering, after which it was ready for use.

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7.4 Motility Assessments

Motility assessments were made within 4 minutes of the service being collected and just over 1 hour later for services which had been incubated at 37°C. The procedure for each assessment was as follows:

(i) A clean warm glass rod was dipped into the service or incubated sample and a drop of semen mounted under a glass cover slip on a slide resting on a warming stage maintained at 35-37°C;

(ii) the motility was microscopically assessed after the sample had been mounted for about 1 minute (x75 magnification), all assessments being made by the one technician;

The ratings adopted were:-

0 No visibly motile sperm

+ Few sperm exhibiting motility

1 Slow motility but no wave motion

1+ Slight wave motion

2 Slight improvement over 1+

2+ Moderate wave motion or good motility in the sperm of a thin service

3 Strong wave motion with vigorous swirling

3+ Very rapid wave motion with rapid swirling or rapid motility exhibited by all the sperm of a thin service.

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7.5 pH Measurements

The "Copenhagen Radiometer" used for measuring pH required at least 3.5 mls. of semen and measurements could not be determined for all services. The initial pH was determined 5 minutes after collection and the final pH was determined after 1 hour's incubation at 37°C. The procedure for each reading was as follows:-

(i) 4 mls of undiluted semen were pipetted into a small plastic bowl and the electrodes immersed.

(ii) After the reading was recorded the sample was poured into a narrow-bore 10 ml. pyrex test tube and placed in the incubator, or poured away;

(iii) after each reading, the electrodes were rinsed with warm distilled water and then dried;

(iv) the calibration of the meter was checked with a standard buffer after every 4 readings.

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7.6 Initial Fructose Concentration

The details of the calibration of the Hilger Biochem absorptiometer are reported in Appendix 4. To measure the initial concentration of fructose:

(i) 0.1 ml of undiluted semen was pipetted with a dry micropipette into 0.9 mls distilled water.

(ii) 2 mls of 2% $ZnSO_4 . 7H_2O$ and 2 mls of 0.1 N.NaOH were added to deproteinise the sample, and samples left until collections had been completed;

(iii) the samples were held in a water bath at 100°C for 2 minutes and filtered hot using Whatman No. 1 papers;

(iv) 1.5 mls. of 0.1% resorcinol in ethanol and 4.5 mls of 35% hydrochloric acid were added to 1.5 mls of the clear filtrate;

(v) the resulting mixture was heated in a water bath at 80-85°C for 10 minutes and then rapidly cooled in tap water;

(vi) the intensity of the resulting colour re-action was measured in the absorptiometer with a green filter (No. 49);

(vii) after every 5 readings, the absorptiometer was checked against a standard blank.

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7.7 Measurement of Sexual Response

Since the experimental design necessitated the restraining of a bull prior to service, an accurate assessment of re-action time could not be determined but the collector recorded the time at which he considered the bull would have served had he not been restrained. The interval

between taking a bull from his stall and estimating the time when a service could be collected was termed the re-action time.

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7.8 Summary of Semen Assessments

The procedure adopted with each service as soon as it arrived in the laboratory was:-

(i) the measurement of semen volume and an assessment of appearance;

(ii) sampling for the initial motility assessment and live-dead count;

(iii) sampling for sperm concentration and initial fructose concentration;

(iv) if the remainder was 4 mls or more the initial pH was read;

and (v) the service was incubated for one hour at 37°C and final motility and pH change assessed.

Each of the first four stages were completed within 5 minutes of a service being collected. One technician measured pH, another the live-dead and motility assessments and the researcher measured volume, sperm and fructose concentration and assessed semen appearance.

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PART IV

RESULTS

At the commencement of the trial each of the 2 groups comprised 7 Jersey, 2 Ayrshire and 2 Friesian bulls. In the final analyses of the data 2 bulls were discarded from GROUP I and 1 bull from GROUP 2. Reference to Appendix I will show that 1 Jersey bull in each group (Nos. 393 and 359) had exceptionally low sperm output. Live-dead counts showed that these 2 bulls had abnormally low numbers of live sperm indicating faulty spermatogenesis. Despite the abnormalities in spermatogenesis, the libido of the bulls was satisfactory. Semen output did not decline and the initial concentration of seminal fructose was exceptionally high. An Ayrshire bull (No. 16) was excluded from analyses on data for GROUP I. This bull had an exceptionally vigorous service pattern, making good collections difficult to obtain and several collections were missed.

Terms used in the results include:-

(i) Total Sperm Output per Bull per Collection Day which is the sum of the sperm content of the 2 services

i.e. Total Sperm Output = (Volume 1st Serv x Sperm Conc. 1st Serv) + (Volume 2nd Serv x Sperm Conc. 2nd Serv)

(ii) Total Semen Volume per Bull per Collection Day which is the sum of the volume of the 2 services

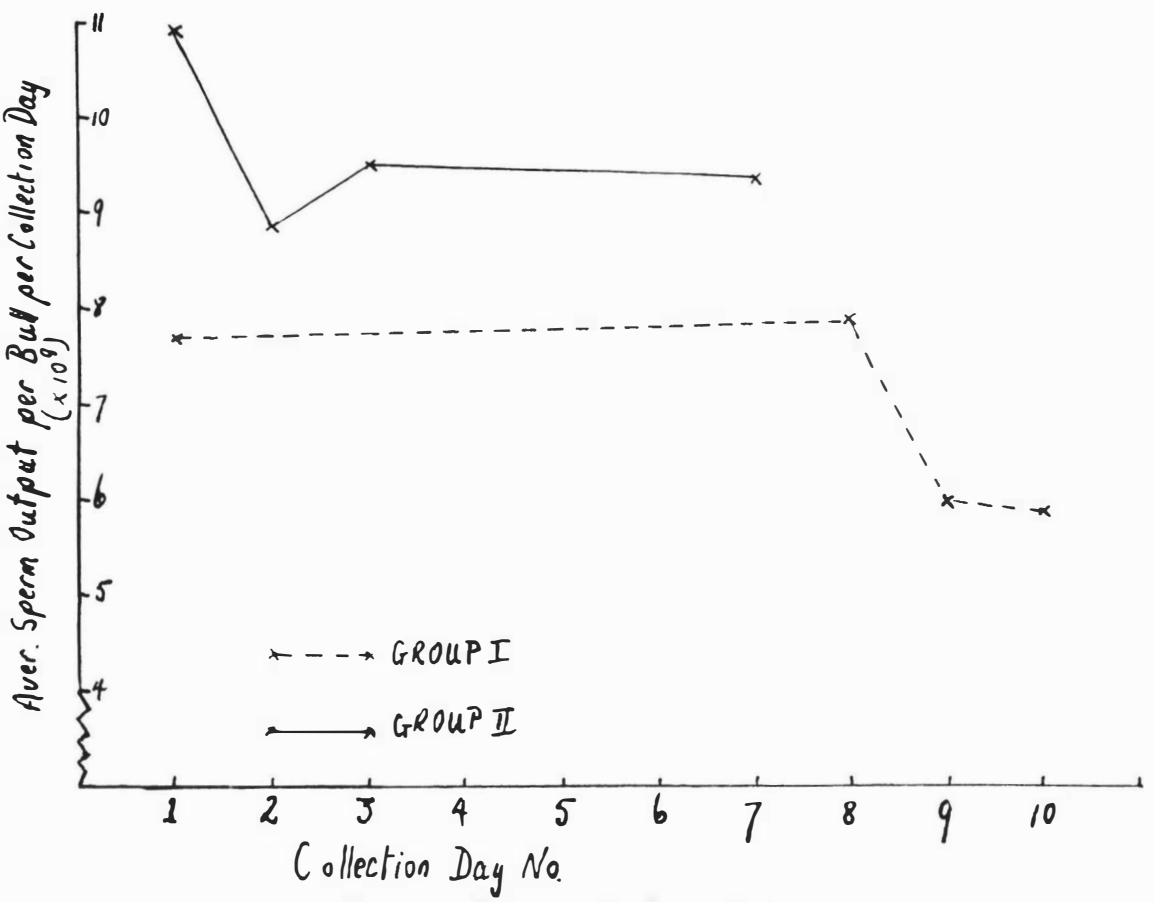

Fig.I: Aver. Sperm Output Per Bull Per Collection Day When The Pregnant Teaser was Used With Each Group.

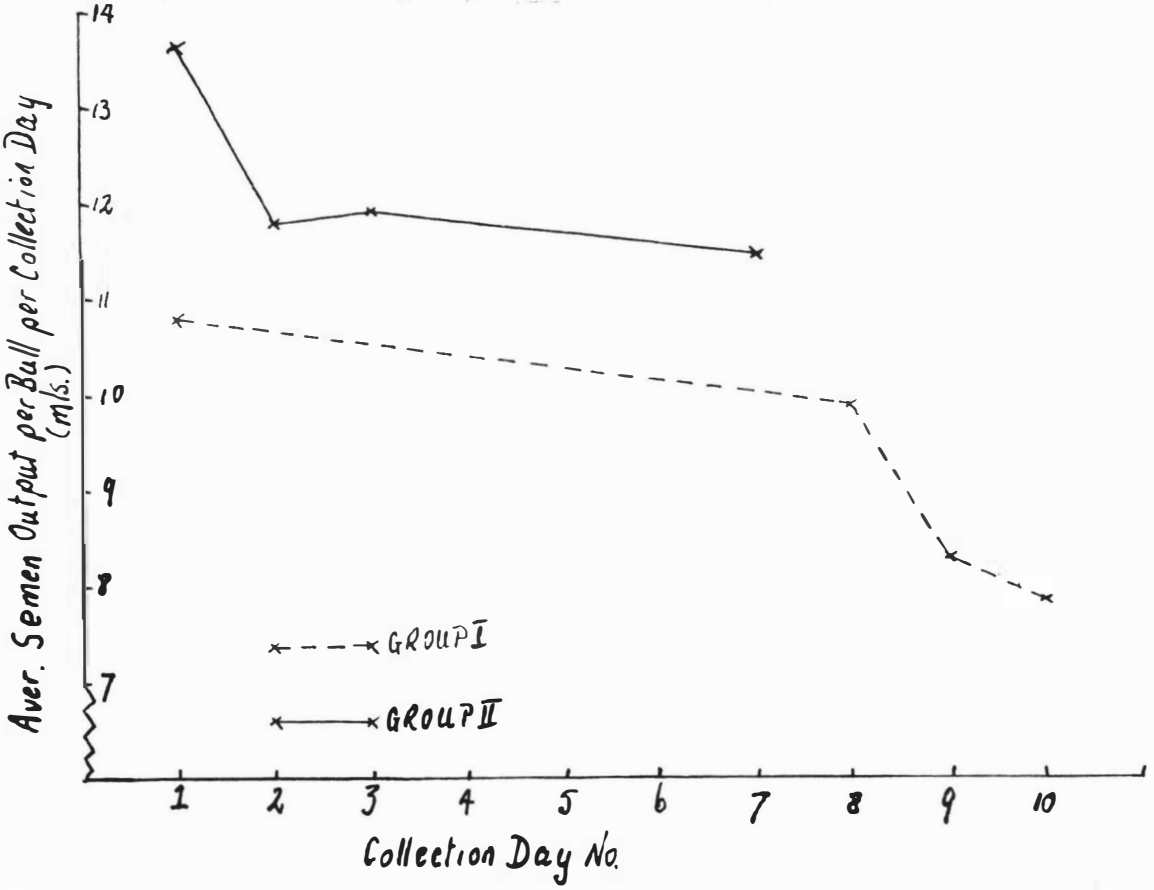

Fig.II: Aver. Semen Output Per Bull Per Collection Day When

CHAPTER 8

Day to Day Variation in Sperm Output and Semen Volume

During the trial period, a pregnant trained teaser-cow was used with each group of bulls on 4 collection days. Since a standardised pre-collection routine was adhered to throughout the trial and the same team of technicians prepared and collected each bull, it was considered that the data collected would indicate whether or not significant between day differences in sperm output and semen volume had occurred during the trial period. Analyses on previous data (Appendix V) suggested that such differences were of no real importance. Figs. 1 and 2 show the respective average sperm output and average semen volume per bull per collection day when the pregnant cow was used as the teaser animal.

Bull No. 406 (GROUP II) was excluded when developing the graphs and from the analyses, as data was incomplete on 2 days. Bull No. 354 (GROUP I) had incomplete data for Collection Day No. 10 but average figures obtained from the outputs of this bull on the other 3 days were substituted and the total degrees of freedom in the analyses reduced by 1.

The results of the analyses (Tables 4 and 5) showed that both groups had experienced unknown variables which produced between day differences in total sperm output which were significant at the 5% level of probability.

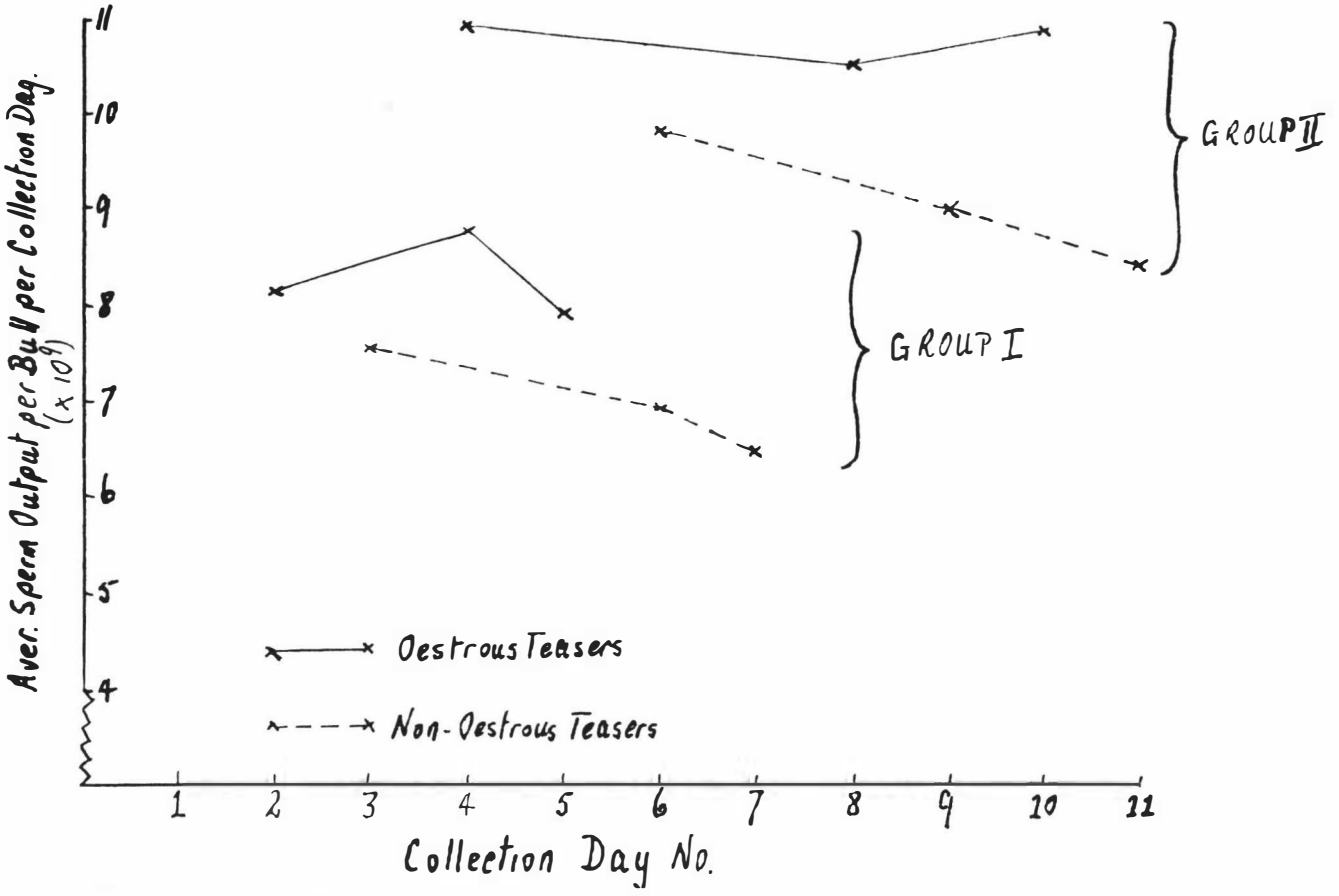

Fig.III: AVER. SPERM OUTPUT PER BULL PER COLLECTION DAY WHEN OESTROUS AND NON-OESTROUS TEASER COWS WERE USED.

