A Study On
Some Aspects of Breeding Behaviour
&
Semen Picture of Holstein Friesian Bulls.

Thesis

Submitted to the

RAJENDRA AGRICULTURAL UNIVERSITY, BIHAR

in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE (ANIMAL HUSBANDRY)

1971

BY

Surendra Nath

G. B. V. C., B. V. Sc. & A. H.

Junior Research Fellow, R. A. U.

Post-Graduate Department of Animal Genetics & Breeding,
BIHAR VETERINARY COLLEGE,
P A T N A.

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M E M O R Y O F

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This is to certify that Dr.Surendra Nath,has worked under my supervision and guidance for his Thesis entitled 'A STUDY ON SOME ASPECTS OF BREEDING BEHAVIOUR AND SEMEN PICTURE OF HOLSTEIN FRIESIAN BULLS' for the degree of M.Sc. (A.H.) with Animal Genetics & Breeding as major subject. The materials incorporated in this thesis are his own findings and I have checked-up his results from time to time, during the course of the Academic year, 1970-71.

Yd. R. Mishra

Patna, the 14 March,1972. (H. R. Mishra)

A C K N O W L E D G E M E N T

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CHAPTER I

INTRODUCTION

INTRODUCTION

With the agricultural and industrial advancement as well as the increasing human population, the need of increased milk production is being felt more keenly, as the Indian cattle are well known for their low production. In order to face this challenge it has now become immensely important to increase the production potentiality of our cattle during the shortest period of time.

There are principally two ways to increase the milk yeild of our cattle; firstly to improve the environment to which the animals are subjected and which is totally dependent upon the human consideration and secondly to improve their genetic make-up.

For milk production, the heritability co-efficient usually centres around 0.3, where as the environmental contribution remains around 0.7.

Though the heritability co-efficient for milk yeild is around 0.3, it is not of less importance, as without having genetic potentiality no individual can produce more, even after providing the best environment, i.e. the genetic potentiality puts a ceiling upon the production level of the individuals.

The milk production of our cattle can be increased through breeding at varying rates depending upon the type of breeding followed. Out of the three systems of breeding that are usually advised under

our existing conditions-viz.Selective breeding,Grading-up and Cross-breeding, only the last in the list, i.e. Cross-breeding of Indian cattle to exotic stocks is now being vigorously recommended for boosting up milk production during the shortest period of time. It has been observed that on a rough estimate, the present milk production level of our Indian cattle can be doubled in hundred years through Selective breeding and in fifty years through Grading-up where as Cross-breeding can do the job within less than 10 years.

In pursuance of this recommendation the exotic bulls of different cattle breeds are being imported for breeding in the different states. In Bihar also,Holstein,Ayrshire and Jersey bulls have been introduced in different cross breeding zones.

Although much work has been done on semen picture and breeding behaviour of the bulls belonging to these breeds in their native countries, such information are available only to a very limited extent under the environmental conditions of our country.

Though much work has been done on these two aspects i.e. breeding behaviour and semen picture, of different breeds of exotic bulls, it has been reported by various workers that these do not show the same results under different environmental conditions under which the bulls are reared. It seems that the environmental

variables like, temperature, rainfall, humidity as well as different managemental conditions have important bearings on these (Erb et al. 1942; Phillips et al., 1943; Lasley & Bogart, 1943; Swanson & Herman, 1944; Salisbury, 1944; Anderson, 1945; Mercier & Salisbury, 1946; Bane, 1954; Hultnas, 1967).

Since such informations are of great help in assessing the overall usefulness of these bulls, a study on some aspects of breeding behaviour and semen picture of Holstein bulls has been undertaken as detailed under the subsequent chapters.

* * *

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Review of the available literature has been resorted to under the headings :

(A) Breeding behaviour,

(B) Semen picture and its variations.

(A) BREEDING BEHAVIOUR :

The breeding behaviour includes sexual behaviour and other behavioural patterns of animal related to reproduction. The term 'Sexual behaviour' consists of the mating desire and the ability to copulate. Potency means ability to copulate normally and ejaculate viable spermatozoa (Shukla, 1956).

The breeding behaviour is an activity and hence it necessarily envolves physiological function and reception of stimuli through sense organs.

The intensity of sexual behaviour expressed by the male may not reflect the potential intensity, because the latter is greatly modified by extroceptive factors impinging on the male (Hafez, 1962).

Sexual behaviour is one of the main events subserving reproduction which perhaps has more direct and important effects than any other. This phenomenon has been termed as behavioural chain

of sequances which encomparises courtship, errection and protrusion, mounting, intromission, ejaculatory thrust and ejaculate and dismounting (Schein & Hale,1965). These are regarded as typical male behaviour during copulation (Hafez et al.,1969). The manifestation of copulation in bull varies from full to no expression. Sex libido of bulls is a mixed reaction of physiological developments governed by heredity. The arousal of sex desire in Holstein bulls was first observed by Baker et al.(1955 a) at an age of 29 weeks.

The time taken by bull from its release to the cow till it donates semen and dismounts is termed the reaction time. All stages of sex behaviour are included in it. The sexual instict and the mounting capacity of the bull affects reaction time (Donham et al. 1931).

Active spermatozoa production (as evidenced by the presence of mature spermatozoa in the testis) starts at the age of 7½ - 8½ months and correspond with the period of first sexual interest as indicated by the willingness of the bull to mount a restrained heifer (Phillips & Andrews,1936; Hooker,1944).

Over periods of 8-9 days the bulls though let out singly performed 98-99 matings, an average of 10.9 - 12.4 a day. The number of matings per cow were reported to be 2 to 11. The intensive sexual activity of bulls under conditions of natural mating

was according to them, wasteful; the bulls became exhausted and a great many matings remained ineffective (Verevkina et al.1938).