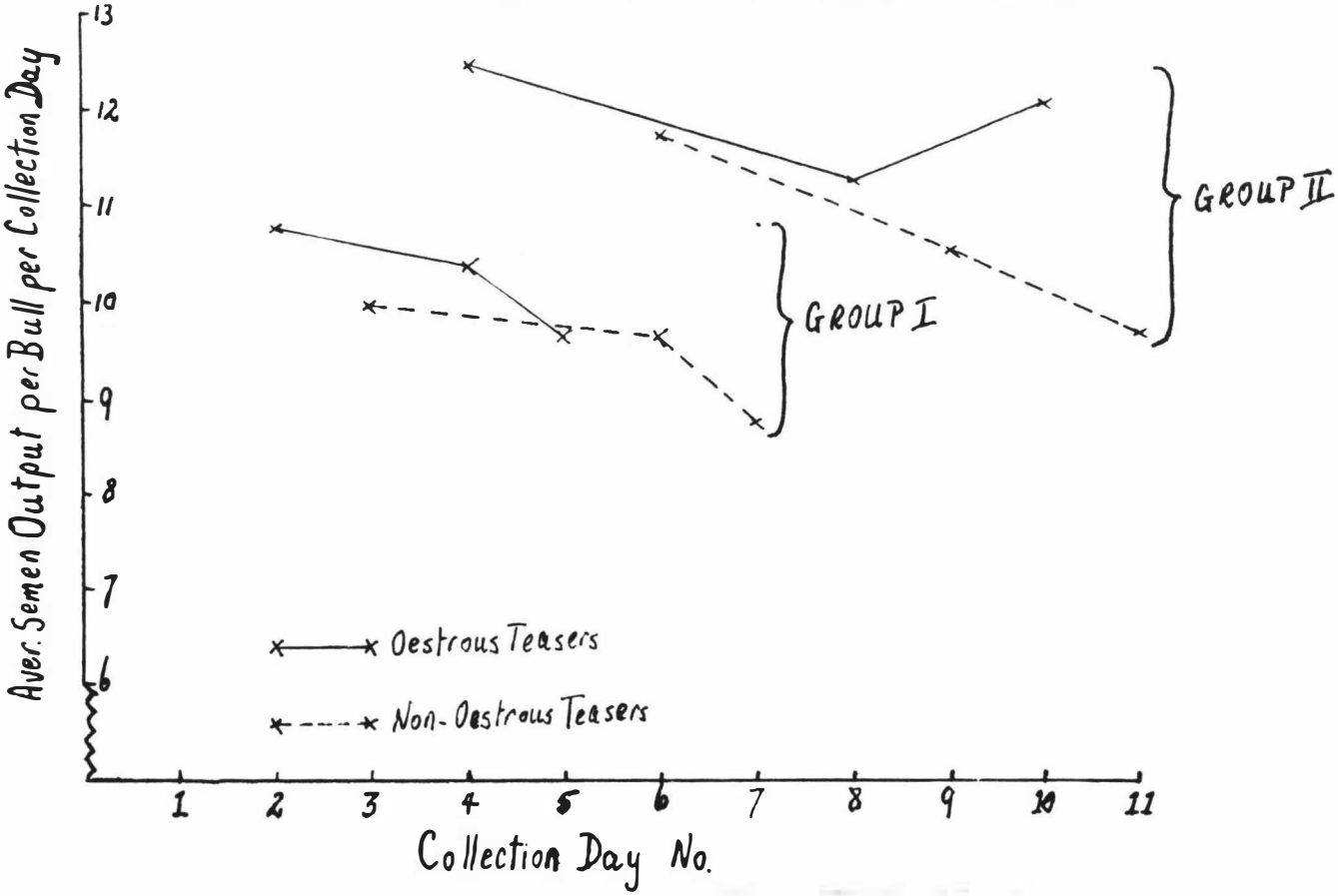

Fig.IV: AVER. SEMEN OUTPUT PER BULL PER COLLECTION DAY WHEN

Source of Variation	d.f.	Sums of Squares	Mean Square	F. Calc.	F.Reqd.	Test of Significance
Between Bulls	8	110.19	13.77	5.60	2.38 3.41	**
Between Days	3	31.75	10.58	4.30	3.03 4.76	*
Error	23	56.67	2.46			
Total	34	198.61				

Table 4: Analysis of Variance for Between Day Differences of The Total Sperm Output When The Pregnant Teaser Was Used with GROUP I.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	Test of Significance
Between Bulls	8	263.28	32.91	20.96	2.36 3.36	**
Between Days	3	21.56	7.19	4.58	3.01 4.72	*
Error	24	37.76	1.57			
Total	35	322.6				

Table 5: Analysis of Variance For Between Day Differences of The Total Sperm Output When the Pregnant Teaser Was Used with GROUP II.

These levels of significance in between day differences in total sperm output were not expected. Further analyses were therefore carried out on the data collected on the days when oestrous teasers and when non-oestrous teasers were used. Implicit in these analyses was variations due to the use of a different cow each day and between day effects, if such existed, could be confounded with cow effects. The analyses (Tables 6-9) showed that there were no significant differences between "cow-days"

in the total sperm output when either the between oestrous or between non-oestrous "cow-day" comparisons were made.

Source of Variation	d.f.	Sums of Squares	Mean Square	F. Calc.	F. Reqd.	Test of Significance
Between Bulls	8	137.13	17.14	8.74	2.59 3.89	**
Between Cows	2	3.58	1.79	0.91	3.63 6.23	N.S.
Error	16	31.36	1.96			
Total	26	172.07				

Table 6: Analysis of Variance For The Total Sperm Collected From Group I when Oestrous Teaser Cows Were Used.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	Test of Significance
Between Bulls	9	272.61	30.29	8.56	2.46 3.60	**
Between Cows	2	0.72	0.36	0.1	3.55 6.01	N.S.
Error	18	63.66	3.54			
Total	29	336.99				

Table 7: Analysis of Variance For The Total Sperm Collected From GROUP II, when Oestrous Teaser Cows Were Used.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	Test of Significance
Between Bulls	8	91.29	11.41	7.98	2.59 3.89	**
Between Cows	2	5.36	2.68	1.87	3.65 6.23	N.S.
Error	16	22.91	1.43			
Total	26	119.56				

Table 8: Analysis of Variance For The Total Sperm Collected from GROUP I When Non-Oestrous Teaser Animals were Used.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc	F.Reqd.	Test of Significance
Between Bulls	9	179.15	19.9	5.38	2.46 3.60	**
Between Cows	2	9.90	4.95	1.34	3.55 6.01	N.S.
Error	18	66.64	3.7			
Total	29	255.67				

Table 9: Analysis of Variance For The Total Sperm Collected From GROUP II When Non-Oestrous Teaser Animals Were Used.

Analysis of the data for semen volume when each group was collected over the pregnant teaser cow indicated that neither group exhibited between day differences which were significant at the 5% level of probability. (Tables 10 and 11)

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	Test of Significance
Between Bulls	8	182.36	17.5	3.0	2.36 3.36	*
Between Days	3	48.45	16.15	2.77	3.01 4.72	N.S.
Error	24	139.98	5.83			
Total	35	370.79				

Table 10: Analysis of Variance for the Total Volume of Semen Collected From GROUP I When The Pregnant Teaser Was Used.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	Test of Significance
Between Bulls	8	550.63	68.83	21.24	2.36 3.36	**
Between Days	3	25.67	8.56	2.64	3.01 4.72	N.S.
Error	24	77.70	3.24			
Total	35	654.0				

Table 11: Analysis of Variance for the Total Volume of Semen Collected From GROUP II When the Pregnant Teaser Was Used.

Figs 1-4 showed that total sperm and semen output were highest at the commencement of the trial. Regression analyses were therefore calculated to:

(i) determine whether these apparent declines in output were significant,

and (ii) calculate correction factors for use in the main analyses if a decline proved significant.

The data comprised the collections made when the pregnant teaser

and when the non-oestrous teasers were used.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	Test of Significance
Regression	1	14.39	14.39	2.58	4.14 7.47	N.S.
Error	33	183.92	5.57			
Total	34	198.31				

Table 12: Regression Analysis on Total Sperm Collected From GROUP I When The Pregnant Teaser Was Used.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	Test of Significance
Regression	1	11.25	11.25	1.14	4.11 7.39	N.S.
Error	36	357.79	9.94			
Total	37	369.04				

Table 13: Regression Analysis on Total Sperm Collected from GROUP II When The Pregnant Teaser Was Used.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	Test of Significance
Regression	1	5.13	5.13	1.12	4.24 7.77	N.S.
Error	25	114.43	4.58			
Total	26	119.56				

Table 14: Regression Analysis on Total Sperm Collected From GROUP I When Non-Oestrous Teaser Cows Were Used.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	Test of Significance
Regression	1	9.89	9.89	1.13	4.20 7.64	N.S.
Error	~~2~~ 28	245.78	8.78			
Total	29	255.67				

Table 15: Regression Analysis on Total Sperm Collected From GROUP II When Non-Oestrous Teaser Cows Were Used.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	Test of Significance
Regression	1	33.22	33.22	3.33	4.14 7.47	N.S.
Error	33	329.8	9.99			
Total	34	363.02				

Table 16: Regression Analysis on Total Volume of Semen Collected from GROUP I When the Pregnant Teaser Cow Was Used.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	Test of Significance
Regression	1	21.29	21.29	1.13	4.11 7.39	N.S.
Error	36	680.21	18.89			
Total	37	701.5				

Table 17: Regression Analysis on Total Volume of Semen Collected From GROUP II When the Pregnant Teaser Was Used.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	Test of Significance
Regression	1	5.58	5.58	0.89	4.24 7.77	N.S.
Error	25	157.17	6.29			
Total	26	162.75				

Table 18: Regression Analysis on Total Volume of Semen Collected From GROUP I When Non-Oestrous Teaser Cows Were Used.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	Test of Significance
Regression	1	22.39	22.39	2.08	4.20 7.64	N.S.
Error	28	302.21	10.79			
Total	29	324.6				

Table 19: Regression Analysis on Total Volume of Semen Collected From GROUP II When Non-Oestrous Teaser Cows Were Used.

Tables 12-19 indicated that the apparent declines in sperm output and semen volume were not statistically significant. In light of these results, and because the data collected when oestrous cows were used as teasers did not exhibit any marked decline, regression analyses on the "oestrous-teaser" data were not considered necessary. Because these regression analyses indicated that there had not been significant declines in sperm output and semen volume, correction factors were not developed for use in the major analyses.

CHAPTER 9

Treatment Effects on Total Sperm Output, Total Semen Volume and Average Sperm Concentration

9.1 Treatment Effect on Total Sperm Output

Data was incomplete for Bull No. 406 (GROUP II on Collection Day No. 2.) The average sperm output for this bull calculated from the other 2 collection days when oestrous teasers were used was substituted for Day No. 2 and the total degrees of freedom in the analysis reduced by 1.

The F values for the first order interactions were calculated by dividing each first order mean square by the second order mean square. The F ratio for the test of treatment effects was calculated as either

$$\frac{\text{Treatment M.S.}}{\text{Cow x Treat.M.S.}} \quad \text{or} \quad \frac{\text{Treatment M.S.}}{\text{Bull x Treat.M.S.}}$$

depending upon which first order treatment inter-action mean-square was the greater. With GROUP I, the former test applied and in GROUP II the latter. The degrees of freedom for the denominators were 2 and 9, but the conservative degrees of freedom (2) were used in both cases when F tables were consulted to test the levels of significance. These tests were conservative estimates of the level of significance of treatment differences, but since only 3 cows were used as teasers by each group of bulls, such conservatism was considered necessary when interpreting the results.

COW.NO. / BULL NO.		354	115	351	365	129	518	322	52	364	Total
2	Oestrous	12.1	9.1	3.5	5.4	9.7	7.7	7.9	9.9	8.0	73.3
	Non-Oest.	11.1	6.4	3.5	6.8	8.3	6.7	9.0	9.0	7.3	68.1
Cow Total		23.2	15.5	7.0	12.2	18.0	14.4	16.9	18.9	15.3	141.4
3	Oestrous	12.4	7.8	3.5	9.0	8.9	7.4	8.5	12.7	8.9	79.1
	Non-Oest.	9.9	7.2	2.3	6.0	7.1	6.7	7.1	8.6	7.7	62.6
Cow Total		22.3	15.0	5.8	15.0	16.0	14.1	15.6	21.3	16.6	141.7
5	Oestrous	11.0	6.0	3.0	11.3	8.2	7.3	5.9	9.7	9.0	71.4
	Non-Oest.	8.1	2.4	2.8	9.0	7.3	7.2	6.7	8.1	6.7	58.3
Cow Total		19.1	8.4	5.8	20.3	15.5	14.5	12.6	17.8	15.7	129.7
Oestrous Total		35.5	22.9	10.0	25.7	26.8	22.4	22.3	32.3	25.9	223.8
Non-Oest. Total		29.1	16.0	8.6	21.8	22.7	20.6	22.8	25.7	21.7	189.0
Grand Total		64.6	38.9	18.6	47.5	49.5	43.0	45.1	58.0	47.6	412.8

Table 20: Total Sperm ($x10^9$) Collected From Bulls of GROUP I When Oestrous and Non-Oestrous Teaser Cows Were Used.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	d.f.for Test	Test of Significance
Between Bulls	8	219.64	27.46	10.25	2.59 3.89	8;16	**
Between Cows	2	5.21	2.61	0.97	3.63 6.23	2;16	N.S.
Between Treatments	1	22.43	22.43	11.99	18.51 98.49	1;2	N.S.
Bulls x Cows	16	42.85	2.68	3.77	2.33 3.37	16;16	**
Bulls x Treats.	8	8.78	1.09	1.54	2.59 3.89	8;16	N.S.
Cows x Treats.	2	3.73	1.87	2.63	3.63 6.23	2;16	N.S.
Bulls x Cows x Treats.	16	11.42	0.71				
Total	53	314.06					

Table 21: Analysis of Variance For the Total Sperm Output From GROUP I when Oestrous and Non-Oestrous Teaser Cows Were Used.

COW NO. / BULL NO.		19	406	122	159	304	397	561	12	391	705	Total
4	Oestrous	17.7	8.6	8.7	14.8	11.5	6.4	7.4	16.2	7.0	11.2	109.5
	Non-Oest.	11.8	5.1	9.2	14.5	9.9	6.6	10.3	13.2	6.1	11.5	98.2
Cow Total		29.5	13.7	17.9	29.3	21.4	13.0	17.7	29.4	13.1	22.7	207.7
2	Oestrous	16.8	9.6	9.0	13.9	12.8	8.2	6.9	10.7	7.0	11.0	105.9
	Non-Oest.	13.6	6.3	14.3	7.2	6.8	5.2	6.9	12.3	6.4	11.0	90.0
Cow Total		30.4	15.9	23.3	21.1	19.6	13.4	13.8	23.0	13.4	22.0	195.9
6	Oestrous	16.6	7.6	12.1	10.2	9.7	7.7	11.9	15.3	5.8	13.8	108.7
	Non-Oest.	12.0	6.2	10.2	9.6	9.8	6.3	4.6	9.9	5.9	9.7	84.2
Cow Total		28.6	13.8	22.3	19.8	18.5	14.0	16.5	25.2	11.7	23.5	192.9
Oestrous Total		51.1	23.8	29.8	38.9	34.0	22.5	26.2	40.2	19.8	36.0	324.1
Non-Oest. Total		37.4	17.6	33.7	31.3	26.5	18.1	21.8	35.4	18.4	32.2	272.4
Grand Total		88.5	43.4	63.5	70.2	60.5	40.4	48.0	75.6	38.2	68.2	596.5

Table 22: Total Sperm ($x10^9$) Collected From Bulls of GROUP II When Oestrous and Non-Oestrous Teaser Cows Were Used.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	d.f. for Test	Test of Significance
Between Bulls	9	419.52	46.61	13.02	3.18 5.35	9;9	**
Between Cows	2	6.12	3.06	1.08	3.55 6.01	2;18	N.S.
Between Treatments	1	44.55	44.55	12.44	18.51 98.49	1;2	N.S.
Bulls x Cows	18	51.05	2.84	0.61	2.26 3.21	18;17	N.S.
Bulls x Treats.	9	32.22	3.58	0.77	2.50 3.68	9;17	N.S.
Cows x Treats.	2	4.49	2.25	0.48	3.59 6.11	2;17	N.S.
Bulls x Cows x Treats.	17	79.28	4.66				
Total	58	637.21					

Table 23: Analysis of Variance For The Total Sperm Output From GROUP II When Oestrous and Non-Oestrous Teasers Were Used.

Note: Treatment F Ratio = $\frac{\text{Treatment M.S.}}{\text{Bull x Treat. M.S.}}$ but 1;2 d.f. used for testing significance

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Both groups showed highly significant differences between bulls in sperm output. Neither group showed significant variations in the total sperm output for each cow used in the oestrous and non-oestrous condition. Similarly neither of the first order interactions involving treatment attained significance at the 5% level of probability in either group. Bulls of GROUP I experienced a highly significant interaction with the cows they used as teasers.

In determining which bulls of GROUP I produced the significant bull x cows interaction the following calculation was used:

$$\text{Standard Error of}\left(\begin{matrix}\text{Oestrous + Non-Oestrous}\\ \text{Total Per Bull Per Cow}\end{matrix}\right) = \sqrt{2 \times \text{Error M.S.}}$$

$$= \sqrt{2 \times 0.71}$$

$$d_{0.5} = t_{16df}\sqrt{2 \times S.E.}$$

$$= 2.12 \times \sqrt{2.84}$$

$$= 3.58$$

Those bulls which showed cow total sperm output differences of 3.58×10^9 or greater not shown by the other bulls would have contributed to this interaction. Bull No. 115 showed a much lowered output when COW No. 5 was used. Although reference to the appendices will show that the collector noted that for some unknown reason the service behaviour was abnormal for the first service collected when this cow was in oestrous, no reason was suggested for the exceptionally low non-oestrous total. In contrast, Bull No. 365 showed a marked increase in output when Cow No. 5 was used. No logical reasons can be suggested as to why these bulls produced the interactions with this cow.

In comparison with non-oestrous teasers, the use of oestrous teasers increased the total sperm output by 18.4% in GROUP I and 18.7% in GROUP II. Because only three cows were used as teasers with each group and conservative measures were adopted in determining levels of significance, the treatment effect was not significant at the customary 5% level of probability in either group.

	GROUP I			GROUP II		
	Mean	± Stand. Error	Coeff. Var.	Mean	± Stand. Error	Coeff. Var.
Oestrous Teasers	8.3	± 0.5	31.4%	10.8	± 0.6	31.5%
Non-Oestrous Teasers	7.0	± 0.4	30.0%	9.1	± 0.6	33.0%

Table 24: Mean Sperm Output ($x10^9$) and Coefficient of Variation Per Bull Per Collection Day When Oestrous and Non-Oestrous Cows Were Used.

A graph of the distribution for t(2) showed that the level of probability for treatment differences was 8% in GROUP I and 7.5% in GROUP II. Since there were limited degrees of freedom through only using three cows with each group, the data for each cow in the oestrous and non-oestrous condition were averaged and combined.

	GROUP I			GROUP II			
COW NO.	2	3	5	4	2	6	Total ± S.E.
TREATMENT							
Oestrous	8.14	8.79	7.93	10.95	10.59	10.87	57.27 ± 1.1
Non-Oest.	7.57	6.96	6.48	9.82	9.00	8.42	48.25 ± 1.1
Total	15.71	15.75	14.41	20.77	19.59	19.29	105.52

Table 25: Average Sperm Output ($x10^9$) Per Bull Per Cow Used As An Oestrous and a Non-Oestrous Teaser.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	Test of Significance
Between Cows	5	17.02	3.40	16.82	5.05 10.97	**
Between Treatments	1	6.78	6.78	33.5	6.61 16.26	**
Error	5	1.01	0.20			
Total	11	24.81				

Table 26: Analysis of Variance of Average Sperm Output Per Bull Per Cow Used as an Oestrous and Non-Oestrous Teaser.

The analysis presented in Table 26 indicated that when the treatment effect was compared over 6 oestrous--non-oestrous replications, the use of oestrous teasers had produced an increase in total sperm output which was significant at the 1% level of probability.

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9.2 Treatment Effect on Total Volume of Semen

As with the data for total sperm output an average figure derived from the semen output of Bull No. 406 on Collection Days Nos 8 and 10 was substituted for missing data on Day No. 2, and total degrees of freedom for GROUP II reduced by 1. In testing the significance of the treatment effect, calculation procedures were identical to those used in testing the significance of the treatment effect on total sperm output.

COW NO. \ BULL NO.		354	115	351	365	129	518	322	52	364	Total
2	Oestrous	10.1	14.8	11.5	10.8	16.5	5.6	8.6	12.0	7.2	97.1
	Non-Oest.	9.6	10.3	11.1	11.6	13.6	5.3	10.8	11.1	6.5	89.9
Cow Total		19.7	25.3	22.6	22.4	30.1	10.9	19.4	23.1	13.7	187.0
3	Oestrous	9.4	9.5	12.8	11.5	15.6	5.1	8.7	13.6	7.5	93.5
	Non-Oest.	10.0	9.7	9.8	10.7	12.2	4.9	9.4	12.8	7.1	86.6
Cow Total		19.4	19.2	22.6	22.2	27.8	10.0	18.1	26.4	14.4	180.1
5	Oestrous	8.9	7.7	9.5	12.3	14.7	5.1	8.2	12.1	8.0	86.5
	Non-Oest.	6.9	5.0	10.4	11.7	12.1	5.6	8.5	10.3	7.7	78.7
Cow Total		15.8	12.7	19.9	24.0	26.8	10.7	16.7	22.9	15.7	165.2
Oestrous Total		28.4	32.0	33.8	34.6	46.8	15.8	25.5	37.7	22.5	277.1
Non-Oest. Total		26.5	25.0	31.3	34.0	37.9	15.8	28.7	34.7	21.3	255.2
Grand Total		54.9	57.0	65.1	68.6	84.7	31.6	54.2	72.4	43.8	532.3

Table 27: Total Volume of Semen Collected From Bulls of GROUP I When Oestrous and Non-Oestrous Teaser Cows Were Used.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Recd.	d.f. for Test	Test of Significance
Between Bulls	8	332.48	41.56	15.62	2.59 3.89	8;16	**
Between Cows	2	13.8	6.90	2.59	3.63 6.23	2;16	N.S
Between Treatments	1	8.88	8.88	4.02	18.51 98.49	1;2	N.S.
Bulls x Cows	16	42.59	2.66	3.17	2.33 3.37	16;16	*
Bulls x Treats	8	17.64	2.21	2.63	2.59 3.89	8;16	*
Cows x Treats	2	0.02	0.01	0.12	3.63 6.23	2;16	N.S.
Bulls x Cows x Treats	16	13.51	0.84				
Total	53	423.92					

Table 28: Analysis of Variance For The Total Volume of Semen Collected From GROUP I When Oestrous and Non-Oestrous Teaser Cows Were Used.

COW NO. \ BULL NO.		19	406	122	158	304	397	561	12	391	703	Total
4	Oestrous	17.3	9.9	15.8	17.6	12.0	11.9	6.4	16.9	6.4	10.1	124.3
	Non-Oest.	11.3	6.5	15.0	18.8	13.4	12.7	8.7	15.4	5.9	9.8	117.5
Cow Total		28.6	16.4	30.8	36.4	25.4	24.6	15.1	32.3	12.3	19.9	241.8
2	Oestrous	15.2	9.9	11.6	14.6	15.5	12.5	5.7	12.9	6.2	8.7	112.8
	Non-Oest	12.2	6.7	15.6	12.2	9.4	11.5	9.6	12.9	5.8	9.5	105.4
Cow Total		27.4	16.6	27.2	26.8	24.9	24.0	15.3	25.8	12.0	18.2	218.2
6	Oestrous	12.4	9.8	16.9	14.7	10.8	14.1	10.4	14.5	5.2	11.6	120.4
	Non-Oest.	10.5	7.2	12.2	12.7	11.2	11.1	5.6	12.1	5.0	8.8	96.4
Cow Total		22.9	17.0	29.1	27.4	22.0	25.2	16.0	26.6	10.2	20.4	216.8
Oest. Total		44.9	29.6	44.3	46.9	38.3	38.5	22.5	44.3	17.9	30.4	357.5
Non-Oest. Total		34.0	20.4	42.8	43.7	34.0	35.3	23.9	40.4	16.7	28.1	319.3
Grand Total		78.9	50.0	87.1	90.6	72.3	73.8	46.4	84.7	34.5	58.5	676.8

Table 29: Total Volume of Semen Collected From Bulls of GROUP II When Oestrous and Non-Oestrous Teaser Cows Were Used.

Source of Var.	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	d.f. for Test	Test of Significance
Between Bulls	9	554.57	61.62	27.15	2.46 3.60	9;9	**
Between Cows	2	19.73	9.87	2.07	19.00 99.00	2;2	N.S.
Between Treatments	1	24.32	24.32	5.10	18.51 98.49	1;2	N.S.
Bulls x Cows	18	40.70	2.26	0.59	2.26 3.21	18;17	N.S.
Bulls x Treats	9	20.41	2.27	0.60	2.50 3.68	9;17	N.S.
Cows x Treats	2	9.54	4.77	1.26	3.59 6.11	2;17	N.S.
Bulls x Cows x Treats	17	64.55	3.80				
Total	58	733.82					

Table 30: Analysis of Variance For The Total Volume of Semen Collected From GROUP II When Oestrous and Non-Oestrous Teaser Cows Were Used.

These analyses proved that the use of an oestrous teaser did not significantly increase the total volume of semen collected from either group of bulls. The significant first order inter-actions in GROUP I both involved bulls. Calculation similar to that used in total sperm analyses showed that in GROUP I some bulls with oestrous--non-oestrous differences of 4.8 mls or greater, not common to all bulls, would have produced the significant bulls x treatment inter-action. Again, Bull No. 115 was involved primarily because an exceptionally low volume of semen was collected when Cow No. 5 was used as a non-oestrous teaser. No logical reason can be suggested. Bull No. 129 would have contributed to the inter-action because his semen output showed a greater increase than other bulls but a consistent increase occurred with each oestrous teaser.

Bulls which showed a variation of 3.9 mls in the total semen output for each cow not shown by other bulls, produced the significant bulls x cows inter-action. This inter-action was primarily due to the lowered semen output of Bull No. 115 when Cow No. 5 was used as a teaser.

	GROUP I			GROUP II		
	Mean	± Stand. Error	Coeff. Var.	Mean	± Stand. Error	Coeff. Var.
Oestrous Teasers	10.3	± 0.6	30.2%	11.9	± 0.7	30.2%
Non-Oest. "	9.5	± 0.5	26.4%	10.6	± 0.6	32%

Table 31: Mean Semen Output (mls.) and Coefficient of Variation Per Bull Per Collection Day When Oestrous and Non-Oestrous Teasers Were Used.

Table 31 indicated that the use of oestrous cows as teasers had produced apparent increases in the mean semen output of the bulls in both groups. These outputs represented 8.6% and 12.0% increases over

the volume of semen collected when non-oestrous cows were used in GROUPS I and II respectively. Within either group these increases were not significant at the 5% level but, as with the data on total sperm output, the pooling of results from both groups showed that there was a significant increase in semen volume when non-oestrous cows were used as teasers. (Table 33)

COW NO. / TREATMENT	GROUP I			GROUP II			TOTAL ± S.E.
	2	3	5	4	2	6	
Oestrous	10.79	10.39	9.61	12.43	11.28	12.04	66.54 ± 1.15
Non-Oestrous	9.99	9.62	8.74	11.75	10.54	9.64	60.28 ± 1.15
TOTAL	20.78	20.01	18.35	24.18	21.82	21.68	126.82

Table 32: Average Semen Volume (mls) Per Bull Per Cow Used As An Oestrous and A Non-Oestrous Teaser.

Source of Var.	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	Test of Significance
Between Cows	5	9.59	1.92	8.73	5.05 10.97	*
Between Treatments	1	3.27	3.27	14.86	6.61 16.26	*
Error	5	1.11	0.22			
Total	11	13.97				

Table 33: Analysis of Variance of Average Semen Output per Bull per Cow Used As An Oestrous and A Non-Oestrous Teaser.

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9.3 Treatment Effect on The Average Sperm Concentration

In deriving this data presented in Tables 34 and 36 the following calculation was used:

$$\text{Aver. Sperm Conc. per Bull per Collection Day} = \frac{\text{Total Sperm Output}}{\text{Total Semen Output}}$$

The analyses indicated that the apparent increases in sperm concentration shown by each group when oestrous cows were used as teasers were not significant, but pooling the data from both groups showed that the increase in the average sperm concentration was significant at the 5% level of probability.

COW NO. / BULL NO.		354	115	351	365	129	518	322	52	364	Total
2	Oestrous	1198	615	304	500	588	1575	919	825	1111	7435
	Non-Oestrous	1156	621	315	586	610	1264	853	811	1123	7319
Cow Total		2354	1236	619	1086	1198	2839	1752	1636	2234	14754
3	Oestrous	1319	821	273	785	571	1451	977	934	1219	8348
	Non-Oestrous	990	742	235	561	582	1367	755	672	1085	6989
Cow Total		2309	1563	508	1344	1153	2818	1732	1606	2304	15337
5	Oestrous	1236	779	316	919	558	1431	720	802	1125	7886
	Non-Oestrous	1174	480	269	769	603	1286	788	750	870	6989
Cow Total		2410	1259	585	1688	1161	2717	1508	1552	1995	14875
Oest. Total		3753	2215	893	2202	1717	4257	2616	2561	3455	23669
Non-Oest. Tot.		3320	1843	819	1916	1795	3917	2376	2233	3078	21297
Grand Total		7073	4058	1712	4118	3512	8174	4992	4794	6533	44966

Table 34: Average Sperm Concentration (x 10^6 per ml) of The Total Sperm Output From Bulls of GROUP I When Oestrous and Non-Oestrous Teaser Cows Were Used.

Source of Variation	Deg. of F.	Sums of Squares	Mean Sq.	F.Calc.	F.Reqd.	d.f. for Test	Test of Significan
Between Bulls	8	5,242,407	655,300	59.96	2.59 3.89	8;16	**
Between Cows	2	10,518	5,259	0.24	19.0 99.0	2;2	N.S.
Between Treatments	1	104,192	104,192	4.75	18.51 98.49	1;2	N.S.
Bulls x Cows	16	174,851	10,928.2	1.06	2.33 3.37	16;16	N.S.
Bulls x Treatments	8	36,165	4,520.6	0.44	2.59 3.89	8;16	N.S.
Cows x Treatments	2	43,860	21,930	2.12	3.63 6.23	2;16	N.S.
Bulls x Cows x Treats	16	165,726	10,357.9				
Total	53	5,777,719					

Table 33: Analysis of Variance For the Average Sperm Concentration For GROUP I When Oestrous and Non-Oestrous Teaser Cows Were Used.

BULL NO / COW NO.		19	406	122	158	304	397	561	12	591	705	Total
4	Oestrous	1025	869	551	841	958	558	1156	959	1094	1109	9098
	Non-Oest.	1044	785	613	771	739	520	1184	857	1034	1173	8720
Cow Total		2067	1654	1164	1612	1697	1058	2340	1816	2128	2282	17818
2	Oestrous	1105	970	776	952	826	656	1211	829	1129	1264	9818
	Non-Oest.	1115	940	917	590	723	452	719	953	1103	1158	8670
Cow Total		2220	1910	1693	1542	1549	1108	1930	1782	2232	2422	18388
6	Oestrous	1339	776	716	694	898	548	1144	917	1115	1190	9335
	Non-Oest.	1115	861	856	756	875	568	821	818	1180	1102	8932
Cow Total		2454	1637	1552	1450	1773	1114	1965	1735	2295	2292	18267
Oestrous Tot.		3437	2615	2043	2487	2682	1740	3511	2705	3338	3563	28151
Non-Oest. Tot.		3274	2586	2386	2117	2337	1540	2724	2628	3317	3433	26322
Grand Total		6741	5201	4409	4604	5019	3280	6235	5333	6655	6996	54473

Table 36: Average Sperm Concentration (x 10^6 per ml) of The Total Sperm Output From Bulls of GROUP II When Oestrous and Non-Oestrous Teaser Cows Were Used.

Source of Var.	Deg. of F.	Sum of Squares	Mean Square	F.Calc.	F.Reqd.	d.f. for Test	Test of Significance
Between Bulls	9	2,149,120	238,791	17.27	3.18 5.35	9;9	••
Between Cows	2	9,019	4,510	0.38	3.55 6.01	2;18	N.S.
Between Treatments	1	55,754	55,754	4.05	18.51 98.49	1;1	N.S.
Bulls x Cows	18	214,435	11,913	1.09	2.26 3.21	18;17	N.S.
Bulls x Treats	9	124,410	13,823	1.27	2.50 3.68	9;17	N.S.
Cows x Treats	2	14425	7,213	0.66	3.59 6.11	2;17	N.S.
Bulls x Cows x Treats	17	185,265	10,898				
Total	58	2,752,428					

Table 37: Analysis of Variance For The Average Sperm Concentration for GROUP II When Oestrous and Non-Oestrous Teaser Cows Were Used.

Note: Bull No. 406 Cow No. 4. Oestrous - substituted data, so total d.f. reduced by one.

Treatment F. Ratios = $\frac{\text{Treatment M.S.}}{\text{Bull x Treat. M.S.}}$ but 1;2 d.f. used for testing significance.

COW NO. / TREATMENT	GROUP I 2	3	5	GROUP II 4	2	6	TOTAL ± S.E.
Oestrous	826	928	876	910	972	934	5446 ± 370 %
Non-Oestrous	813	777	777	872	867	893	4999 ± 370 %
Total	1639	1705	1653	1782	1839	1827	10445

Table 38: Average Sperm Concentration (x 10^6 per ml) of Total Sperm Output Per Bull Per Cow When Used As An Oestrous and A Non-Oestrous Teaser.

Source of Var.	Deg. of F.	Sum of Squares	Mean Square	F.Calc.	F.Reqd.	Test of Significance
Between Cows	5	19,063	3,813	2.80	5.05 10.97	N.S.
Between Treatments	1	16,651	16,651	12.23	6.61 16.26	*
Error	5	6,809	1,362			
Total	11	42,523				

Table 39: Analysis of Variance of Average Sperm Concentration of Total Sperm Output Per Bull Per Cow When Used As An Oestrous and A Non-Oestrous Teaser.

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9.4 Service by Treatment Interactions

The major aim of the experiment was to determine the effect of oestrous teasers on the total sperm output per collection day. Since 2 services were collected from each bull on each collection day, analyses to determine whether there was any inter-action between the first and

second service and treatment was carried out. Only one replication was analysed for each group of bulls. A replication in this case was regarded as the data collected when a cow was first used as an oestrous teaser and later as her own non-oestrous control. The replication in each group selected for analysis was that replication which showed the greatest apparent treatment effect in total sperm output. In GROUP I this occurred when Cow No. 3 was used as a teaser and for GROUP II it was Cow No. 6.

Tables 40 and 41 indicated that in these replications, neither group experienced interactions between the treatments imposed and either of the two services collected from each bull on either of the collection days involved.

Neither group showed significant differences in the sperm content of the first services and second services, but the highly significant bulls x services inter-action in GROUP II arose because individual bulls differed in the sperm content of their services.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	d.f. for Test	Test of Significance
Between Bulls	8	44.59	5.57	2.92	3.44 6.03	8;8	N.S.
Between Services	1	1.40	1.40	0.73	5.32 11.26	1;8	N.S.
Between Treatments	1	7.57	7.57	5.11	161 4,052	1;1	N.S.
Bulls x Servs	8	15.27	1.91	2.55	3.44 6.03	8;8	N.S.
Bulls x Treats	8	2.57	0.32	0.43	3.44 6.03	8;8	N.S.
Servs x Treats	1	1.48	1.48	1.97	5.32 11.26	1;8	N.S.
Bulls x Servs x Treats	8	5.98	0.75				
Total	35	78.86					

Table 40: Analysis of Variance of Sperm ~~Concentration~~ Content of Each Service Collected from Each Bull of GROUP I When Cow No. 3 Was Used As An Oestrous and A Non-Oestrous Teaser.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	d.f.for Test	Test of Signific
Between Bulls	9	64.98	7.22	1.0	3.18 5.35	9;9	N.S.
Between Services	1	12.1	12.1	1.67	5.12 10.56	1;9	N.S.
Between Treatments	1	18.5	18.5	7.71	161 4052	1;1	N.S.
Bulls x Services	9	65.07	7.23	6.08	3.18 5.35	9;9	**
Bulls x Treatments	9	11.93	1.33	1.12	3.18 5.35	9;9	N.S.
Servs x Treatments	1	2.4	2.4	2.01	5.12 10.56	1;9	N.S.
Bulls x Servs x Treats	9	10.7	1.19				
Total	39	185.66					

Table 41: Analysis of Variance of Sperm Content ($x\ 10^9$) of Each Service Collected From Each Bull of GROUP II When Cow No. 6 Was Used As An Oestrous and A Non-Oestrous Teaser.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	d.f. for Test	Test of Significanc
Between Bulls	8	62.34	7.79	14.16	3.44 6.03	8;8	**
Between Services	1	1.32	.1.32	3.77	5.32 11.26	1;8	N.S.
Between Treatments	1	1.32	1.32	2.40	5.32 11.26	1;8	N.S.
Bulls x Services	8	2.78	0.35	0.78	3.44 6.03	8;8	N.S.
Bulls x Treats	8	4.39	0.55	1.22	3.44 6.03	8;8	N.S.
Servs x Treats	1	0.01	0.01	-	5.32 11.26	1;8	N.S.
Bulls x Servs X Treats	8	3.61					
Total	35	75.77					

Table 42: Analysis of Variance of Semen Volume (mls) of Each Service Collected From Each Bull of GROUP I When Cow No. 3 Was Used As An Oestrous and A Non-Oestrous Teaser.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	d.f.for Test	Test of Significance
Between Bulls	9	78.39	8.71	2.62	3.18 5.35	9;9	N.S.
Between Services	1	2.7	2.7	0.81	5.12 10.56	1;9	N.S.
Between Treatments	1	14.39	14.39	24.39	5.12 10.56	1;9	**
Bulls x Services	9	30.01	3.33	4.62	3.18 5.35	9;9	*
Bulls x Treats	9	6.18	0.59	0.82	3.18 5.35	9;9	N.S.
Serve x Treats	1	0.01	0.01	0.14	5.12 10.56	1;9	N.S.
Bulls x Serve x Treats	9	6.44	0.72				
Total	39	138.12					

Table 43: Analysis of Variance of Semen Volume (mls) of Each Service Collected From Each Bull of GROUP II When Cow No. 6 Was Used As An Oestrous and A Non-Oestrous Teaser.