Exercise leads to greater vitality and ejaculation of better semen samples (Conn,1946). Improper feeding leads to weak spermatozoa ejaculation and mated to poor cows, it leads to abortion or weak calves. Feeding of animal protein was found to increase fertility and vitality. Young bulls under two years of age should not be over-worked and Conn(1946) recommended one mating per week. Long rest was also found to be deterimental to working bulls. A bull not in use for 7 - 10 days continuously may provide a dead sample. Use of bulls before meals and judicious handling for less refractory service should be practised. Temperaments of bulls were classified by him as - the nervous, the sulky, the treacherous (slaughter of these bulls was recommended) and the placid. These may be due to heredity or due to environment or might be due to both.

According to Sarthou - Mountegou (1950) loss of sex-libido in bull used for artificial insemination was due to stimulus provided in natural mating by oestrous secretions from the vagina of the cow being absent. To preserve libido in the bull, cows in oestrous were suggested to be used. As an alternative oestrous secretions stored at low temperature and smeared on a cow in an oestrous or dummy could be used for collection.

Collins et al.(1951) found that sexual excitment was increased by restraining the bulls and except in the case of the percentage of motile spermatozoa significant increase in semen quality was recorded.

Smith (1951) with large number of bulls of different breeds found 10% of the bulls to be erratic in their behaviour during semen collection, usually they were in the older animals first used for artificial insemination after relatively along periods of natural service. Bulls of Hereford and North Devon were found to be the most docile with the longest reaction time at service; whereas the shortest reaction time was recorded in Friesian herd. The more intractable bulls were generally to be in the dairy.

No significant difference in the reaction time in Kumauni Hill bulls was noticed by Prabhu & Guha (1951).

Unrestrained sexual excitment, viz. one or two false mounts prior to the first ejaculate resulted in better semen production. Inverse relationship between fructose content and spermatozoandensity of semen was recorded. As a routine practice one false mount was recommended for stud bulls (Branton et al., 1952).

Average reaction time of the Kumauni bulls was found to be 74.34 seconds by Mukherjee & Bhattacharya (1952). The reaction time of the bulls were noted to be 67.23, 63.07, 73.42 seconds in spring, summer and autumn respectively. Significant

differences between the bulls and seasons were noted by them.

On the influence of the sexual excitment upon quality and quantity of seminal ejaculate by dairy bulls, Ishei & Okamoto (1953) found volume, spermatozoal motility, survival, concentration and total number of spermatozoa were the least in the first ejaculate, there being no significant difference between the second and third ejaculates.

On the effect of sexual stimulation prior to service on the behaviour and conception rate of bull, average conception rate was found to be 60.8% when kept on a regimen of incomplete sexual stimulation prior to semen collection over a five month period by Kerruish (1955). He also showed the average value to be 69.5% for the same bulls kept on a regimen of intensive sexual stimulation during the five month period. Marked improvement in sexual behaviour was also noticed by him.

While studying the growth of Holstein bulls and its relation to sperm production, Baker et al.(1955 a) found that age and body weight were highly significant up to 2 years, but beyond that age the relationship was less significantly marked. Highly significant correlation of the logarithm of weight in pounds with heart girth up to and over 47 inches were found by them to be + 0.967 and + 0.959 respectively. Correlation of sperm production per ejaculation with age were + 0.514 for all bulls

and + 0.499 within bulls. The correlations for bulls with 3 x weekly semen collections were highly significantly different from those of the 2 x weekly bulls, but did not differ significantly from those of once weekly bulls. Correlations of sperm production with weight were + 0.533 for all bulls and + 0.501 within bulls. Twentyfive percent of the variation in sperm concentration per ejaculation of the growing bull, could be attributed to growth associated with increasing age. Age and weight were almost of equal value in predicting sperm production, but higher correlations would be necessary in practice. They suggested that more accurate prediction would be obtained with more frequent collection than those obtained for the bulls on 3 x weekly collection.

Baker et al. (1955 b) showed the effect of frequency of ejaculation on the semen production, seminal characteristics and libido of bulls during first post- puberal year. Overt expression of sexual interest was shown at a mean age of 29.4 weeks and the bulls first ejaculated at mean age of 38.9 weeks. The values for all semen characters were lowest in the first quarter of the year; differences in improvement between values in each succeding quarter were significant. There was no appreciable difference in semen volume, percentage motile sperm, sperm concentration, total sperm per ejaculate and total motile sperm per ejaculate at the various frequencies of collection; differences between bulls within frequencies were

significant. They found semen volume lowest and sperm concentration highest in the group from which collections were made three times a week. The number of trials in which bulls refused to ejaculate was significantly greater in the thrice weekly than in the once or twice-weekly groups.

In Sahiwal breed the average reaction time was noted to be 267.87 seconds and significant breed differences in Indian cattle was found by Bhattacharya & Prabhu (1955 b).

'Potency' has been defined as the ability to copulate and ejaculate normal and viable spermatozoa whereas impotency is the lack of either or both (Shukla,1956). Mating behaviour and sperm-production were observed to be independent of each other. Sexual behaviour was found to be incluenced by (a) Internal factors viz. physiological, psychological and physical conditions of the male, (b) External factors viz. climate, nutrition, exercise and disease, by him.

Disposition of the bulls were found to be dependent to some extent on the age and breed of the bulls (Fraser,1957). Young dairy type and beef bulls were reported to be of mild temperament whereas adult dairy bulls showed fluctuating termperament and aggressive dispositions. Adult Beef bulls were observed to be docile. Libido and threat in environment were reported to be the cause of this neurosis.

Working on the psychology of bulls relating to mating behaviour, Trautwein et al.(1958) discussed the effect of environment,disease,climate, nutrition and weather on the mating behaviour.

Kalev & Venkov (1959) concluded that semen characters like ejaculate volume, spermatozoal concentration resistance, pH and the percentage of pathological spermatozoa were better when reaction time was longer.

Fraser (1960) revealed that reaction time was found to be affected by a great number of factors. Beef bulls over 4 years showed a significantly greater reaction time than similarly aged dairy bulls. Bulls were classified in six different behaviour groups. Only bulls over 4 years of age and those showing exaggerated apprehension regarding service differed significantly in behaviour; their differences being highly significant.