As with the data relating to the sperm content of each service, the only significant interaction was that between the services and the bulls of GROUP II. In this instance, however, the interaction was only significant at the 5% level.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	d.f.for Test	Test of Significance
Between Bulls	8	383.20	47.90	12.38	3.44 6.03	8;8	**
Between Services	1	18.35	18.35	3.86	161 4052	1;1	N.S.
Between Treatments	1	20.10	20.10	4.22	161 4052	1;1	N.S.
Bulls x Services	8	30.95	3.87	3.65	3.44 6.03	8;8	*
Bulls x Treats	8	10.04	1.26	1.19	3.44 6.03	8;8	N.S.
Serv x Treats	1	4.76	4.76	4.49	5.32 11.26	1;8	N.S.
Bulls x Serv x Treats	8	8.53	1.06				
Total	35	475.93					

Table 44: Analysis of Variance of Sperm Concentration ($x\ 10^6$/ml) of Each Service Collected From Each Bull of GROUP I When Cow No. 3 Was Used As An Oestrous and A Non-Oestrous Teaser.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	d.f. for Test	Test of Significance
Between Bulls	9	153.30	17.03	3.94	3.18 5.35	9;9	*
Between Services	1	72.90	72.90	6.02	161 4052	1;1	N.S.
Between Treatments	1	8.84	8.84	0.73	161 4052	1;1	N.S.
Bulls x Services	9	38.88	4.32	0.98	3.18 5.35	9;9	N.S.
Bulls x Treats	9	18.14	2.02	0.46	3.18 5.35	9;9	N.S.
Serv. x Treats	1	12.10	12.10	2.75	5.12 10.56	1;9	N.S.
Bulls x Serv. x Treats	9	39.60	4.4				
Total	39	343.76					

Table 45: Analysis of Variance of Sperm Concentration (x 10^6/ml) of Each Service Collected From Each Bull of GROUP II When Cow No. 6 Was Used As An Oestrous and A Non-Oestrous Teaser.

The analysis on sperm concentration indicated that in the 2 replications examined the service x treatment inter-actions were not significant at the 5% level of probability.

Tables 40-45 indicated that although significant first order inter-actions occurred between bulls and services in one or other of the 2 groups for the 3 factors analysed, neither group had significant inter-actions between the treatments and bulls or treatments and services. These results showed that with the bulls and cows used in this trial, the use of an oestrous teaser cow did not influence the sperm content, semen volume or sperm concentration of the first service to a significantly greater

extent that the second service.

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CHAPTER 10

Treatment Effects on Laboratory Tests Applied and Sexual Response.

10.1 Initial Fructose Concentration

The technique for measuring the initial concentration of seminal fructose was not satisfactorily standardised until the latter part of the trial. The only oestrous--non-oestrous replication with complete data was when Cow No. 2 was used as a teaser with GROUP II. This was the only data analysed.

In this replicate there was a significant decrease in the initial concentration of seminal fructose when the cow was used as an oestrous teaser. Since data from only 1 cow was analysed this result has been treated with caution. There was also an indication that the average fructose concentration of the second service was greater than the concentration of the first service although the difference was not significant at the usually accepted 5% level of probability. ($0.05 < P < 0.10$)

TREATMENT	BULL NO.	19	406	122	158	304	397	561	12	391	703	Tptal
Oestrous	First	2.58	4.05	4.80	7.15	6.85	6.85	6.55	3.90	2.90	4.25	49.86
	Second	4.23	7.50	6.05	7.68	7.50	7.85	4.50	7.15	3.90	6.70	63.14
Total		6.81	11.55	10.85	14.83	14.35	14.70	11.05	11.05	6.88	10.95	113.00
Non-Oest.	First	2.43	4.32	8.08	8.97	8.68	8.08	8.30	5.85	5.75	5.65	66.11
	Second	5.53	5.43	10.50	7.85	6.05	10.50	7.00	8.68	5.65	5.32	72.51
Total		7.96	9.75	18.58	16.82	14.73	18.58	15.30	14.53	11.40	10.97	138.62
First Serv. Total		5.01	8.37	12.88	16.12	15.53	14.93	14.85	9.75	8.65	9.88	115.97
Sec. Serv. Total		9.76	12.93	16.55	15.53	13.55	18.35	11.50	15.83	9.63	12.02	135.65
Grand Total		14.77	21.30	29.63	31.65	29.08	33.28	26.35	25.58	18.28	21.90	251.62

Table 46: Initial Concentration of Seminal Fructose (mgrms/ml) in Services Collected From Bulls of GROUP II When Cow No. 2 Was Used as an Oestrous and A Non-Oestrous Teaser.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	d.f. for Test	Test of Significance
Between Bulls	9	81.02	9.00	3.69	3.18 5.35	9;9	*
Between Services	1	9.68	9.68	3.97	5.12 10.56	1;9	N.S.
Between Treatments	1	16.41	6.41	8.64	5.12 10.56	1;9	*
Bulls x Services	9	21.94	2.44	3.13	3.18 5.35	9;9	N.S.
Bulls x Treats	9	17.10	1.90	2.44	3.18 5.35	9;9	N.S.
Servs x Treats	1	1.18	1.18	1.51	5.12 10.56	1;9	N.S.
Bulls x Servs x Treats	9	7.05	0.78				
Total	39	154.38					

Table 47: Analysis of Variance of The Initial Concentration of Seminal Fructose in Services Collected From Bulls of GROUP II When Cow No. 2 Was Used as An Oestrous and A Non-Oestrous Teaser.

10.2. Percentage of Live Sperm

Since live-dead estimates were made on both of the services collected from each bull each collection day, the replication in each group in which the use of an oestrous cow had produced the greatest increase in sperm output was analysed. For GROUP I this resulted when Cow No. 5 was used, and with GROUP II with Cow No. 6.

The analyses showed that there were significant differences between bulls. Differences between the 2 services and between treatments were small and did not approach significance. Thus, the quality of the services for the bulls used in this trial was not influenced by the physiological condition of the teaser when quality was measured by a live-dead count of the sperm.

	BULL NO	354	115	351	365	129	518	322	52	364	Total
TREATMENT	SERVICE										
Oestrous	First	74	78	36	74	78	70	40	77	81	608
	Second	90	74	43	70	72	76	64	86	80	655
	Total	164	152	79	144	150	146	104	163	161	1263
Non-Oest.	First	80	72	44	78	80	79	51	71	84	639
	Second	80	72	36	80	80	80	67	81	75	651
	Total	160	144	80	158	160	159	118	152	159	1290
First Service Total		154	150	80	152	158	149	91	148	165	1247
Sec. Service Total		170	146	79	150	152	156	131	167	155	1306
Grand Total		324	296	159	302	310	305	222	315	320	2553

Table 48: The Percentage of Live Sperm in Services Collected From Bulls of GROUP I When Cow No. 3 Was Used As An Oestrous and A Non-Oestrous Teaser.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	d.f. for Test	Test of Significance
Between Bulls	8	6228	778.5	12.23	3.44 6.03	8;8	**
Between Services	1	97	97.0	1.52	5.32 11.26	1;8	N.S.
Between Treatments	1	21	21.0	0.64	161 4052	1;1	N.S.
Bulls x Services	8	509	63.63	3.46	3.44 6.03	8;8	*
Bulls x Treats	8	196	24.5	1.33	3.44 6.03	8;8	N.S.
Servs x Treats	1	33	33	1.80	5.32 11.26	1;8	N.S.
Bulls x Servs x Treats	8	147	18.38				
Total	35	7231					

Table 49: Analysis of Variance of The Percentage of Live Sperm in Services Collected From Bulls of GROUP I When Cow No. 3 Was Used As An Oestrous and A Non-Oestrous Teaser.

BULL NO.		19	406	122	158	304	397	561	12	391	703	Total
TREATMENT												
Oestrous	First	80	62	77	84	73	79	75	85	78	81	774
	Second	77	74	74	77	65	81	78	80	68	77	751
	Total	157	136	151	161	138	160	153	165	146	158	1525
Non-Oest.	First	74	62	77	81	55	82	72	82	60	87	732
	Second	68	64	85	72	76	79	88	70	75	90	765
	Total	142	126	162	153	131	161	160	152	135	177	1497
First Serv. Total		154	124	154	165	128	161	147	167	138	168	1506
Second Serv. Total		145	138	159	149	141	160	166	150	141	167	1516
Grand Total		299	262	313	314	269	321	313	317	279	335	3022

Table 50: The Percentage of Live Sperm in Services Collected From Bulls of GROUP II When Cow No. 6 Was Used As An Oestrous and A Non-Oestrous Teaser.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	d.f. for Test	Test of Significance
Between Bulls	9	1322	146.9	3.85	3.18 5.35	9;9	*
Between Services	1	3	3.0	0.04	161 4052	1;1	N.S.
Between Treatments	1	20	10.0	0.26	161 4052	1;1	N.S.
Bulls x Services	9	344	38.2	0.62	3.18 5.35	9;9	N.S.
Bulls x Treats	9	307	34.1	0.56	3.18 5.35	9;9	N.S.
Serve x Treats	1	78	78.0	1.27	5.12 10.56	1;9	N.S.
Bulls x Serve x Treats	9	552	61.3				
Total	39	2470					

Table 51: Analysis of Variance of The Percentage of Live Sperm Collected From Bulls of GROUP II When Cow No. 6 Was Used As An Oestrous and A Non-Oestrous Teaser.

10.3 Initial Motility Estimate.

A commonly used measure for the assessment of semen quality is a subjective estimate of the initial motility of a service. In order to analyse the motility data recorded during the trial, a simple conversion of the motility rating was developed.

Motility Rating	Converted Rating
0	0
+	1
1	2
1+	3
2	4
2+	5
3	6
3+	7

Table 52: Motility Ratings Used For Routine Semen Assessment and The Converted Ratings Used in The Analyses.

The analyses showed that the use of an oestrous teaser did not appear to influence the quality of the semen as assessed by converted motility estimates. Whereas bulls of GROUP II showed little variation between treatments, the bulls of GROUP I showed quite marked but irregular effects. In GROUP I there was a significant bull x treatment inter-action.

	BULL NO.	354	115	351	365	129	518	322	52	364	Total
TREATMENT	SERVICE										
	First	6	6	2	5	5	6	3	5	6	44
Oestrous	Second	7	6	3	6	6	5	4	6	6	49
	Total	13	12	5	11	11	11	7	11	12	93
	First	5	5	3	6	5	6	3	5	7	45
Non-Oest.	Second	6	5	2	6	6	6	4	6	6	47
	Total	11	10	5	12	11	12	7	11	13	92
First Serv. Total		11	11	5	11	10	12	6	10	13	89
Second Serv. Total		13	11	5	12	12	11	8	12	12	96
Grand Total		24	22	10	23	22	23	14	22	25	185

Table 53: The Converted Motility Assessment of Services Collected From Bulls of GROUP I When Cow No. 3 Was Used As An Oestrous and A Non-Oestrous Teaser.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	d.f. for Test	Test of Significance
Between Bulls	8	51.1	6.39	15.59	3.44 6.03	8;8	**
Between Services	1	1.4	1.4	3.41	5.32 11.26	1;8	N.S.
Between Treatments	1	0.0	0.0	0.0	5.32 11.26	1;8	N.S.
Bulls x Services	8	3.3	0.41	2.1	3.44 6.03	8;8	N.S.
Bulls x Treats	8	2.7	0.34	1.7	3.44 6.03	8;8	N.S.
Serve x Treats	1	0.2	0.2	1.0	5.32 11.26	1;8	N.S.
Bulls x Serve x Treats	8	1.6	0.2				
Total	35	60.3					

Table 54: Analysis of Variance of The Converted Motility Assessment of Services Collected From Bulls of GROUP I When Cow No. 3 Was Used As An Oestrous and A Non-Oestrous Teaser.

BULL NO.		19	406	122	158	304	397	561	12	591	703	Total
TREATMENT												
	First	6	4	6	7	5	5	5	7	6	6	57
Oestrous	Second	6	3	5	6	4	6	6	6	5	6	53
	Total	12	7	11	13	9	11	11	13	11	12	110
	First	6	3	6	5	3	6	5	5	4	7	50
Non-Oest.	Second	6	3	6	5	4	5	6	5	3	7	50
	Total	12	6	12	10	7	11	11	10	7	14	100
First Serv. Total		12	7	12	12	8	11	10	12	10	13	107
Second Serv. Total		12	6	11	11	8	11	12	11	8	13	103
Grand Total		24	13	23	23	16	22	22	23	18	26	210

Table 55: The Converted Motility Assessment of Services Collected From Bulls of GROUP II When Cow No. 6 Was Used As An Oestrous and A Non-Oestrous Teaser.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	d.f. for Test	Test of Significance
Between Bulls	9	36.5	4.06	4.32	3.18 5.35	9;9	*
Between Services	1	0.4	0.4	1.0	161 4052	1;1	N.S.
Between Treatments	1	2.5	2.5	2.66	5.12 10.56	1;9	N.S.
Bulls x Services	9	2.6	0.29	1.00	3.18 5.35	9;9	N.S.
Bulls x Treats	9	8.5	0.94	3.24	3.18 5.35	9;9	*
Serve x Treats	1	0.4	0.4	1.38	5.12 10.56	1;9	N.S.
Bulls x Serve x Treats	9	2.6	0.29				
Total	39	53.5					

Table 56: Analysis of Variance of The Converted Motility Assessment of Services Collected from Bulls of GROUP II When Cow No. 6 Was Used As An Oestrous and A Non-Oestrous Teaser.

10.4 Initial pH and pH Change

As some bulls had low semen output, pH assessments could not be conducted on all services. The only data subjected to analyses were for the initial pH and the pH change after 1 hour's incubation at 37°C. Only one replicate was analysed in each group. Complete data was available for 6 bulls of GROUP I when Cow No. 2 was used and for 7 bulls of GROUP II when Cow No. 6 was used.

The analyses showed that in neither group did the use of an oestrous teaser appear to significantly alter the initial pH of the semen. It was of interest to note that in GROUP I, the initial pH of the first service was significantly lower than the pH of the second service. This service difference was not significant at the 5% level of probability in GROUP II ($0.05 < P < 0.10$). The highly significant interaction (bulls x services) in GROUP II was due to Bull No. 304. Whereas the pH of the first service of all other GROUP II bulls was less than the pH of their second services, the pH levels for Bull No. 304 were reversed.

The analyses for the pH change after 1 hour's incubation showed that in spite of the difference in the initial pH of the first and second services, the difference between services in pH change after incubation was not significant.

Whereas GROUP II did not exhibit significant differences in the pH change between the bulls, GROUP I showed highly significant differences between bulls. Bull No. 351 (GROUP I) showed extremely small changes in pH of semen after incubation. As for initial semen pH, the treatments imposed did not significantly alter the pH change of the incubated samples of semen collected from bulls of either group.

BULL NO			115	351	365	129	322	52	Totals
TREATMENT SERVICE									
Oestrous	First	Initial	6.45	6.65	6.50	6.55	6.45	6.50	39.10
		Final	5.95	6.55	6.05	6.15	6.00	5.95	36.65
		Change	0.50	0.10	0.45	0.40	0.45	0.55	2.45
	Second	Initial	6.75	6.90	6.60	6.25	6.30	6.65	40.05
		Final	6.15	6.80	6.05	5.55	6.35	6.10	37.00
		Change	0.60	0.10	0.55	0.70	0.55	0.55	3.05
	Total	Initial	13.20	13.55	13.10	12.80	13.35	13.15	79.15
		Final	12.10	13.35	12.10	11.70	12.35	12.05	73.65
		Change	1.10	0.20	1.00	1.10	1.00	1.10	5.50
Non-Oestrous	First	Initial	6.55	6.60	6.75	6.55	6.70	6.50	39.65
		Final	6.00	6.50	6.35	6.15	6.25	5.95	37.20
		Change	0.55	0.10	0.40	0.40	0.45	0.55	2.45
	Second	Initial	6.65	6.80	6.65	6.75	6.80	6.65	40.50
		Final	6.05	6.75	6.10	6.35	6.40	6.20	37.85
		Change	0.60	0.05	0.55	0.40	0.40	0.45	2.45
	Total	Initial	13.20	13.40	13.40	13.30	13.50	13.15	79.95
		Final	12.05	13.25	12.45	12.50	12.65	12.15	75.05
		Change	1.15	0.15	0.95	0.80	0.85	1.00	4.90

Table 57: Initial pH, Final pH and pH Change of Semen Collected from Bulls of GROUP I When Cow No. 2 Was Used As An Oestrous and A Non-Oestrous Teaser.

Source of Var.	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	d.f. for Test	Test of Significance
Between Bulls	5	0.133	0.133	7.82	5.05 10.97	5;5	*
Between Services	1	0.107	0.107	6.29	6.61 16.26	1;5	N.S.
Between Treats	1	0.027	0.027	1.93	6.61 16.26	1;5	N.S.
Bulls x Serve	5	0.085	0.017	0.77	5.05 10.97	5;5	N.S.
Bulls x Treats	5	0.070	0.014	0.64	5.05	5;5 10.97	N.S.
Serve x Treats	1	0.004	0.004	0.18	6.61 16.26	1;5	N.S.
Bulls x Serve x Treats	5	0.109	0.022				
Total	23	0.535					

Table 58: Analysis of Variance of The Initial pH of Semen Collected From Bulls of GROUP I When Cow No. 2 Was Used As An Oestrous and A Non-Oestrous Teaser.

BULL NO.			19	122	159	304	397	12	703	Total
TREATMENT	SERVICE									
Oestrous	First	Initial	6.35	6.40	6.30	6.50	6.40	6.30	6.30	44.55
		Final	5.80	6.05	5.95	5.85	5.85	5.75	5.70	40.95
		Change	0.55	0.35	0.35	0.65	0.55	0.55	0.60	3.60
	Second	Initial	6.65	6.60	6.65	6.45	6.70	6.45	6.45	45.95
		Final	6.35	6.20	6.00	6.85	6.35	5.90	6.00	42.65
		Change	0.30	0.40	0.65	0.60	0.35	0.55	0.45	3.30
	Total	Initial	13.00	13.00	12.95	12.95	13.10	12.75	12.75	90.50
		Final	12.15	12.25	11.95	11.70	12.20	11.65	11.70	85.60
		Change	0.85	0.75	1.00	1.25	0.90	1.10	1.05	6.90
Non-Oestrous	First	Initial	6.45	6.45	6.45	6.85	6.40	6.40	6.40	45.40
		Final	5.90	6.00	6.05	6.60	5.95	5.85	5.80	42.15
		Change	0.55	0.45	0.40	0.25	0.45	0.55	0.60	3.25
	Second	Initial	6.60	6.65	6.60	6.50	6.65	6.55	6.55	46.10
		Final	6.15	6.30	6.35	6.05	6.30	6.00	6.00	43.15
		Change	0.45	0.35	0.25	0.45	0.35	0.55	0.55	2.95
	Total	Initial	13.05	13.10	13.05	13.35	13.05	12.95	12.95	91.50
		Final	12.05	12.30	12.40	12.65	12.25	11.85	11.80	85.30
		Change	1.00	0.80	0.65	0.70	0.80	1.10	1.15	6.20

Table 59: Initial pH, Final pH and pH Change of Semen Collected From Bulls of GROUP II When Cow No. 6 Was Used As An Oestrous and A Non-Oestrous Teaser.

Source of Var.	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Recd.	d.f.for Test	Test of Significance
Between Bulls	6	0.076	0.013	0.50	4.28 8.47	6;6	N.S.
Between Services	1	0.158	0.158	6.08	5.99 13.74	1;6	*
Between Treats	1	0.036	0.036	2.00	161 4052	1;1	N.S.
Bulls x Services	6	0.156	0.026	8.67	4.28 8.47	6;6	**
Bulls x Treats	6	0.031	0.005	1.67	4.28 8.47	6;6	N.S.
Servs x Treats	1	0.018	0.018	6.00	5.99 13.74	1:6	*
Bulls x Servs x Treats	6	0.020	0.003				
Total	27	0.495					

Table 60: Analysis of Variance of Initial pH of Semen Collected From Bulls of GROUP II When Cow No. 6 Was Used As An Oestrous and A Non-Oestrous Teaser.

Source of Var.	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	d.f. for Test	Test of Significance
Between Bulls	5	0.601	0.120	17.14	5.05 10.97	5;5	**
Between Services	1	0.015	0.015	1.00	161 4052	1;1	N.S.
Between Treatments	1	0.015	0.015	1.00	161 4052	1;1	N.S.
Bulls x Servs	5	0.033	0.007	2.33	5.05 10.97	5;5	N.S.
Bulls x Treats	5	0.018	0.004	1.33	5.05 10.97	5;5	N.S.
Servs x Treats	1	0.015	0.015	5.00	6.61 16.26	1;5	N.S.
Bulls x Servs x Treats	5	0.016	0.003				
Total	23	0.713					

Table 61: Analysis of Variance of the pH Change of Semen Collected from Bulls of GROUP I After Incubation at 37°C for One Hour.

Source of Var.	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	d.f. for Test	Test of Significanc
Between Bulls	6	0.1011	0.0168	1.01	4.28 8.47	6;6	N.S.
Between Services	1	0.0129	0.0129	1.24	5.99 13.74	1;6	N.S.
Between Treatments	1	0.0175	0.0175	1.05	5.99 13.74	1;6	N.S.
Bulls x Servs	6	0.0621	0.0104	0.75	4.28 8.47	6;6	N.S.
Bulls x Treats	6	0.1000	0.0167	1.21	4.28 8.47	6;6	N.S.
Servs x Treats	1	0.0	0.0	0.0	5.99 13.74	1;6	N.S.
Bulls x Servs x Treats	6	0.0825	0.0138				
Total	27	0.3761					

Table 62: Analysis of Variance of The pH Change of Semen Collected From Bulls of GROUP II After Incubation at 37°C for One Hour.

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10.5 Re-Action Time

The measurement for re-action time used during the trial was highly subjective and could have been biased in favour of either the oestrous or non-oestrous cows. While these estimates were considered too subjective to warrant statistical analysis, the following observations were made from the results presented in Tables 63 and 64:

(a) the re-action times for first services were always longer than for second services, irrespective of the physiological state of the teaser;

and (b) the re-action times varied considerably between bulls; and (c) the re-action times of bulls in both groups were less when oestrous teasers were used. The response varied considerably between bulls.

COW NO.	TREAT	SERV.	BULL NO. 354	115	351	365	129	518	322	52	364	Total
2	Oest.	First	5.0	1	4	2	1.0	2	2	10	2	29.0
		Second	0.5	1	2	4	1.5	1	1	4.5	1	16.5
		Total	5.5	2	6	6	2.5	3	3	14.5	3	45.5
	Non-Oest.	First	5	1.5	3	5	3	5	3	5.5	2	33.0
		Second	3	3	2	3	2	2	3	3.0	3	24.0
		Total	8	4.5	5	8	5	7	6	8.5	5	57.0
3	Oest.	First	4	2	5	5	1	2	5	1	2	27.0
		Second	2	1	2	1	1	2	2	7	2	20.0
		Total	6	3	7	6	2	4	7	8	4	47.0
	Non-Oest.	First	5	5	4	4	1	2.5	3	10	3.0	37.5
		Second	3	3	3	5	1	2.0	3	7	1.5	28.5
		Total	8	8	7	9	2	4.5	6	17	4.5	66.0
5	Oest.	First	3	1.5	1.0	1	5	0.5	1.5	1	1	15.5
		Second	2	2.5	1.5	3	1	2.0	2.0	4	2	20.0
		Total	5	4.0	2.5	4	6	2.5	3.5	5	3	35.5
	Non-Oest.	First	5	5	4	5	5	5	3	5	1	38.0
		Second	3	2	3	3	2	5	2	5	2	27.0
		Total	8	7	7	8	7	10	5	10	3	65.0
First Service Total			27.0	16.0	21.0	22	16.0	17	17.5	32.5	11.0	180.0
Second Service Total			13.5	12.5	13.5	19	8.5	14	13.0	30.5	11.5	136.0
Grand Total			40.5	28.5	34.5	41	24.5	31	30.5	63.0	22.5	316.0
Oestrous Total			16.5	9.0	15.5	16	10.5	9.5	13.5	27.5	10.0	128.0
Non-Oestrous Total			24.0	19.5	19.0	25	14.0	21.5	17.0	35.5	12.5	188.0

Table 63: Re-Action Times (mins) For Services Collected From Bulls of GROUP I When Presented with Oestrous and Non-Oestrous Teaser Cows.

BULL NO.

COW NO.	TREAT	SERV.	19	406	122	158	304	397	561	12	391	705	Total
4	Oest.	First	2.0	1	1.0	3	1.5	1.0	3	1	3	1.5	18.0
		Second	0.5	1	0.5	5	1.0	0.5	3	1	1	1.0	14.5
		Total	2.5	2	1.5	8	2.5	1.5	6	2	4	2.5	32.5
	Non-Oest.	First	5	6	3.0	5	3	3	5	4	5	5	44.0
		Second	3	4	1.5	4	3	3	3	4	3	3	31.5
		Total	8	10	4.5	9	6	6	8	8	8	8	75.5
2	Oest.	First	1	0.5	0.5	1	5.0	3	3	0.5	3	3	20.5
		Second	2	1.0	1.0	1	1.5	3	3	1.0	3	7	23.5
		Total	3	1.5	1.5	2	6.5	6	6	1.5	6	10	44.0
	Non-Oest.	First	5	5	5	5	5	5	5	5	8	8	54.0
		Second	3	3	7	3	3	3	5	1	3	7	38.0
		Total	8	8	12	8	8	8	10	6	9	15	92.0
6	Oest.	First	1.0	5.0	1.5	5.0	1.0	2	3	2	5	2.0	27.5
		Second	0.5	2.5	1.0	1.5	1.5	2	4	1	1	4.0	19.0
		Total	1.5	7.5	2.5	6.5	2.5	4	7	3	6	5	46.5
	Non-Oest.	First	6	5	5	5.0	5	5	5	6	6	7.5	55.5
		Second	5	3	10	3.5	4	4	4	3	3	4.0	43.5
		Total	11	8	15	8.5	9	9	9	9	9	11.5	99.0
First Service Total			20.0	22.5	16.0	24.0	20.5	19.0	24.0	18.5	28.0	27.0	219.5
Second Service Total			14.0	14.5	21.0	18.0	14.0	15.5	22.0	11.0	14.0	26.0	170.0
Grand Total			34.0	37.0	37.0	42.0	34.5	34.5	46.0	29.5	42.0	53.0	389.5
Oestrous Total			7.0	11.0	5.5	16.5	11.5	11.5	19.0	6.5	16.0	18.5	122.0
Non-Oestrous Total			27.0	26.0	31.5	25.5	23.0	23.0	27.0	23.0	26.0	34.5	266.5

Table 64: Re-Action Times (mins.) For Services Collected From Bulls of GROUP II When Presented with Oestrous and Non-oestrous Teaser Cows.

PART V

GENERAL DISCUSSION, SUMMARY AND CONCLUSIONS

CHAPTER 11

The Discussion and Interpretation of Results

In collecting the data for the trial reported in this thesis, 22 bulls were selected and divided into 2 groups. Each bull had 2 services collected every third day over a period of almost 5 weeks. The 2 groups were collected on different days. Each cow was used as an oestrous teaser before being used as her own non-oestrous control 3 or 6 days later. The variables so introduced, although mentioned in the section outlining the experimental design, deserve further comment in view of the results of the analyses of the data. The discussion has therefore been dealt with in 3 main sections:-

(a) the adequacy of the experimental design;

(b) the effects of the use of oestrous teasers on:

(i) total sperm output, semen output and sperm concentration

and (ii) other semen characteristics and sexual response;

and (c) the implications of the treatment effects.

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11.1 The Adequacy of the Experimental Design

The bulls included in the trial were a selected group. None of the bulls had previously exhibited abnormal sexual behaviour. Because of the adoption of a standardised teasing routine, only bulls which would respond to this routine were suitable. The reasons for standardising the teasing routine were outlined in the section detailing the experimental design. Nonetheless, the conclusions from this trial are only applicable to mature bulls which have not shown abnormal collection

behaviour, which are on a similar collection roster and which receive pre-collection preparation similar to that adopted during the trial.

The use of 2 groups which were collected on different days and the extension of the experimental period over a series of collection days introduced a time variable. Preliminary analyses (Appendix V) had indicated that a group of mature Jersey bulls did not exhibit significant between day variations in the sperm output of their first service during the spring mating period. One of the 2 groups of Jersey bulls showed significant day-to-day variations in semen volume but reference to the tables in Appendix V will show that the semen output of the particular group was remarkably constant except for one day when one bull showed a marked increase. Variables which could have modified day-to-day variations in these preliminary analyses may have arisen from the use of different teaser cows in a series of collection days, the uncontrolled pre-collection preparation and different technicians teasing or making the collections.

The lack of variation in sperm output by a group of bulls during the regular collection period has been noted at the Newstead A.B. Centre (Shannon, P. Personal communication) and Bonadonna (1956) considered that only climatic extremes produced marked variations. No authors of papers reviewed in connection with the trial mentioned the significance of these day to day variations and none employed a control group of bulls to take account of the variation.

During the current trial, each group of bulls used a trained teaser (Cow No. 1) on 4 collection days. Analyses of this data showed that each group had exhibited significant variations in total sperm output,

but not in semen output. No known factors were operating to produce these variations in total sperm output but regression analyses showed that the variation was not due to a significant decline over the experimental period.

Although these results arose from the analyses of data collected on only 4 days, there was no reason to suspect that the unknown factors producing this variation would not act in a random manner if they acted throughout the remainder of the trial, and therefore, even though both groups showed significant variations in total sperm output on these 4 days, the significance of treatment effects need not be modified.

A second feature of the time variation in the experimental design was that a cow was always used as an oestrous teaser before being used as her own non-oestrous control 3 or 6 days later. Any decline in output could inflate the treatment difference. Reference to Figs 1-4 will show that each group did exhibit an apparent decline in sperm and semen output. However, regression analyses showed that these apparent declines were not statistically significant. In view of these results, it was not considered necessary to develop correction factors when interpreting treatment effects.

If a cow had been a satisfactory teaser in the oestrous condition but had been over-restless as a non-oestrous teaser, a treatment effect in favour of the oestrous condition could have resulted. This problem of suitability was not encountered in the current trial as no cow proved too restless when used as either an oestrous or non-oestrous teaser. (Appendix II).

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11.2 Treatment Effects on Total Sperm Output, Semen Output and Average Sperm Concentration.

Of the total of 22 bulls included in the trial, 3 were discarded because of faulty or insufficient data. This resulted in GROUP I comprising 9 bulls and GROUP II comprising 10 bulls. The accompanying table shows the average % increases when oestrous teasers were used for bulls of each group and for the 19 bulls included in the analyses. The individual analyses for each group of bulls showed that these increases were not significant, but on pooling the data from both groups the increase in total sperm output was highly significant at the 1% level of probability and the increases in semen volume and average sperm concentration significant at the 5% level.

	Sperm Output	Semen Output	Sperm Concentration
GROUP I	18.4%	8.6%	11.1%
GROUP II	19.0%	12%	6.9%
GROUPS I AND II	18.7%	10.3%	8.9%

Table 65: Average Increases in Total Sperm Output, Semen Volume and Average Sperm Concentration When Oestrous Teasers Were Used With GROUPS I AND II.

Since the sperm content of an ejaculate and the contribution of the epididymal secretions only represent a minor fraction of the volume of an ejaculate (Salisbury and Van Demark (1961)), the increase in semen volume due to the greater sperm output resulting from the use of an oestrous teasers would only be slight. The actual increase in volume obtained in this trial suggests that the use of oestrous teasers increased

the accessory gland secretions besides increasing the total sperm output. An interesting observation was the fact that whereas GROUP I showed the greater percentage increase in sperm concentration, and GROUP II showed the greater increase in semen output, over-all both groups showed similar increases in sperm output.

The results of this trial were in contrast to those reported by James (1958). James considered that his results "did not suggest that there was any stimulating effect in collecting semen by using an oestral cow as a decoy". Several points are worthy of note in this work reported by James. They are:

(i) only 2 oestrous cows were used and each bull had his first 4 services collected over one oestrous cow on one day and his second 4 services collected over the other oestrous cow on another day. 8 Services were collected each day, the remaining 4 on any one day being collected over a non-oestrous teaser. 4 of the 8 bulls used showed a marked increase and 2 bulls a decrease in the sperm output of the first 4 services when an oestrous teaser was used suggesting that there was a marked variation in the response to the treatment involved;

(ii) the changing of the teaser after 4 services had been collected could have confounded results as Almquist and Hale (1956) reported that changing teasers mid-way through a series of collections could re-vitalise or alter the sexual responsiveness of a bull;

(iii) the pre-collection routine was not mentioned and neither was re-action time measured. The bulls may have needed less preparation when collected over an oestrous cow than when collected over a non-oestrous cow. Any difference in preparation could have nullified the treatment differences.

In the current trial, a teaser was not changed on any one collection day, comparisons were made by using 3 cows with each group and a standardised pre-collection routine was adopted. These 3 points stress the marked differences in the design of the 2 trials. Nonetheless, the results of James' work showed that the use of oestrous teasers had increased the total sperm content of the first 4 services collected from each of the 8 bulls by 12.2%, the total semen output by 11.4% and the average sperm concentration by 2.9%. Only totals for the first 4 or second 4 services were presented by James, so comparisons of the first 2 services cannot be made. In the current trial both the first and second services showed increases when oestrous teasers were used but subsequent services may not have shown this response.

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11.3 Treatment Effects on Other Semen Characteristics

The use of oestrous teasers did not significantly influence either the % live sperm or the initial motility rating of the services collected from the bulls. The extra sperm output did not appear to be associated with any variation in the quality of the services collected as assessed by these 2 estimates.

The use of an oestrous cow as a teaser produced a significant decline in the initial fructose concentration of semen. The data analysed to determine this result were obtained from only 1 group of bulls for only 1 oestrous—non-oestrous comparison. The decline in fructose concentration was probably related to the increase in sperm concentration as both Branton et. al (1952) and Bishop et. al (1954)

reported that the initial fructose concentration bore an inverse relationship to sperm concentration.

Neither the initial pH of the services nor the pH change after 1 hour's incubation at 35°C showed any significant variation due to the treatments applied to the bulls.

Because the measure of sexual response used in the current trial was highly subjective, the data were not subjected to statistical analyses. Nevertheless, the data indicated that the use of oestrous teasers did produce an apparent reduction in the re-action time although the degree of response varied between bulls. This result could be interpreted as contrary to conclusions reached by Almquist and Hale (1956) who considered that smearing oestrous mucus on the rump of a teaser did not alter the cow's stimulus value as measured by the re-action time of the bulls. No data was published to support this claim. On the other hand Walton (1960) considered that a receptive oestrous female had the greatest excitatory value to males but did not suggest how the oestrous condition achieved its effect.