Bhatia (1960) working on reaction time of bulls found the average reaction time of Hariyana, Jersey, Sindhi and Gir bulls to be 8.63, 30-60, 5.5 and 208.43 seconds respectively.

Crombach (1961) concluded that it was possible to obtain more and better sperm by preparing the bull before allowing him to serve. A preparation consisting of a false mount following restraint for 5 minutes prior to service, proved to be the most effective. When two successive services were required, it was

found that the second ejaculate could also be improved by preparation. Once output performance had been improved by preparation, the improvement was maintained when preparatory procedures were discontinued even for a period of several months. According to him teaser had no marked influance on sperm production. Once the bull was willing to serve the effect of teaser was negligible.

Bhatnagar & Lohia (1961) found reaction time to be 18.0 ± 1.30 minutes and recorded significant effect of months on it.

Almquist et al. (1963) studied the sperm output and sexual behaviour of Holstein bulls from 2 to 3 years of age, when collected from puberty at high frequency continuously or when given prolonged sexual rest. Changes in mean ejaculate characteristics from 101-104 to 153-156 weeks of age for 1x and 6x bulls were; semen volume 4.6 to 6.2 and 3.5 to 4.5 ml.; sperm concentration 1529 to 1599 and 1160 to 1057 x 10^6 per ml. and total sperm per ejaculate 6908 to 9717 and 4127 to 4727 x 10^6.

Reaction time in Khillari bulls was found to range between 2.40 seconds to a few minutes by Kodagali (1963).

Singh & Prabhu (1963) found significant breed difference in reaction time of Hariana & Kumauni bulls. Significant effect of frequency of ejaculation on reaction time was also noticed.

Sinha (1964) recorded average reaction time in Tharparker, Hariana and Taylor bulls as 10.08, 4.71 and 11.31 minutes respectively and noted variations in reaction time among the bulls, breeds and seasons.

Abdel-Raouf (1965) working with Swedish-Red and White breed between the ages of 9 and 15 months found wide variations in sexual behaviour of bulls widely. Bulls held together with heifers or mates of the same sex, showed precocious signs of interest. Permitting the young bull to observe mating carried out by another bull, or leaving the bull with a heifer in an open yard appeared to have stimulating effect on sexual behaviour. Volume of the ejaculate, motility and number of spermatozoa were reported to increase with the age, while protoplasmic droplets diminished with age.

Hale (1966) studied the effect of visual stimuli on reproductive behaviour of bulls. Visual stimulation did not seem to be essential for normal reproductive development in the bull. Lack of vision resulted in the initiation of sexual activity, an initial impairment of orientation, and a reduction in the ability to detect and respond to novel stimulation; these effects were observed also in temporarily blind folded normal bulls. Subsequent behavioural responses were essentially unimpaired. The bulls did

not use odour to detect the presence of other animals, the essential cues were either visual or tactile.

Sinha & Prasad (1966) compared the first and second ejaculate collected within short intervals of time in Tharparker bulls : each pair of ejaculates being collected within 30 minutes of each other. No significant difference in reaction time was noted.

Tomar et al.(1966) observed that reaction time and pH varied significantly between months and seasons in Hariana and Murrah bulls.

In Hariana breed, Tripathi & Prabhu (1966) found increase of reaction time with age; bulls of 12 years being significantly slower than those of 4,5,7 and 8 years of age.

Leidl & Biegert (1967) working with Spotted Mountain Cattle (German Simmenthal) found that libido commenced at 256 and 274 days and errection at 264 and 315 days; the first ejaculate was obtained at 345 and 355 days.

Ejaculate volume increased with age and with duration of use; no significant trend in sperm concentration and motility was noticed. The percentage of abnormal spermatozoa differed significantly between bulls, but not due to duration of uses (Chominat & Agache, 1967).

Kozlo (1967) studied the effect of exercise on

reproduction and sexual reflexes in the bulls. Out of ten adult bulls kept untethered on pasture, five were exercised daily by an electrically driven apparatus. Reaction time before ejaculation was less, ejaculate volume and motility, resistance, servival rate and resistance to freezing of spermatozoa were greater and the number of discarded ejaculates was lower for the exercised than for the pastured.

Kodagali (1967) found average reaction time to be 2 and 3 minutes for Girs and Jaffari (Jaferabadi) bulls respectively.

Ionova (1968) made observation on the reproductive function of young bulls between 3 and 18 months of age. The bulls were trained to serve an artificial vagina at 10 months of age. Average ejaculate volume was 1.2, 2.7 and 4.1 ml. at 11.15 and 18 months of age respectively and the corresponding sperm motilities were 74.61 and 75% respectively, showing thereby the semen of young bulls was suitable for use in artificial insemination.

Summer et al.(1968) found that courtship and mating behaviour of bulls and rams followed a characteristic pattern with preleminary activities begining with the proestrous female. When more than one female was in oest-rous the bulls and rams spent more time with those more recently in oestrous. After several matings sexual interest for a particular female declined,

but upon discovery of another female in oestrous libido was rapidly regained. The average number of mating per oestrous female for bulls was 1.73 and for rams was 4.03. The largest number of mating observed in a single dayfor a bull was 10 and for a ram was 29.

Environmental temperature was found to significantly affect semen quality. Extreme high and low temperature reduced semen quality, libido and fertility (Miliecvic et al.,1968).

The sex-libido and reaction time in Tharparker bulls was found by Ansari (1970) to be 4.37 ± 0.06, 4.23 ± 0.08 and 4.25 ± 0.08 in spring,summer and rainy respectively. Sex-libido was higher in spring, a little less during the rainy and least during the summer. A significant differences between the bulls and seasons were observed. The average reaction time was estimated to be 434 ± 19.55 seconds. A highly significant difference in reaction time amongst bulls and seasons were observed.

Mishra et al.(1971) studied the breeding behaviour and some economic characters in Tharparker bulls. They observed that out of 55 bull calves 69% were grouped under A, 12.7%in group B, 12.7% in group C and 56% in group D.

As regards the reaction towards female 78.2% of the bulls were found to be Active; 16.4% Dull and 5.4% Shy. Average reaction time was found to be 5.14 ± 0.49 minutes.

B. SEMEN PICTURE & ITS VARIATIONS :

Semen ejaculate constitutes of two components-Seminal plasma and spermatozoa. Seminal plasma is a composite fluid secreated by epididymis,seminal vessicles,Prostate and Cowper's glands. Secreation from the seminal vessicles forms nearly 55% of the total volume of bull semen. Spermatozoa constitute about 10% of the total volume of the semen. Spermatozoa are produced in the seminiferous tubules of testis. Production cycle of sperm goes on continuously.

The important contribution of semen is to provide spermatozoa for fertilisation of female gamets-ovum. Seminal plasma with all its favourable biochemical compositions nourishes the sperm cells. The semen picture varies with species,breed,age,season and the nutritional standard etc. of the individual. The semen picture constitute study of the following characteristics :-

COLOUR :

Colour and consistency of ejaculates have got a special significance to indicate the quantity of the semen. Normal colour of semen is from greyish white to yellowish white. Individual bulls have characteristic semen colour. Colour and consistency of an ejaculate have been reported to be dependent upon the concentration of the spermatozoa. The higher the concentration the thicker the ejaculate.

VOLUME :

Volume of the ejaculate is most important characteristic for extensive utilisation in Artificial Insemination. The volume of the semen per ejaculate varies from breed to breed and within a breed from bull to bull. The genetic basis of ejaculate volume does exhist. Younger bulls have lower ejaculate volume and it increases gradually with an increase in the age but beyond certain age the level, is maintained.

HYDROGEN-ION-CONCENTRATION (pH) :

pH of semen depends upon ionic concentration and buffering capacity of various compounds present in the plasma. The pH of semen samples from clinically normal bull is slightly acidic and on storage the pH diclines generally towards more acidic side. Highly motile semen is acidic while semen with poor concentration of spermatozoa or semen having poor motility is generally alkaline.

MOTILITY :

Motility of spermatozoa is an index of their activities. There is no clear evidence that initial motility can be closely correlated with fertility and studies have yielded negative or indefinite results. Moreover, Erb et al.(1950),Stone et al.(1950) and Bishop et al.(1955) found initial motility to be more closely correlated with other semen characters than with fertility. The heritability of initial motility of bull semen was estimated by Zelfel (1964) to be 0.501. A number of variables have been reported

to effect the motility of semen.

PERCENTAGE OF DEAD SPERMS :

Physical and chemical activity of the semen depends upon the proportion of live and dead sperms. The motility and the proportion of live and dead spermatozoa are very important factors for evaluating semen sample. Lasley et al.(1942) first discovered the use of eosin and opal blue for differentiation of live and dead spermatozoa. Semen samples having less than 50% of live spermatozoa were stated to affect fertility. Lasley and Bogart(1943) showed a linear relationship with motility, which was directly related to fertility. Semen with more than 30% initial dead spermatozoa may not be good for preservation. No effect of age has been reported on the percentage of live and dead spermatozoa. Seasons have been found to affect the live percentage.

CONCENTRATION OF SPERMATOZOA :

Accurate determination of spermatozoa per unit volume of semen was an important consideration in determining the optimum dilution ratio for artificial insemination. It was also helpful in determining the fertility of a bull. It had been reported that the number of spermatozoa per unit volume of semen differs in bulls, and the different ejaculates of any bull vary widely in this respect. Seasonal variation in sperm concentration was not found to be significant. The age of the bull did not seem respon-

-sible for an alteration in sperm density of semen(Lepard et al., 1941).

Sciuchetti (1938) investigated the fertility of some bulls of Brown Swiss and Simmental breeds and the number of spermatozoa per 1 mm^3 of semen varied between 5800 and 547000 in the ejaculate and from 387000 to 2330000 in the content of the ampullae. The proportion of abnormal spermatozoa from the ampullae ranged between 19.68% and 43.75% that in the sample taken from the epididymis ranged from 13.07% to 45.33%. He further reported that the morphology of the spermatozoa and the variation of sperm heads were the best indicators of the fertilising properties of the semen; number and motility of spermatozoa, as well as the volume and properties of the ejaculate must also be considered.

Weatherby et al. (1940) studied the ability of dairy bulls to with-stand regular service for artificial insemination during one year. In 362 semen samples, obtained from five dairy bulls by means of the artificial vagina, he found the volumes of ejaculates decreased while concentration and longevity (Av.14 days) remained fairly constant in a bull ejaculated every 3rd day for 11½ months. An animal ejaculated once a day for 57 days showed no marked variation in weekly volume of semen, but concentration varied from 2179 million spermatozoa per cm^3 in the 4th week to none in five ejaculates obtained in the 9th week (Av.for the 57days period, 114 million spermatozoa per cm^3). Sperm longevity increased

up to the 5th week decreasing there after to a very low level. He further reported that after 24-day rest, the same animal was ejaculated once weekly for the next 11 months, during which period concentration averaged 308 million per cm^3 higher and longevity 8 days longer than in the 24-hourly ejaculates. When collections were made twice weekly, one immediately after the other, in two animals average volume and motility of the second collections were greater, while concentration was less than the first collection in one, greater in the other. The correlation between concentration and longevity in 87 semen samples was significant(0.47). The feeding of sprouted oats to the bulls had no significant effect on quantity and/or quality of their semen.

Smith & Asdell (1940) studied the buffering capacity of bull semen of 2 Holstein bulls and found that the semen was well buffered in the region of pH 4.0 to 5.5 and again at pH 9 & 10 and relatively poorly buffered in the pH range of 6 to 9. He observed that the buffering capacity apparently decreased with storage. He found that the curves for neutralisation and buffering capacity of the seminal vesicle fluid were so similar to those for semen, that it was thought that the former fluid contributed much to the buffering capacity of the semen.