Hart et al (1946) suggested that the stimulating effect of an oestrous teaser on sexually slothful bulls was the result of an olfactory stimulus peculiar to the oestrous condition. In view of this suggestion and the conclusive results of the current trial, 2 possible reasons can be suggested as to why the oestrous mucus applied by Almquist and Hale produced no measurable effect. Firstly, the oestrous mucus may have been applied after any peculiar odour had been lost, and secondly, an oestrous cow may produce a stimulating effect because of other factors which act together with or apart from an oestrous odour associated with the mucus.

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11.4 The Implications of The Treatment Effects

Hale and Almquist (1960) have defined the pre-collection sexual preparation of a bull as "the prolonging of the period of stimulation beyond that adequate for mounting and ejaculation. This can be achieved only if the teaser or stimulus animal presented provides adequate stimulation for ejaculation during the preparation period". Sexual preparation is not to be confused with sexual stimulation. "The function of optimal stimulation is to obtain an ejaculation in the shortest possible time. The function of preparation is to provide high-quality semen containing the greatest possible number of sperm per ejaculate". (Hale and Almquist, (1960)).

If re-action time is regarded as a measure of the stimulus value of a teaser, the results of the current trial suggested that a cow in the oestrous condition had a greater stimulus value than the same cow in the non-oestrous condition. In view of this greater stimulus value and the resulting increase in sperm output, a corollary to the definitions of Almquist and Hale can be developed:- "When bulls are subjected to a fixed period of sexual preparation, the effectiveness of this preparation on sperm output is greatest when teasers with a high stimulation value are used".

Table 2 effectively showed that sexual preparation increased the sperm content, the semen volume and the sperm concentration of a service but the mechanisms involved in producing these effects have not been investigated. Hafs et. al (1962) showed that decreasing increments in sperm output result with increasing intensities of preparation. Semen volume and sperm concentration contribute equally at lower intensities

but sperm concentration showed smaller increments with the increased intensity of preparation.

In the current trial the increase in sperm output probably resulted because the bulls showed a more rapid response to the oestrous teasers than to the non-oestrous teasers. This resulted in the bulls being frustrated for a longer period of time and, because of the teasers' improved stimulus value, this frustration was intensified. The important factor in sexual preparation is to achieve a degree of frustration in the bull. This leads to the conclusion that "frustration is intensified, as by the use of oestrous teasers, mature bulls will show an increase in sperm output which will be the product of similar increases in semen volume and sperm concentration". This increase will be obtained in 2 ways:

(i) by the removal of more sperm from the epididymus;

and (ii) by a greater outpouring of fluid from the accessory sexual glands which will contribute the greater part of the increase in semen volume.

The question arose as to how the oestrous condition of the teasers used improved their stimulus value. Two theories can be advanced in explanation. They are:-

(i) Almquist and Hale (1956), Hafs et. al (1959), Rowson (1959) and Walton (1960) all reported that when bulls were being collected at greater frequencies, a change in the pre-collection routine, in teaser animals or in the site of collections could decrease the re-action time. An oestrous cow may have produced a stimulating variation in the teasing routine and in consequence the sperm output of the bulls was significantly increased. However, each cow was only used twice and merely changing the

teaser did not influence sperm output in the current trial as the analyses showed that there were no significant differences in the sperm output when different cows were used. Each cow had a greater stimulus value as an oestrous teaser and if the oestrous condition was the stimulating variation, then the continuous use of oestrous teasers would probably reduce their initial effectiveness;

(ii) Oestrous cows produce an "unconditioned" response as distinct from a "conditioned" response in the sense of the terms as used by Pavlov. Hart et al (1946) suggested that this response is associated with an oestrous odour, but the comments of Almquist and Hale (1956) suggest that oestrous mucus may not possess this odour. The results of the current trial suggest that the smearing of oestrous mucus on the rump of a teaser should be repeated to test whether odours associated with oestrous mucus do produce the "unconditioned" response obtained by using oestrous cows. Despite the statement by the Milk Marketing Board (1954) that the presence of an oestrous cow excited the bulls in a barn, no increased excitement prior to service was noticed amongst either group of bulls in the current trial. If an oestrous odour had permeated the barn, the bulls should have been stimulated prior to being introduced to the teaser but none of the bulls showed a noticeable response until introduced to the teaser. This suggested that the stimulating property or properties possessed by all the oestrous cows used in the current trial only produced a response when the bulls were in very close proximity to the teaser. Since the treatment x cows interaction was not significant in either group of bulls, all the cows produced this "unconditioned" response in mature bulls only when each cow was in oestrus.

As only 3 cows were used with each group over a 4½-5 week period,

the first hypothesis could not be tested. None of the bulls had experienced natural mating with a herd of cows for at least a year and for most bulls it was a longer period. This would reduce the likelihood that the response to an oestrous cow was the revitalising of a "conditioned"reflex. Most bulls can be trained to collections being taken over a non-oestrous teaser, another bull or even an inanimate dummy, but this development of a "conditioned" response does not preclude the possibility that a property or properties possessed by an oestrous cow can produce an "unconditioned" response in mature bulls.

Several practical benefits arise from the results of the current trial. They are:-

(i) because of the increased sperm output resulting when oestrous teasers were used, a greater coverage could be obtained with the top sires from an Artificial Breeding Centre;

(ii) the peak demand experienced by the 2 Centres in New Zealand could be satisfied with fewer bulls;

and (iii) where despatch schedules are critical during this peak period, more bulls could be collected in a shorter period of time.

The significance of these benefits may be modified because of management problems and the nature of the response. Thus:-

(i) if the bulls showed a response to the oestrous teasers because of a stimulating variation in the preparation routine, the continued use of oestrous teasers could reduce the improved stimulation value of the oestrous cows;

(ii) the continuous use of oestrous teasers could result in some bulls refusing to be collected over a non-oestrous teaser;

and (iii) since 2 or 3 cows are required as teasers each day during

the spring peak a large population of cows would have to be available from which to select oestrous cows. All the cows in this population would have to be suitable for use as teasers.

If the first 2 problems did not eventuate the third problem could be surmounted by either:-

(i) developing a satisfactory method for the control of the oestrous cycle, thus reducing the cow numbers required to provide oestrous teasers;

or (ii) the repeating of the trial conducted by Almquist and Hale (1956) to re-investigate whether the smearing of oestrous mucus on the rump of a teaser significantly improved the stimulus value.

The latter possibility offers the greater scope for the application of the results of the current trial to routine preparation procedures of mature bulls standing at an Artificial Breeding Centre.

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CHAPTER 12

General Summary and Conclusions

(a) 12 mature Jersey, 4 mature Friesian and 5 mature Ayrshire bulls, none of which had previously exhibited abnormal sexual behaviour, were selected from the sires standing at the Awahuri Artificial Breeding Centre. The bulls were divided into 2 groups which were collected on different days.

(b) The collection frequency maintained throughout the experimental period was 2 collections per bull every third day. Each bull was subjected to 10 minutes sexual preparation prior to the collection of the first service and then received 10 minutes rest before being subjected to 7 minutes preparation prior to the second collection.

(c) To determine the significance of the effects produced by using oestrous cows as teasers, 3 oestrous cows were used with each group. Each cow was used as an oestrous teaser before being used as her own non-oestrous control 3 or 6 days later.

(d) The use of oestrous cows as teasers produced average increases per bull per collection day of 18.7% in total sperm output, 10.4% in semen volume and 8.9% in average sperm concentration when comparisons were made with collections taken over the same cows in the non-oestrous condition. The increase in total sperm output was significant at the 1% level of probability and the increases in semen volume and sperm concentration were significant at the 5% level.

(e) The use of oestrous cows as teasers:-

(i) was not associated with any significant alteration in either the initial motility assessment or the % live sperm;

(ii) was associated with a significant decline in the initial

concentration of seminal fructose in both of the services collected from a bull on any one collection day;

(iii) was not associated with measurable changes in either the initial pH of a service or the pH change after 1 hours incubation at 37°C;

(iv) produced an apparent decline in re-action time.

(f) The conclusion drawn was the under controlled pre-collection routines the use of oestrous cows as teasers produced a significant increase in sperm output because of the improved stimulus value of the teaser. The improved stimulus value may have been due to:-

(i) a stimulating variation from the normal teasing environment;

or (ii) properties of the oestrous condition which produced an "unconditioned" response in the bulls used.

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APPENDICES

Appendix I. Detailed Results of The Semen Volume, Sperm Concentration and The Sperm Content of Each of the Two Services Collected from Each Bull Each Collection Day.

Appendix II. The Description and Behaviour of The Cows Used as Teasers.

Appendix III. The Calibration of The Absorptiometer for Measuring Sperm Concentration.

Appendix IV. The Calibration of The Absorptiometer for Measuring The Initial Fructose Concentration of Semen.

Appendix V. The Day-to-Day Variation in Sperm Output and Semen Volume in Bulls Under Routine Management Conditions During The 1961 Spring Mating Season at The Awahuri Artificial Breeding Centre.

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BULL NO:- 354 GROUP:-I

BREED:- Jersey AGE:-8yrs

Collect. Number	Collect. Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot.Sp/Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	9	1	1	2.9	1535	4452		
			2	2.1	719	1510	5962	5.0
2	9	2(O)	1	4.7	1374	6458		
			2	5.4	1050	5670	12128	10.1
3	9	2(N)	1	4.5	1325	5963		
			2	5.1	1002	5110	11073	9.6
4	6	3(O)	1	3.9	1293	5043		
			2	5.5	1341	7376	12419	9.4
5	10	5(O)	1	4.0	1293	5172		
			2	4.9	1196	5860	11032	8.9
6	6	3(N)	1	4.4	915	4026		
			2	5.6	1050	5880	9906	10.0
7	10	5(N)	1	2.9	1228	3561		
			2	4.0	1147	4588	8149	6.9
8	10	1	1	3.0	1244	3732		
			2	3.7	1180	4366	8098	6.7
9	10	1	1	3.5	1034	3619		
			2	4.2	1034	4343	7962	7.7
10	3	1	1					
			2					

Collection No 10 Bull had high rectal temperature and sore feet.

BULL NO:- 115 GROUP:-I

BREED:- Friesian AGE:-12yrs

Collect. Number	Collect. Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot.Sp/Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	11	1	1	9.6	695	6672		
			2	9.4	404	3798	10470	19.0
2	11	2(O)	1	8.5	711	6044		
			2	6.3	492	3100	9144	14.8
3	11	2(N)	1	5.5	606	3333		
			2	4.8	630	3024	6357	10.3
4	5	3(O)	1	5.5	915	5033		
			2	4.0	695	2780	7813	9.5
5	5	5(O)	1	3.1	1002	3106		
			2	4.6	622	2861	5967	7.7
6	5	3(N)	1	4.7	816	3835		
			2	5.0	679	3395	7230	9.7
7	5	5(N)	1	2.2	452	994		
			2	2.8	500	1400	2394	5.0
8	6	1	1	4.5	873	3929		
			2	3.7	719	2660	6589	8.2
9	1	1	1	3.3	177	584		
			2	3.2	1188	3802	4386	6.5
10	2	1	1	1.4	323	452		
			2	3.1	727	2254	2706	4.5

Collection Number 3 Both services appeared to be below usual standard
Collection Number 5 First service appeared to be below usual standard
Collection Number 10 First service appeared to be below usual standard

BULL NO:- 351 GROUP:-I

BREED:- Jersey AGE:-11yrs

Collect. Number	Collect. Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot. No./Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	4	1	1	5.5	315	1733		
			2	4.5	307	1382	3115	10.0
2	4	2(O)	1	5.3	258	1367		
			2	6.2	347	2151	3518	11.5
3	6	2(N)	1	5.5	331	1821		
			2	5.6	291	1630	3451	11.1
4	8	3(O)	1	5.8	275	1595		
			2	7.0	275	1925	3520	12.8
5	4	5(O)	1	5.1	275	1403		
			2	4.4	372	1637	3040	9.5
6	8	3(N)	1	5.0	242	1210		
			2	4.8	218	1046	2256	9.8
7	4	5(N)	1	5.3	177	938		
			2	5.1	372	1897	2835	10.4
8	5	1	1	4.2	258	1084		
			2	5.0	339	1695	2779	9.2
9	2	1	1	4.3	128	550		
			2	5.5	275	1513	2063	9.8
10	1	1	1	4.8	48	230		
			2	4.9	193	946	1176	9.7

BULL NO:- 365 GROUP:-I

BREED:- Jersey AGE:-8yrs

Collect. Number	Collect. Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot.Sp/Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	1	1	1	7.1	638	4530		
			2	8.0	776	6208	10738	15.1
2	1	2(O)	1	5.9	201	1186		
			2	4.9	856	4194	5380	10.8
3	3	2(N)	1	5.5	484	2662		
			2	6.1	679	4142	6804	11.6
4	9	3(O)	1	5.7	820	4674		
			2	5.8	743	4309	8983	11.5
5	7	5(O)	1	6.6	923	6092		
			2	5.7	915	5216	11308	12.3
6	9	3(N)	1	4.8	388	1862		
			2	5.9	695	4101	5963	10.7
7	7	5(N)	1	4.7	630	2961		
			2	7.0	856	5992	8953	11.7
8	7	1	1	5.0	557	2785		
			2	7.0	679	4753	7538	12.0
9	6	1	1	6.1	508	3099		
			2	4.5	776	3492	6591	10.6
10	4	1	1	4.0	331	1314		
			2	6.8	840	5712	7026	10.8

BULL NO:- 129 GROUP:-I

BREED:- Friesian AGE:-7yrs

Collect. Number	Collect. Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot.Sp/Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	3	1	1	8.0	727	5816		
			2	6.9	372	2567	8383	14.9
2	3	2(O)	1	7.8	646	5039		
			2	8.7	533	4637	9676	16.5
3	5	2(N)	1	7.5	800	6000		
			2	6.1	380	2318	8318	13.6
4	10	3(O)	1	7.0	719	5033		
			2	8.6	452	3887	8920	15.6
5	1	5(O)	1	6.8	654	4447		
			2	7.9	428	3381	8228	14.7
6	10	3(N)	1	6.2	711	4408		
			2	6.0	444	2664	7072	12.2
7	1	5(N)	1	6.1	760	4636		
			2	6.0	436	2616	7252	12.1
8	2	1	1	6.1	856	5222		
			2	7.7	460	3542	8764	13.8
9	8	1	1	5.0	751	3755		
			2	6.0	404	2424	6179	11.0
10	11	1	1	2.5	1058	2645		
			2	6.0	663	3978	6623	8.5

BULL NO:- 518 GROUP:-I

BREED:- Jersey AGE:-10yrs

Collect. Number	Collect. Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot.Sp./Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	2	1	1	2.0	1616	3232		
			2	3.0	1196	3588	6820	5.0
2	2	2(O)	1	2.9	1600	4640		
			2	2.7	1131	3054	7694	5.6
3	4	2(N)	1	2.3	1462	3363		
			2	3.0	1107	3321	6684	5.3
4	2	3(O)	1	2.1	1430	3003		
			2	3.0	1478	4434	7437	5.1
5	6	5(O)	1	2.1	1705	3581		
			2	3.0	1228	3684	7265	5.1
6	2	3(N)	1	2.4	1406	3374		
			2	2.5	1333	3333	6707	4.9
7	6	5(N)	1	2.4	1268	3043		
			2	3.2	1293	4138	7181	5.6
8	1	1	1	3.6	1616	5818		
			2	4.0	947	3788	9606	7.6
9	11	1	1	2.9	1616	4686		
			2	3.0	1212	3636	8322	5.9
10	6	1	1	1.6	1333	2133		
			2	3.0	1576	4728	6861	4.6

BULL NO:- 322 GROUP:-I

BREED:- Jersey AGE:-9yrs

Collect. Number	Collect. Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot.Sp/Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	6	1	1	4.7	873	4103		
			2	5.1	646	3295	7398	9.8
2	6	2(O)	1	3.0	1252	3756		
			2	5.6	743	4161	7917	8.6
3	7	2(N)	1	5.5	970	5335		
			2	5.3	687	3641	8976	10.8
4	3	3(O)	1	4.8	1131	5429		
			2	3.9	776	3026	8455	8.7
5	3	5(O)	1	2.4	756	1814		
			2	5.8	711	4124	5938	8.2
6	3	3(N)	1	4.5	808	3636		
			2	4.9	711	3484	7120	9.4
7	3	5(N)	1	3.0	840	2520		
			2	5.5	768	4224	6744	8.5
8	4	1	1	5.2	1042	5418		
			2	5.5	663	3647	9065	10.7
9	4	1	1	4.7	907	4263		
			2	2.6	323	840	5103	7.3
10	9	1	1	4.7	638	2999		
			2	5.2	840	4368	7367	9.9

BULL NO:- 52 GROUP:-I

BREED:- Ayrshire AGE:-9yrs

Collect. Number	Collect. Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot.Sp/Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	5	1	1	4.9	768	3763		
			2	6.1	663	4044	7807	11.0
2	5	2(O)	1	6.0	1156	6936		
			2	6.0	500	3000	9936	12.0
3	1	2(N)	1	6.0	907	5442		
			2	5.1	695	3545	8987	11.1
4	1	3(O)	1	6.3	1147	7226		
			2	7.3	743	5424	12650	13.6
5	8	5(O)	1	6.2	873	5413		
			2	5.9	731	4313	9726	12.1
6	1	3(N)	1	6.3	808	5090		
			2	6.5	533	3465	8555	12.8
7	8	5(N)	1	4.6	915	4209		
			2	6.2	630	3906	8115	10.8
8	3	1	1	7.4	1083	8014		
			2	6.0	663	3978	11992	13.4
9	9	1	1	5.0	816	4080		
			2	5.7	566	3226	7306	10.7
10	7	1	1	4.3	638	2743		
			2	5.6	808	4525	7268	9.9