Anderson(1941) made further investigations on the

semen of bulls and found that the volume of the ejaculate showed a tendency to be less in abnormal bulls, but there was no hard and fast line between the normal and abnormal bulls. Variation in volume was probably due to individuality, age and management. Bulls of beef breeds had smaller ejaculates than dairy bulls. He could not determine the nature of seasonal influance. The proportion of abnormal spermatozoa was about 10% for normal and 25% for abnormal bulls. He got no evidence that sperm quality was affected by frequency of collection or interval since last collection. Examination of clinically abnormal bulls demonstrated that sperm production was seriously affected a long time before there was any clinical evidence of disease. In an attempt to correlate sperm quality with the grade of the bulls' fertility,it appeared that non of the criteria commonly used was adequate to determine variation in grade of fertility though a type of sperm with certain general features,which could be readily recognised, was associated with fertility. He suggested that when a single ejaculate was classed as good, the bull could probably be regarded as fertile,but one poor ejaculate should by no means condemn a bull and in such case repeated tests at intervals should be made.

Rimoldi & Brigatti (1941) studied the variation in the pH of bull semen in relation to sexual activity and to individual

characteristics. He collected semen from 30 bulls by artificial vagina or by massage and found the average pH of semen collected by massage was higher (7.85) than that of semen collected by artificial vagina (6.89). In both cases the pH tended to increase with successive ejaculations. No relationship could be found between variations in pH and the age or breed of the sire. A correlation existed between number of sperm and pH, i.e., the denser the sperm, the lower the pH, though there was an innate tendency for the semen of individual bulls to either acid or alkaline.

Weatherby et al.(1942) made comparison of first and second semen collections from dairy bulls. He found that average volume was 3.1; 3.4 and 3.7 cm^3 for the 1st Vs. 5.4; 6.4 and 6.9cm^3 for the 2nd; Average concentration was 985, 907 and 1916 million sperms per cm^3 Vs. 990, 1223 and 1527; longevity was 9,7 and 12 days Vs. 10, 11 & 16.

Erb et al. (1942) studied the seasonal variation in semen quality of the dairy bull. Analysis of variance in various characteristics of semen production revealed highly significant differences between bulls and between months for all the factors studied except pH. Average semen volume and initial motility were least in July,August and September. The average concentration of spermatozoa and total sperm per ejaculate were at maxima during

April, May and June. No significant seasonal variations in pH were observed. The quality of the semen studied was, therefore, significantly superior in spring and inferior in summer, while the semen produced in autumn and winter did not vary significantly from mean.

Anderson (1942) studied the clinical significance of the hydrogen-ion-concentration of the semen of the bull. He found the mean pH of 30 ejaculates from 15 bulls with epididymitis to be 7.618 ± 0.07 (range 6.53 to 8.39) and the semen contained either no spermatozoa or a few which were lifeless or showed feeble activity. Only 6% of the ejaculates of affected bulls had a pH lower than 7.00. Six ejaculates from 3 bulls with small testis had a mean pH of 7.73 (range 7.01 to 8.02) and spermatozoa were absent. The semen of three bulls with clinically normal testis and epididymis, but bad sperm producer also gave a nutral or alkaline reaction. In sterile bulls the pH of successive ejaculates become increasingly alkaline.

Blome (1942) evaluated the activity of the bull sperm by means of the microscope and suggested a method of determining sperm motility by means of the undulations of the semen. Three drops of undiluted semen were spread by means of a glass rod, 3 mm. in diameter on a slide heated to body temperature and examined without coverglass at a magnification of 20 to 50 times. The author distinguished 4 degrees of motion :- (1) Intense whirling (+++);

(2) Slow waves (++); (3) No mass movement, movement of individual sperm only (+); (4) No motion (0).

Phillips et al.(1943) reported marked decrease in breeding efficiency in terms of fertile matings in bulls during the summer months. Significant differences were observed between seasons in volume, total sperms, abnormal head, abnormal middle piece,and abnormal neck of the sperm in bull semen.

Lasley (1944) determined the relationship between spermatozoan motility and the percentage of live spermatozoa and fertilising capacity of the bull semen. The percentage of live sperm as determined by the opal-blue eosin staining method was highly correlated with the percentage of motile sperm as determined by the haemocytometer and with the percentage of progressively motile sperm. A few minutes after collection there was an average of 16.9% dead sperm, 19% non-motile live sperm, 12.5% weekly motile and 51.5% progressively motile.

Swanson & Herman (1944) studied the seasonal variation in semen quality of some Missouri Dairy bulls. An analysis was made of the monthly variation in the semen quality of one Guernsey, 9 Holstein Friesian, and 3 Jersey bulls used in the University of Missouri herd. The average age of the bulls was 6 years. Monthly variation in volume,concentration and percentage of total abnormal spermatozoa were not statistically significant. The pH of the semen

was significantly lower in summer than in autumn. Initial motility and useful viability were lower in winter than in spring and summer. No important seasonal effects were observed in the semen of the young bulls, and the results were interpreted as being largely due to the adverse effect of winter weather on the physical well being and sexual activity of the aged bulls.

Anderson (1945) studied the seasonal variations in the reproductive capacity of the bull regarding mating desire (percentage possible ejaculates), and in pH, sperm density, volume and motility of sperm and were compared with the groups of meteorological data for the same period. The combined data for the years and farms were also compared. The differences between bulls and between months were highly significant. In general, mating desire and semen quality was highest during January-February and September-October, when temperature and hours of sun shine were highest and rainfall, humidity lowest. However, there was considerable variation between years, between bulls and between farms. The modification of the basic rhythm by nutritional factors is discussed. Service rates for the same period showed roughly the same trend as those for semen quality specially motility.

Mercier & Salisbury (1946) studied the effects of seasons on the spermatogenic activity and fertility of Holstein

Friesian and Guernsey bulls and found that the differences between bulls in every semen characters studied (Volume, Percentage motile sperm, Motility rate, Concentration, Total sperm per ejaculate, Tailless heads, True abnormal, Days between collections, Methylene blue reduction rate and Fertility) except motility were so great as to indicate that the probability of their being due to chance variation was less than one in 100. Differences between months were significant or highly significant except for motility rating and total number of sperm per ejaculate, but variations in collection frequency, which was not rigorously controlled, may have obscured a possible seasonal effect on sperm total per ejaculate. Differences between first and second ejaculates were significant or highly significant for all characters except the proportion of true abnormals. The bulls differed in their reaction to the same seasonal factors from month to month was shown, for changes in volume, concentration of sperm and proportion of abnormal sperm.

Correlation coefficients between semen characters and fertility for the two breeds, individually and combined were calculated. For the combined breeds concentration of sperms, methylene blue reduction time and proportion of morphologically abnormal sperm were significantly correlated with the fertility level of the same semen samples. The Holstein Friesian showed a positive correlation between the proportion of morphologically

abnormal sperm and relative fertility and the Guernsey a negative correlation. The breed differences were highly significant.

Raps and Cannon (1947) studied the influance of management, breed and season upon the pH of bull semen in Brown Swiss, Guernsey, Holstein Friesian and Short-horn. The effect of differences in management at different studs on the average pH of the semen was significant; differences in pH levels between the first and second ejaculates varied greatly between studs. There was a positive correlation between average semen pH and breeds. There was a tendency for the semen pH of individual bulls to remain constant and there were defenite fluctuations in semen pH which was maximal in February and minimal in May.

Madden et al.(1947) found significant correlation between initial motility and initial percent of live spermatozoa and also between longevity and initial percent of live spermatozoa.

Mukherjee & Bhattacharya (1947) studied the seasonal variations on seminal characteristics of Kumauni bulls and found that the semen quality was superior in spring than that of summer.

Rao & Hart (1948) observed the motility of bovine spermatozoa and the following grades based on the amount of disturbance in the microscopic field, were used to indicate motility intensity of semen samples and are applicable to moderate dilutions

with Egg yolk citrates: Good-when 80% of sperms were alive and the majority in maximal motility; Medium-when 60% are alive and a large proportion in maximal motility; Fair-when 50% are alive and a small proportion in maximal motility; Poor-when 30% are alive but few or non in maximal motility; Dead-when there is no movement in the microscopic field.

Romijin (1948 b) determined the pH of bull semen with a glass electrode to an accuracy of 0.02 pH units. The good quality of semen showed an average pH of 6.65, compared with 7.03 for semen of inferior quality, storage at a temperature of 38°C (with protection from the air) decreased the pH of good semen by 0.49 pH units during the first hour; the corresponding decrease for inferior quality semen was 0.21 pH units. After three hours the decreaseswere 0.65 and 0.28 pH units respectively. He suggested that pH determinations should be used along with other methods of investigations for the evaluation of semen.

Lewis (1948) observed the effect of seasons on spermatogenesis and fertility of dairy bulls under Michigan condition. A comparison was also made between spermatogenesis and fertility of the Holstein and Guernsey bulls. The Holstein bulls surpassed the Guernsey in spermatozoa concentration, total spermatozoa and initial motility. The percentage of non-returns

from the Holstein bulls averaged 6.3% higher than that from the Guernsey bulls. The Guernsey bulls produced a greater average volume of semen. Partial correlation indicated that both light and temperature might be positively correlated with spermatogenesis with light exerting the greater effect. Spermatogenic activity of both breeds, reached a peak during the spring and was depressed during the fall and winter months. Peak fertility in Holstein occured in March and April. Breeding results from both breeds were poorer in winter and summer than during the other seasons.

Shukla & Bhattacharya (1949) studied the semen characteristics of Indian breeds of livestocks regarding appearance, volume, motility, sperm concentration, total number of sperms per ejaculate, pH and percentage of abnormal sperm. The mean volume of Kumauni bull semen was considerably lower than that of the Hariana and Sahiwal bulls viz. 2.00 ± 0.10 cm^3 per ejaculate Vs. 3.16 ± 0.20 cm^3 and 3.80 ± 0.35 cm^3 for Hariana and Sahiwal respectively. They suggested that the differences may be due to differences in body size of the breeds. Sperm concentration was also lower in the Kumauni bulls (in millions), 25- 1950 per cm^3 Vs. 575- 1879 per cm^3 in the Hariana and 420-2020 per cm^3 in the Sahiwal.

Rao (1950) made a study on semen and fertility in the bull. Semen from 5 Guernsey and 7 Holstein Friesian bulls was collected every third day. The average ejaculate volume of the

Holstein was 1.2 ml. greater than that of the Guernseys. The decrease in the pH of undiluted and diluted semen before and after storage was not as great as that in motility. Spermatozoal motility and longevity were greater after semen examination at 100°F than at room temperature. There was no appreciable difference in semen quality when the diluent used was fresh or stored for 10 days at 35°F. Data showed differences in the reproductive efficiency of bulls used for inseminating the same cow in different years. With young bulls 2.05 inseminations were required per conception Vs. 2.61 with mature bulls.

Mies Filho & De Paulo Graca (1950) studied the influance of season on spermatogensis in imported bulls. From four Friesian bulls 229 semen samples were examined; which were maintained on the same feeding and managemental condition and were housed together in an environmental temperatures which fluctuated with the seasons except for 7-17 hours each day, when it was maintained constant at 20°-24°C. Conception rates obtained with the semen varied according to the seasonsas follows : Summer, 62.5%; Autumn,79.3%; Winter,100%; Spring,79.4%. The average volume and average motility of the semen samples were also highest in winter 4.70 cm^3 and 4.0 respectively and lowest in summer(3.54 cm^3 and 3.2 respectively).

Thomson (1950) made some notes on the management of

bulls in relation to semen production and found wide variations in semen production between individual bulls. It was found that the most effective semen production as characterised by quantity, quality and willingness to serve, was best achieved and maintained by feeding bulls on an adequate well balanced diet. The feeding of extra protein to a bull already receiving a balanced diet tended to cause scouring with an eventual drop of 50% in sperm count. It was observed that too much green food although increased semen quantity, total relative sperm count dropped by 20-50%. Variations in the amount of ration were reflected by variations in semen quantity and sexual behaviour, but it was not untill the bulls began to show general loss of condition that there was a rapid falling off in fertility.