BULL NO:- 364 GROUP:-I

BREED:- Jersey AGE:-10yrs

Collect. Number	Collect. Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot.Sp/Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	7	1	1	4.3	1535	6601		
			2	3.1	727	2254	8855	7.4
2	7	2(O)	1	3.2	1277	4086		
			2	4.0	970	3880	7966	7.2
3	2	2(N)	1	3.5	1357	4750		
			2	3.0	840	2520	7270	6.5
4	11	3(O)	1	4.0	1535	6140		
			2	3.3	840	2772	8912	7.3
5	11	5(O)	1	4.1	1293	5301		
			2	3.9	955	3725	9026	8.0
6	11	3(N)	1	3.2	1180	3776		
			2	3.9	1002	3908	7684	7.1
7	11	5(N)	1	4.3	1067	4588		
			2	3.4	614	2088	6676	7.7
8	8	1	1	3.6	1067	3841		
			2	4.0	743	2972	6813	7.6
9	5	1	1	2.8	1067	2988		
			2	3.0	1002	3006	5994	5.8
10	10	1	1	3.4	1147	3900		
			2	3.5	864	3024	6924	6.9

BULL NO:- 393 GROUP:-I

BREED:- Jersey AGE:-8yrs

Collect. Number	Collect. Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot.Sp/Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	10	1	1	4.2	177	743		
			2	5.0	123	615	1358	9.2
2	10	2(O)	1	4.8	94	451		
			2	4.2	96	403	854	9.0
3	10	2(N)	1	4.1	128	525		
			2	4.6	80	368	893	8.7
4	4	3(O)	1	4.3	48	206		
			2	4.2	56	235	441	8.5
5	2	5(O)	1	4.3	24	103		
			2	3.0	56	168	271	7.3
6	4	3(N)	1	4.6	32	147		
			2	4.8	48	230	377	9.4
7	2	5(N)	1	4.0	48	192		
			2	5.0	76	380	572	9.0
8	9	1	1	7.4	258	1901		
			2	6.3	218	1373	3282	13.7
9	3	1	1	4.5	145	653		
			2	5.0	161	805	1458	9.5
10	5	1	1	4.3	64	275		
			2	4.6	112	515	790	8.9

BULL NO:- 16 GROUP:-I

BREED:- Ayrshire AGE:-10yrs

Collect. Number	Collect Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot.Sp/Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	8	1	1	Lost				
			2	7.6	549	4172		
2	8	2(O)	1	4.9	824	4038		
			2	8.8	500	4400	8438	13.7
3	8	2(N)	1	8.1	963	7800		
			2	8.9	654	5821	13621	17.0
4	7	3(O)	1	Lost				
			2	6.8	436	2965		
5	9	5(O)	1	Lost				
			2	1.5	549	824		
6	7	3(N)	1	2.9	899	2607		
			2	7.5	468	3510	6117	10.4
7	9	5(N)	1	8.7	840	7308		
			2	8.2	452	3706	11014	16.9
8	11	1	1	9.9	792	7841		
			2	7.7	436	3357	11198	17.6
9	7	1	1	8.4	931	7820		
			2	3.0	420	1260	9080	11.4
10	8	1	1	3.0	840	2520		
			2	4.6	760	3496	6016	7.6

Collection No 1 First service lost in the air
Collection No 4 First service lost in the air
Collection No 5 Served cow with first service
Collection No 6 First service not up to standard
Collection No 9 Second service not up to standard

BULL NO:- 19 GROUP:-II

BREED:- Ayrshire AGE:-9yrs

Collect. Number	Collect. Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot.Sp/Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	5	1	1	7.8	1268	9890		
			2	5.9	630	3717	13607	13.7
2	1	1	1	6.4	1058	6771		
			2	4.5	784	3528	10299	10.9
3	1	1	1	5.7	978	5575		
			2	5.8	1050	6090	11665	11.5
4	8	4(O)	1	9.3	1422	13225		
			2	8.0	557	4456	17681	17.3
6	8	4(N)	1	6.3	1374	8656		
			2	5.0	630	3150	11806	11.3
7	9	1	1	6.6	1123	7412		
			2	6.8	792	5386	12798	13.4
8	7	2(O)	1	7.9	1430	11297		
			2	7.3	751	5482	16779	15.2
9	7	2(N)	1	6.1	1535	9364		
			2	6.1	687	4191	13555	12.2
10	9	6(O)	1	7.1	1621	11509		
			2	5.3	963	5104	16613	12.4
11	9	6(N)	1	5.6	1503	8417		
			2	4.9	727	3562	11979	10.5

BULL NO:- 406 GROUP:-II

BREED:- Jersey AGE:-8yrs

Collect. Number	Collect. Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot.Sp/Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	10	1	1	1.9	266	505		
			2	3.8	468	1778	2283	5.7
2	4	1	1	4.7	1228	5772		
			2	3.6	549	1976	7748	8.3
3	4	1	1	3.0	1107	3321		
			2					
4	2	4(O)	1	5.1	1252	6385		
			2					
6	2	4(N)	1	2.9	1107	3210		
			2	3.6	517	1861	5071	6.5
7	3	1	1	4.2	404	1697		
			2	2.1	614	1289	2986	6.3
8	5	2(O)	1	5.5	1390	7645		
			2	4.4	452	1989	9634	9.9
9	5	2(N)	1	3.0	1095	3285		
			2	3.7	808	2990	6275	6.7
10	3	6(O)	1	3.7	1050	3885		
			2	6.1	606	3697	7582	9.8
11	3	6(N)	1	3.5	873	3056		
			2	3.7	856	3167	6223	7.2

Collection Number 1 First service partly lost as a/v was dropped
Collection Number 3 Second service ejaculated into sheath
Collection Number 4 Second service ejaculated into air

BULL NO:- 122 GROUP:-II

BREED:- Friesian AGE:-8yrs

Collect. Number	Collect. Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot.Sp/Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	11	1	1	11.6	646	7494		
			2	9.8	380	3724	11218	21.4
2	7	1	1	8.8	533	4690		
			2	7.9	468	3697	8387	18.7
3	5	1	1	9.5	808	7676		
			2	8.0	521	4168	11844	17.5
4	5	4(O)	1	9.8	590	5782		
			2	6.0	484	2904	8686	15.8
6	5	4(N)	1	8.3	719	5968		
			2	6.7	476	3189	9157	15.0
7	1	1	1	6.9	873	6024		
			2	6.8	598	4066	10090	13.7
8	2	2(O)	1	9.1	955	8691		
			2	2.5	104	260	8951	11.6
9	2	2(N)	1	8.5	808	6868		
			2	7.1	760	5396	14264	15.6
10	4	6(O)	1	8.2	947	7765		
			2	8.7	500	4350	12115	16.9
11	4	6(N)	1	5.2	792	4118		
			2	7.0	873	6111	10229	12.2

BULL NO:- 158 GROUP:-II

BREED:- Friesian AGE:-5yrs

Collect. Number	Collect. Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot.Sp/Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	3	1	1	10.2	947	9659		
			2	10.1	436	4404	14063	20.3
2	10	1	1	11.0	1018	11198		
			2	2.5	299	748	11946	13.5
3	10	1	1	10.6	1034	10960		
			2	9.3	396	3683	14643	19.9
4	1	4(O)	1	10.1	940	9494		
			2	7.5	711	5333	14827	17.6
6	1	4(N)	1	9.8	915	8967		
			2	9.0	614	5526	14493	18.8
7	8	1	1	9.1	899	8181		
			2	8.2	663	5437	13618	17.3
8	11	2(O)	1	8.3	1260	10458		
			2	6.3	541	3408	13866	14.6
9	11	2(N)	1	6.5	735	4778		
			2	5.7	420	2394	7172	12.2
10	5	6(O)	1	8.5	970	5010		
			2	6.2	808	5232	10242	14.7
11	5	6(N)	1	7.5	970	7275		
			2	5.2	452	2350	9625	12.7

BULL NO:- 304 GROUP:-II

BREED:- Jersey AGE:-10yrs

Collect. Number	Collect. Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot.Sp/Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	6	1	1	5.6	1244	6966		
			2	8.4	484	4066	11032	14.0
2	9	1	1	7.5	840	6300		
			2	2.0	646	1292	7592	9.5
3	3	1	1	3.0	881	2643		
			2	4.5	606	2727	5370	7.5
4	3	4(O)	1	4.5	970	4365		
			2	7.5	952	7140	11505	12.0
6	3	4(N)	1	4.4	404	1778		
			2	9.0	899	8091	9869	13.4
7	4	1	1	3.5	1010	3535		
			2	5.8	549	3178	6713	9.3
8	9	2(O)	1	7.6	923	7015		
			2	7.9	735	5807	12822	15.5
9	9	2(N)	1	4.6	492	2263		
			2	4.8	947	4546	6809	9.4
10	8	6(O)	1	2.8	1248	3494		
			2	8.0	776	6208	9702	10.8
11	8	6(N)	1	3.7	646	2390		
			2	7.5	994	7455	9845	11.2

BULL NO:- 397 GROUP:-II

BREED:- Jersey AGE:-11yrs

Collect. Number	Collect. Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot.Sp/Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	1	1	1	7.1	760	5396		
			2	5.5	347	1909	7305	12.6
2	8	1	1	6.8	663	4508		
			2	6.0	300	1800	6308	12.8
3	9	1	1	6.5	743	4830		
			2	6.3	250	1575	6405	12.8
4	7	4(O)	1	5.9	776	4578		
			2	6.0	299	1794	6372	11.9
6	7	4(N)	1	5.9	695	4101		
			2	6.8	372	2530	6631	12.7
7	6	1	1	4.8	646	3101		
			2	5.8	323	1873	4974	10.6
8	4	2(O)	1	6.5	848	5512		
			2	6.0	444	2664	8176	12.5
9	4	2(N)	1	5.1	517	2637		
			2	6.4	404	2586	5223	11.5
10	6	6(O)	1	8.0	654	5232		
			2	6.1	412	2513	7745	14.1
11	6	6(N)	1	4.9	832	4077		
			2	6.2	364	2257	6334	11.1

BULL NO:- 561 GROUP:-II

BREED:- Jersey AGE:-7yrs

Collect. Number	Collect. Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot.Sp/Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	9	1	1	3.3	760	2508		
			2	5.7	1196	6817	9325	9.0
2	6	1	1	3.1	570	1767		
			2	4.8	663	3182	4949	7.9
3	6	1	1	2.7	508	1372		
			2	6.3	1236	4450	5822	6.3
4	10	4(O)	1	4.2	1228	5158		
			2	2.2	1002	2204	7362	6.4
6	10	4(N)	1	4.0	1543	6172		
			2	4.7	881	4141	10313	8.7
7	10	1	1	4.1	784	3214		
			2	4.2	1293	5431	8645	8.3
8	3	2(O)	1	1.5	711	1067		
			2	4.2	1398	5872	6939	5.7
9	3	2(N)	1	2.8	541	1515		
			2	6.8	792	5386	6891	9.6
10	2	6(O)	1	5.0	1268	6340		
			2	5.4	1034	5584	11924	10.4
11	2	6(N)	1	2.2	614	1351		
			2	3.4	970	3298	4649	5.6

BULL NO:- 12 GROUP:-II

BREED:- Ayrshire AGE:-12yrs

Collect. Number	Collect. Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot.Sp/Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	8	1	1	8.6	1018	8755		
			2	6.2	679	4210	12965	14.8
2	3	1	1	8.4	1107	9299		
			2	7.4	594	4396	13695	15.8
3	7	1	1	8.5	1042	8857		
			2	7.5	606	4545	13402	16.0
4	9	4(O)	1	8.7	1164	10127		
			2	8.2	743	6093	16220	16.9
6	9	4(N)	1	8.0	970	7760		
			2	7.4	735	5439	13199	15.4
7	7	1	1	7.1	1050	7455		
			2	7.5	630	4725	12180	14.6
8	8	2(O)	1	5.9	840	4956		
			2	7.0	816	5712	10668	12.9
9	8	2(N)	1	6.8	1103	7500		
			2	6.1	792	4831	12331	12.9
10	11	6(O)	1	7.5	1099	8243		
			2	7.0	727	5089	13332	14.5
11	11	6(N)	1	6.3	970	6111		
			2	5.8	646	3747	9858	12.1

BULL NO:- 391 GROUP:-II

BREED:- Jersey AGE:-9yrs

Collect. Number	Collect. Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot.Sp/Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	7	1	1	3.1	1244	3856		
			2	3.2	1018	3258	7114	6.3
2	2	1	1	2.7	1099	2967		
			2	3.1	959	2973	5940	5.8
3	11	1	1	3.4	1414	4808		
			2	3.4	792	2693	7501	6.8
4	11	4(O)	1	2.9	1196	3468		
			2	3.5	1002	3507	6975	6.4
6	11	4(N)	1	2.3	939	2160		
			2	3.6	1099	3956	6116	5.9
7	5	1	1	2.5	1058	2645		
			2	2.3	1188	2732	5377	4.8
8	10	2(O)	1	2.3	1341	3084		
			2	3.9	994	3877	6961	6.2
9	10	2(N)	1	2.8	1067	2988		
			2	3.0	1123	3369	6357	5.8
10	7	6(O)	1	1.7	1446	2458		
			2	3.5	955	3343	5801	5.2
11	7	6(N)	1	2.5	1123	2808		
			2	2.5	1244	3110	5918	5.0

BULL NO:- 703 GROUP:-II

BREED:- Jersey AGE:-7yrs

Collect. Number	Collect. Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot.Sp/Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	2	1	1	4.2	1406	5905		
			2	6.4	931	5958	11863	10.6
2	5	1	1	5.6	1196	6698		
			2	5.7	703	4007	10705	11.3
3	2	1	1	3.9	1212	4727		
			2	5.0	840	4200	8927	8.9
4	6	4(O)	1	4.8	1454	6979		
			2	5.3	804	4261	11240	10.1
6	6	4(N)	1	4.7	1365	6416		
			2	5.1	1002	5110	11526	9.8
7	11	1	1	5.7	1164	6635		
			2	5.4	646	3488	10123	11.1
8	6	2(O)	1	3.8	1632	6206		
			2	4.9	978	4792	10998	8.7
9	6	2(N)	1	4.5	1552	6984		
			2	5.0	808	4040	11024	9.5
10	1	6(O)	1	5.0	1349	6745		
			2	6.6	1067	7042	13787	11.6
11	1	6(N)	1	4.3	1308	5624		
			2	4.5	899	4046	9670	8.8

BULL NO:- 359 GROUP:-II

BREED:- Jersey AGE:-11yrs

Collect. Number	Collect. Order	Teaser Cow No	Service Number	Volume (mls)	Sperm Conc. ($x10^6$/ml.)	Tot.Sp/Ejac ($x10^6$)	Sp.Output/ Day($x10^6$)	Semen Tot. (mls)
1	4	1	1	4.6	226	1040		
			2	3.5	132	462	1502	8.1
2	11	1	1	3.0	226	678		
			2	4.5	96	432	1110	7.5
3	8	1	1	2.8	120	336		
			2	1.9	331	629	965	4.7
4	4	4(O)	1	4.5	177	797		
			2	4.3	54	232	1029	8.8
6	4	4(N)	1	2.2	146	321		
			2	2.0	210	420	741	4.2
7	2	1	1	1.0	177	177		
			2	4.2	96	403	580	5.2
8	1	2(O)	1	5.2	452	2350		
			2	4.5	96	432	2782	9.7
9	1	2(N)	1	5.3	517	2740		
			2	3.6	339	1220	3960	8.9
10	10	6(O)	1	4.6	266	1224		
			2	3.8	128	486	1710	8.4
11	10	6(N)	1	2.5	80	200		
			2	4.5	40	180	380	7.0

APPENDIX II

The Description and Behaviour of the Cows Used as Teasers

Group I.

Cow No. 1. Trained Friesian teaser from Awahuri A.B. Centre. 5 months pregnant. Used on Collection Days No. 1, 8, 9 and 10. Stood satisfactorily on all days.

Cow No. 2. Small Jersey (T.B. Tag No. 191490) Used as oestrous teaser on Collection Day No. 2, and non-oestrous teaser on Day No. 3. Oestrous condition shown strongly. Stood well on both days. Untrained teaser from Massey College herd.

Cow No. 3. Trained fully-grown Jersey from Awahuri A.B. Centre. Used on Collection Days Nos. 4 and 6. Oestrous condition shown strongly. Stood well on both days.

Cow No. 5. Trained Friesian teaser from Awahuri A.B. Centre. Used on Collection Day Nos. 5 and 7. Possibly completed oestrus just prior to use. Still exhibited turgidity of the uterus and had not ovulated as the termination of the morning's programme. Rather restless as a non-oestrous teaser, but did not impair collections.

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Group II

Cow No. 1. Description as above. Used on Collection Days Nos. 1, 2, 3 and 9. Inclined to be restless on each of the first 3 days.

Cow No. 4. Untrained Ayrshire-cross (T.B. Tag No. 150249) from Massey College herd. Used as a teaser on collection Days Nos. 4 and 6. Stood well as an oestrous teaser but showed slight restlessness as a non-oestrous teaser.

APPENDIX II (CONT)

Cow No. 'X' — Untrained Ayrshire-cross from Massey College Herd. Used as an oestrous teaser on Collection Day No. 5. Bulls showed no marked response; rectal palpation did not confirm strong oestrus and cow was bulling again 3 days later. The data collected was discarded.

Cow No. 2. — Description as above. Used on Collection Days No. 8 and 9. Stood satisfactorily on both days.

Cow No. 6 — Untrained full-grown Jersey (T.B. Tag No. W.83576) from Massey College herd. Used on Collection Days Nos. 10 and 11. Stood well as an oestrous teaser but slightly restless in the non-oestrous state, but did not impair collections.

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APPENDIX III

The Calibration of The Absorptiometer for Measuring Sperm Concentration.

The "Hilger Biochem" absorptiometer used in the current trial was calibrated against counts made with a Fuchs Rosenthal cytometer of depth 0.2 mm and rulings of 1 mm and 0.25 mm. The absorptiometer had, prior to the trial, been calibrated against another absorptiometer which, in turn, had been calibrated by making a large number of haemocytometer counts. The carefully calibrated absorptiometer will hereafter be referred to as the "Newstead absorptiometer".

In conducting the check calibration of the absorptiometer used in the current trial, the following procedure was adopted:-

(i) 0.1 mls. semen was pipetted into 9.9 mls. formalin-saline solution and the absorptiometer reading determined in the manner described in the text.

(ii) 0.1 mls. from the diluted semen sample were removed with a pipette. The first few drops were wiped away before filling the counting chamber of the haemocytometer. The sample was allowed to settle before counting commenced.

(iii) Prior to the addition of each semen sample, the haemocytometer was carefully cleaned, a clean cover-slip applied and carefully rubbed until Newton's rings appeared.