In the case of 2 old bulls, reduction between intervals of semen collection from 7 to 5 and from 7 to 4 days respectively had no effect on semen production, but further curtailment of the rest period resulted in refusal to serve. When semen was obtained, however, it was found to be quite unaffected. The bulls similarly reacted to attempts to increase the number of ejaculates at one collection from 2 to 4 at 7 day-intervals.

As a result of the shortening of the collection interval from 7 to 4 days with 2 young bulls the average semen quantity dropped after 2 months from 8 cm^3 to 3 cm^3, followed at the end of

three month by progressive deterioration of quality. There was however, no disinclination for service. Recovery was complete in 6 weeks, during which time collections were made at 10 day intervals. An increase in the number of ejaculates taken at each collection from 2 to 4 resulted in rapid decrease in quantity after the first month quickly followed by a drop in quality. Slow recovery was noted.

Lasley (1951) studied the spermatozoan motility as a measure of semen quality. He presented the result in which haemocytometer method was used for motility percentage of spermatozoa in bull semen. In 78 ejaculates of fresh bull semen, an average of 64.1% of the spermatozoa were motile and 51.6% were progressively motile. The same ejaculates contained only 34.9% motile and 14.2% progressively motile spermatozoa after a storage period of 4 days at a temperature of 10 and 12°C in Egg-yolk-phosphate buffer solution.

A highly significant correlation was found to exist between the percentage of live,motile and progressively motile spermatozoa in fresh semen. All of these characteristics were also significantly correlated with the percentage of live spermatozoa in semen after storage for 4 days in Egg-yolk-phosphate buffer.However, none of these characteristics were correlated with the percentage of motile spermatozoa after the same storage period.

The average percentage of resistant and progressively motile spermatozoa were almost identical when determined in the

same 63 ejaculates although there was considerable variation in each of these characteristics. For each 10% increase in the percentage of progressively motile spermatozoa, there was a corresponding increase of 5.44% in the percentage of resistant spermatozoa. The percentage of motile spermatozoa in 67 ejaculates of fresh semen was significantly correlated with fertilizing capacity, although this correlation was low (r = 0.314). The correlation between the percentage of progressively motile spermatozoa and fertilizing capacity was not statistically significant (r = 0.167).

Branton et al.(1952) studied semen production, fructose content of semen and fertility of dairy bulls as related to sexual excitement. Three levels of sexual excitement namely unrestrained, one false mount, and two false mounts prior to first ejaculate were related to semen production. It was found that all of these criterion of responses except fertility could be influenced markedly by controlled sexual excitement. There appeared an inverse relationship between fluctose concentration and spermatozoan density of semen.

Bhattacharya & Prabhu (1952) found differences in average volume of semen per collection from animal to animal within the breed. The bull differences amongst the Sahiwal & Tharparker were significant.

Mukherjee & Bhattacharya (1952) studied seasonal variations in semen quality and haemoglobin and cell volume contents

of the blood in 6 Kumauni bulls. On the whole, semen and blood quality were significantly highest in spring (February-April) when air temperature was moderate, rainfall scanty and humidity lowest, and significantly lowest in autumn with high air temperature, humidity and rainfall. There was no significant seasonal variations in average semen volume or total number of sperm. Sex vigour as measured by 'reaction time'(interval between release of bull near cow and moment of ejaculate), did not appear to be related to season or to sperm production.

Branton et al.(1953) reported the relationship between spermatozoal concentration in ejaculated semen and fertility, between numbers of motile spermatozoa, and fertility, that semen sample with a spermatozoal concentration as low as 500 million per ml. would give good fertility results. More uniform fertility results among and within bulls could be obtained when the semen was diluted on the basis of constant number of motile spermatozoa.

Johnston & Branton (1953) studied the effect of seasonal climatic changes on certain physiological reaction, semen production and fertility of dairy bull. They observed marked seasonal differences in semen quality, but no corresponding significance in non-return conception rates could be found.

Schmidt (1954) studied the effect of season on the semen production of bulls. Semen characters were examined in 505

ejaculates of 8 bulls. There were positive correlation between both length of day and atmospheric temperature and (a)Semen character (i.e. spermatozoal concentration total number of spermatozoa per ejaculate, and 50% spermatozoal survival) and (b) fertility, and also between each of the semen characters and fertility. There were negative correlations between relative humidity and (a) semen characters and (b) fertility.

Schindler (1954) compared the conception rate with fresh and stored semen from Friesian,Holstein- Friesian, and grade Damascus x Friesian bulls. Conception rate was highest (60-70%) for March to May and reached their lowest value (below 50%) in September. During August-October spermatozoal longevity was greatest, for May-June 40% motility was maintained for more than 25 days, and it was least in September-October. Spermatozoal concentration was 15-30% above the annual average in June and in some bulls, in July and August, and 10-30%, below the average in September and October. There were no significant monthly differences in ejaculate volume.

Kushwaha et al.(1955) made observations on 6 Murrah buffaloes,4-6 years of age which were used for A.I. Between seasons there were significant differences in reaction time (less in Autumn than in Summer and Spring), initial motility of sperm (lower in Summer than in Spring), sperm concentration (lower in Winter than

in Spring and Summer), and total number of spermatozoa(higher in Summer). The differences in semen volume and pH of semen were not significant.

Rossner (1955) found seasonal variation in all charaters except concentration and forward movement of spermatozoa in which no seasonal changes were observed. Other characters were superior in Winter than that of Summer.

Schmidt et al.(1956) studied the effect of sexual rest on various sperm characters and fertility in breeding bulls. They found best result for semen quality (ejaculate volume, sperm density, and total number of sperms per ejaculate) with a rest for 4 days and for quality (fertilising ability with diluted and undiluted semen and servival time) with a rest for 8 days. Fertility, as measured by the number of inseminations necessary for conception, was best after an interval of 7 or more days.