(iv) With each sample of semen, 2 operators each prepared a slide and each counted ten 0.25 mm x 0.25 mm squares on his particular slide. If the counts of each operator showed a marked variation,

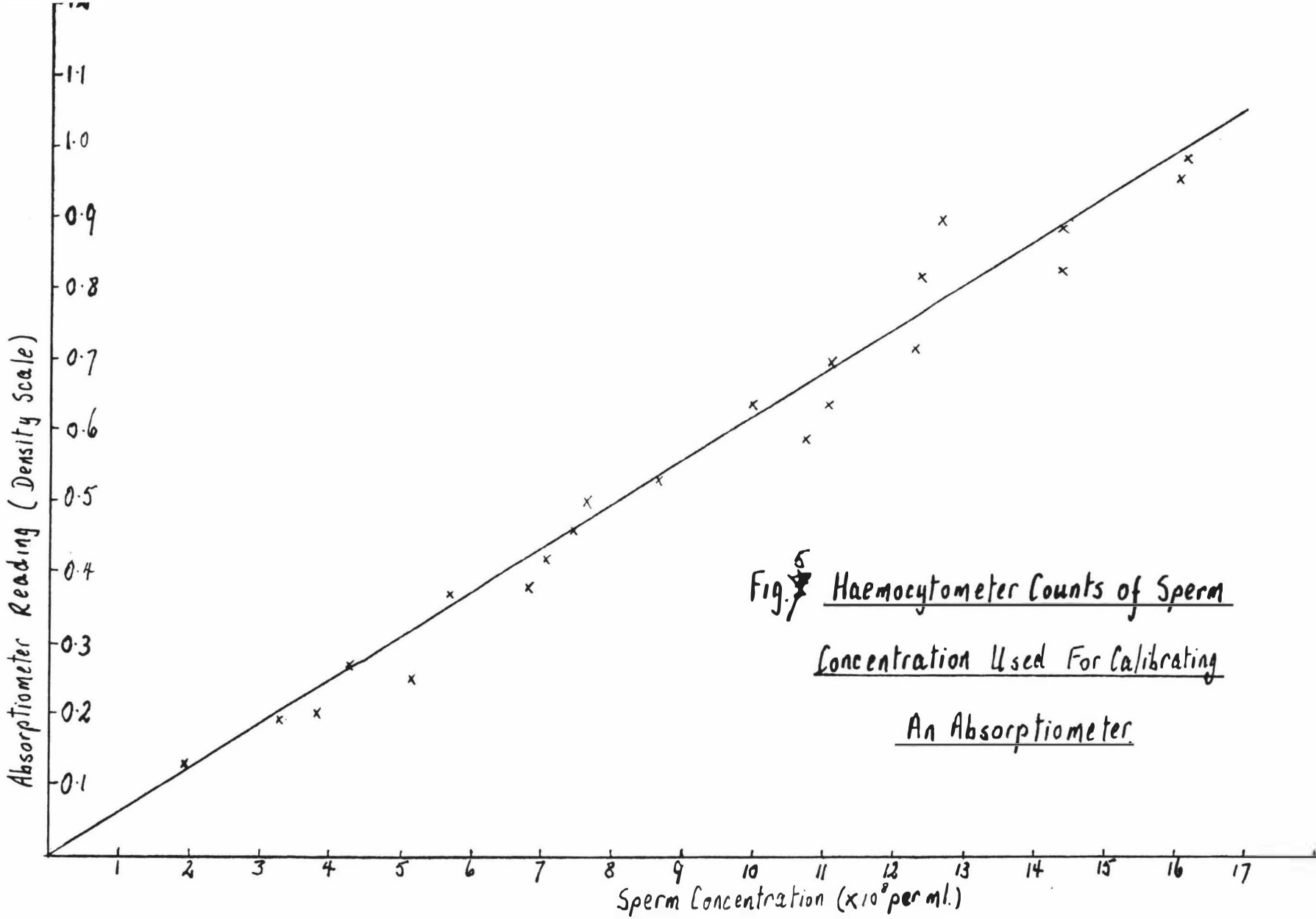

Fig. 6 Haemocytometer Counts of Sperm Concentration Used For Calibrating An Absorptiometer

APPENDIX III (CONT)

the process was repeated. The average of the counts by each operator were graphed against the absorptiometer reading. (Fig. 5)

In the following table the "Newstead Count" is the figure shown on the table prepared for the "Newstead absorptiometer". The regression analysis between the haemocytometer counts and the absorptiometer reading is shown in the accompanying table.

Haemocytometer Count ($\times 10^3$)	Absorptiometer Reading (Density Scale)	Newstead Count ($\times 10^3$)
1.95	0.13	2.10
3.28	0.19	3.06
3.80	0.20	3.25
4.23	0.27	4.36
5.14	0.25	4.03
5.70	0.37	5.97
6.80	0.38	6.13
7.05	0.42	6.77
7.45	0.46	7.42
7.65	0.50	8.07
8.68	0.53	8.55
10.00	0.64	10.32
10.75	0.59	9.52
11.05	0.64	10.32
11.10	0.70	11.40
12.30	0.72	11.61
12.40	0.82	13.23
12.70	0.90	14.52
14.40	0.83	13.39
14.40	0.89	14.36
16.05	0.96	15.48
16.17	0.99	15.97

Table:- Results obtained in Calibrating a "Hilger Biochem" Absorptiometer using a Haemocytometer Counting Chamber.

APPENDIX III (CONT)

Source of Variation	d.f.	Sums of Squares	Mean Square	F. Ratio	Tests of Significance
Regression	1	369.11	369.11	820.24	***
Error	20	8.95	0.45		
Total	21	378.06			

Table:- Regression Analysis of Haemocytometer Count on Absorptiometer Reading.

The regression equation was:-

$$y = 15.66x + 0.42$$

where y = sperm concentration ($\times 10^6$ per ml.) x = absorptiometer reading.

The "Newstead" equation was

$$y = 16.1\ x$$

Since the two equations bore such a close resemblance and the Newstead equation was derived from a greater number of readings, the conversion table which Newstead had developed was used for determining sperm concentration in the current trial.

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APPENDIX IV

The Calibration of the Absorptiometer for Measuring The Initial Fructose Concentration of Semen

Another "Hilger Biochem" absorptiometer similar to that used for measuring sperm concentration was used for determining the initial fructose concentration in each sample of semen. For fructose determinations a green filter (No. 49) was used. It was not until the latter half of the experimental period that satisfactory repeatability was obtained with the standard solutions. Because of this, the results for the first half of the total were discarded.

The standard solutions for the calorimetric assay were prepared from a 0.2% fructose solution. The procedure adopted was:-

(i) 4 test-tubes were labelled A to D. 1.8 mls of distilled water was pipetted into Tube A, and 1 ml into each of tubes B, C and D;

(ii) 0.2 mls of 0.2% fructose solution were pipetted into Tube A, thoroughly mixed, 1 ml of the mixture transferred to Tube B, again mixed, 1 ml transferred to Tube C, and the procedure repeated from Tube C to Tube D.

(iii) 1 ml of the mixed contents of Tube D was discarded, so that finally each tube contained 1 ml of fluid containing 0.2 mgrms, 0.1 mgrms, 0.05 mgrms or 0.025 mgrms of fructose

(iv) 0.5 ml of 2% $ZnSO_4$, 0.5 ml of 0.1 NaOH, 2 mls of 0.1% solution of resorcinol in alcohol and 6 mls of 35% hydrochloric acid were all added to each test tube.

(v) each tube was heated at 80 - 85° C for 10 minutes and then cooled in tap water before being poured into a curette and placed in the absorptiometer.

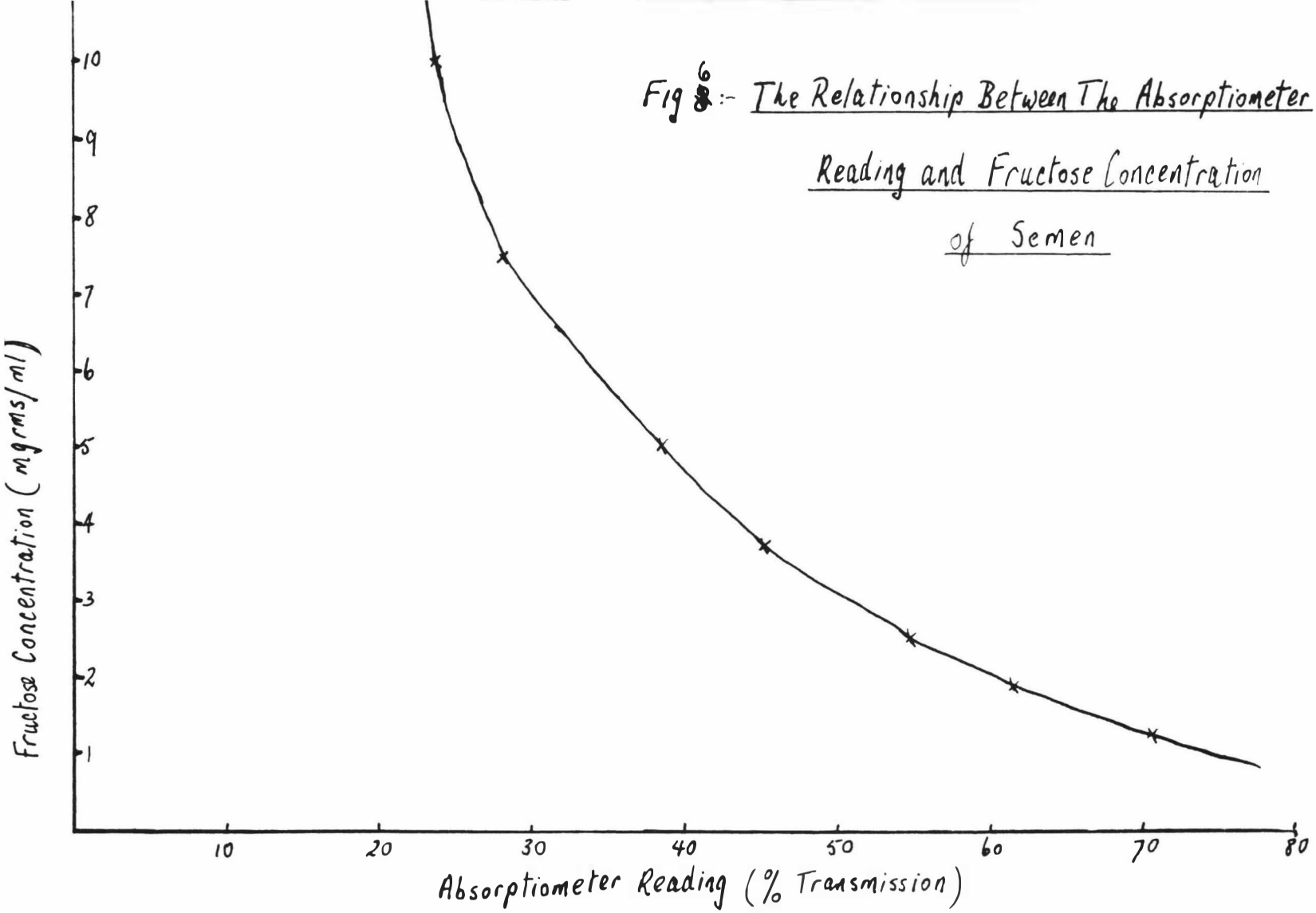

Fig 6 :- The Relationship Between The Absorptiometer Reading and Fructose Concentration of Semen

APPENDIX IV (CONT)

(Vi) the absorptiometer was checked against a re-agent blank containing no fructose.

The fructose concentration was varied to obtain readings over a wider range. The results from which the absorptiometer was calibrated are presented in the accompanying table.

Concentration (mgms/ml.)	10.0	7.5	5.0	3.75	2.5	1.88	1.25
Series I	23.7		38.0		57.0		73.0
II	24.0		37.7		53.0		69.0
III		27.7		45.0		63.0	
IV		28.2		45.2		59.7	
V	25.85	29.0	37.85	45.1	55.0	61.3	71.0
VI	24.0		39.5		55.0		70.0
VII	25.0		40.0		54.0		70.0

Table: The Absorptiometer Readings for Standard Concentrations of Fructose. (Absorptiometer readings recorded from % transmission scale).

APPENDIX V

The Day-to-Day Variation in Sperm Output and Semen Volume in Bulls Under Routine Management Conditions During the 1961 Spring Mating Season at The Awahuri Artificial Breeding Centre.

The following data were abstracted from Station records. The routine management conditions involve the collection of 2 services every third day. In the following data the bulls of each group were collected on the same days but each group was collected on different days. Only the sperm content of the first service was analysed, as although 2 services were collected each collection day, when a second service was not despatched only the volume was recorded. The semen volume for both services was summed to give total semen output from each bull for each collection day.

Bull No. \ Day No.	1	2	3	4	5	6	7	8	9	10	Total
314	15	34	42	27	39	33	20	30	34	27	301
561	74	55	76	50	76	52	37	72	52	75	619
396	63	34	52	35	55	45	41	52	50	63	494
365	47	66	50	43	40	37	16	18	65	40	422
317	21	28	50	20	52	38	71	32	51	47	410
364	68	73	49	32	48	79	29	55	25	52	518
Total	290	290	319	207	310	284	214	259	275	316	2764

Table: Sperm Content ($\times 10^8$) of First Services of 6 Mature Jersey Bulls Over 10 Collection Days. (Group A)

Source of Var.	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	Test of Significance
Between Bulls	5	5903	1181	5.85	2.43 3.46	**
Between Days	9	2545	283	1.4	2.10 2.84	N.S.
Error	45	9100	202			
Total	59	17,548				

Table: Analysis of Variance For Between Day Differences in The Sperm Content of the First Services Collected From Bulls of Group A.

Day No. / Bull No.	1	2	3	4	5	6	7	8	9	10	Total
527	48	29	35	16	25	23	29	25	33	14	267
533	44	52	63	65	25	49	30	55	25	87	496
362	45	58	60	23	43	45	33	40	63	45	455
296	45	34	48	53	48	55	51	53	54	55	501
393	53	44	36	45	25	29	39	51	38	46	404
322	37	71	70	43	76	60	44	63	69	57	590
Total	272	288	312	245	240	261	226	292	272	304	2712

Table: Sperm Content (x 10^8) of First Service of 6 Mature Jersey Bulls over 10 Collection Days (Group B)

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	Test of Significance
Between Bulls	5	5984	1197	7.34	2.43 3.46	**
Between Days	9	1211	135	0.83	2.10 2.84	N.S.
Error	45	7345	163			
Total	59	14540				

Table: Analysis of Variance for Between Day Differences in The Sperm Content of the First Services Collected From Bulls of Group B.

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	Test of Significance
Between Bulls	5	107.86	21.6	16	2.43 3.46	**
Between Days	9	16.1	1.8	1.3	2.10 2.84	N.S.
Error	45	60.69	1.35			
Total	59	184.65				

Table: Analysis of Variance for Between Day Differences in The Total Volume of Semen Collected from Bulls of Group A.

Day No.	1	2	3	4	5	6	7	8	9	10	Total
Bull No.											
314	6.50	7.25	6.75	5.0	7.25	7.25	5.75	6.0	7.75	7.25	66.75
561	8.75	9.0	7.75	8.0	10.0	9.0	9.5	8.75	8.25	8.25	86.25
396	6.25	6.0	7.0	6.5	7.5	7.5	9.0	7.5	8.25	9.0	74.50
365	13.25	11.0	12.0	11.25	9.25	7.5	11.0	10.75	10.5	13.5	110.00
317	8.75	6.25	7.75	7.0	9.0	9.0	9.25	8.5	8.5	8.5	
364	9.25	7.5	8.0	5.75	8.75	6.0	9.25	7.75	9.0	7.75	81.00
Total	52.75	47.0	49.25	43.5	51.75	48.25	52.75	49.25	52.25	54.25	501.00

Table: Total Volume (mls) of Semen Collected From 6 Mature Jersey Bulls Over 10 Collection Days (Group A.)

Day No. / Bull No.	1	2	3	4	5	6	7	8	9	10	Total
527	15.0	15.5	15.0	11.25	12.0	11.5	12.25	12.5	12.0	11.5	126.50
533	6.5	6.75	12.5	8.25	6.25	11.25	7.0	4.75	9.5	11.75	86.50
362	7.75	7.0	11.75	7.75	7.25	7.25	8.0	7.5	8.25	5.75	78.25
296	5.25	5.5	6.0	6.25	5.75	6.25	6.0	6.0	7.75	7.0	62.75
393	11.5	11.25	12.0	10.25	10.25	11.75	11.5	12.75	11.75	12.0	115.00
322	9.25	11.25	13.0	11.0	12.25	11.0	10.0	11.25	12.25	11.75	113.00
Total	55.25	55.25	70.25	54.75	54.75	59.0	54.75	54.75	61.5	59.75	580.00

Table: Total Volume (mls) of Semen Collected From 6 Mature Bulls Over 10 Collection Days. (Group B.)

Source of Variation	d.f.	Sums of Squares	Mean Square	F.Calc.	F.Reqd.	Test of Significance
Between Bulls	5	313.04	62.61	32.61	2.43 3.46	**
Between Days	9	37.29	4.14	2.15	2.10 2.84	*
Error	45	86.25	1.92			
Total	59	436.58				

Table: Analysis of Variance for Between Day Differences in the Total Volume of Semen Collected from Bulls of Group B.

These analyses indicated that during the spring mating season, under conditions of routine management, mature Jersey bulls did not exhibit significant day-to-day variations in the total sperm output of their first services. Whereas Group A did not exhibit significant variations in total semen ~~output~~ volume per collection day, Group B did produce significant variations. Reference to the table will show that this group showed only a light variation except on Day No. 3. The remarkably high output on this day was primarily due to marked increases shown by only 2 bulls - Nos. 533 and 362.

During the spring season, several factors may have been operating to confound true day to day variations. These "other factors" were:-

(i) All the bulls were not collected over the same teaser on any one day, and teasers were altered each collection day.

(ii) A rigid pre-collection teasing routine was not applied to the bulls,

and (iii) The collections and teasing were carried out by a number of technicians.