Zuliani(1957) studied the effect of age and sexual regime on ejaculate volume in Bos taurus (Swiss Brown and Friesian or Holstein Friesian). Average ejaculate volume was 4.99 cm^3; it increased from 3.80 cm^3 at 2 years of age to 6.35 cm^3 at 10 years, after which it declined. Individual and breed differences in volume and relationship of volume to age were, however, considerable. Maximum ejaculate volume was reached at an earlier age in Swiss Brown (6 years) and Friesian (8 years) than in Holstein Friesian

(10 years). The influence of frequency of collection on ejaculate volume was variable according to individual and age, but seemed to be least in Holstein Friesians.

Boyd (1957) studied the factors affecting sperm production by dairy bulls. It was observed that when feeding was good, bulls receiving extra handling plus 'good care' were not noticeably superior to non-handled, poorly treated bulls in ability to ejaculate good quality semen over a 6 month period, although they showed considerably greater libido. When semen was collected twice weekly there was an increase in volume percentage of motile sperm, total sperm count, and total motile sperm count from 10.5 to 16.5 months of age and then a slight decrease between 16.5 and 22.5months. Sperm concentration increased through out the year. Semen volume, sperm concentration and total sperm decreased in successive ejaculates when 10 ejaculates were collected within 90 minutes. Average sperm concentration and percentage of motile sperm were restored to predepletion level within 4 days after partial exhaution and within 7 days after severe depletion of sperm. The daily rate of sperm production in the 2 year old Holsteins was 1949 billion.

Hafs et al.(1958) noted significant variation among the bulls but did not observe any variation in initial motility of spermatozoa between breeds.

Hafez & Bonadonna (1958) studied the effect of place of origin on the seasonal variation in semen production in Friesian bull. Semen was collected twice every 5-7 days. They obtained average ejaculate volume 5.5 cm^3 in Carnation, 3.3 cm^3 in Canadian, 4.5 cm^3 in Dutch and 4.7 cm^3 in the German strain. There was a slight increase in ejaculate volume during summer and autumn in all strains except the Carnation in which no seasonal changes in quantity or quality of semen could be observed. They found considerable differences in the extent of seasonal fluctuations. Semen volume was higher, and the motility lower, in the second than in the first ejaculate.

Hafez & Bonadonna (1959) studied strain differences and seasonality of semen production in Friesian bulls. Seasonal variation in semen characters were studied for 10 years. Ejaculate volume was higher in the 'Carnation' than in the 'Tedesco' strain. Some bulls showed greater seasonal fluctuations in semen production than others; individual differences in the effect of age on semen production were also observed.

Brown (1959) found the adverse effects of temperature on semen quality. Variation was observed by bulls, months and years on semen quality. Semen quality varied inversely with the environmental temperature.

Basirov (1960) invested the basic causes of seasonal

variation in quantity and quality of bulls and buffalo bull semen in Azerbaijan. Bulls and Buffalo bulls were kept in light dry barns or outside in the dry, sub-tropical, semidesert condition and were fed either green or concentrate ration. The atmospheric temperatures were -8° to $+2^{\circ}C$, $+10^{\circ}$ to $+28^{\circ}C$ and $+40^{\circ}$ to $+50^{\circ}C$. The degrees of libido and semen characters were not appreciably different in the two lower temperature groups. Libido and ejaculations were inhibited in all buffaloes at $+40^{\circ}$ to $+50^{\circ}C$ and were greatly reduced in the bulls. After bathing libido and semen characters were normal. Libido and semen characters were better in bulls kept on the concentrate ration than in those on grass and the former ejaculated 4 to 5 times in succession and the later only once.

Marandici and Lopozan (1960) studied the dead and abnormal spermatozoa in fresh bull semen and found that there was a close correlation ($r = 0.95$) between percentage motile and percentage unstained spermatozoa. There were 19.2% more motile than unstained spermatozoa.

Okamoto et al. (1962) studied the influence of high environmental temperatures on the semen quality of dairy bulls, and the effect of cooling the scrotum and concluded that scrotal cooling did not maintain spermatogenesis satisfactorily in high environmental temperatures but helped to maintain semen quality.

Hiroe et al.(1965) found the effect of nutrition on the characteristics of bull semen. Holstein bulls of different age group were studied and he found the quality and volume of semen from those bulls were in positive correlation with the live weight gain.

Dmitrieve (1965) studied the change in the semen quality of bulls in different seasons with regard to the volume, survivability,concentration of spermatozoa and the number of ejaculates rejected for poor quality. Significantly more ejaculates were rejected in winter than in summer, the difference was attributed to the difference in nutrition.

Wiltbank et al. (1965) studied the relationship between measures of semen quality and fertility in bulls mated under natural conditions. Pregnancy rate for bulls with good quality semen (93% motility) was 67% Vs. 45% for bulls with poor quality semen (71% motility). Correlations were seen among semen characters and fertility. The later was most highly correlated with motility(0.40), percentage of live normal spermatozoa (0.39) and percentage abnormal spermatozoa (-0.35).

Barbulescu et al.(1965) studied the effect of breed age and frequency of use on some characters of bull semen. Ejaculate volume was affected by breed,age affected all the semen characters

and the effect of sperm concentration and viability were less than the other characters. Intensity of use did not influence semen characters.

Miasnicov (1966) evaluated the fertilising capacity of bull semen and no relation was found between ejaculate volume and conception rate. The percentage of motile spermatozoa after storage was related to conception rate.

Rosenhalm & Mackle (1967) found no effect of season on semen quality.

Dessouky & Juma (1968) studied seasonal variation in semen characteristics of Friesian bulls in Iraque with regard to the volume, density, pH and mass and individual sperm motility. With the exception of mass sperm motility of European Friesian semen none of the characters was significantly affected by the age of bull or by season. All the semen characters under study were closely correlated with each other.

Nadaraja (1968) studied the semen characteristics of Jersey, Ayrshire and Friesian bulls. Motility was 4.1 $\pm$ 0.4, 3.2 $\pm$ 0.48 and 3.8 $\pm$ 0.54 and sperm concentration ($x10^6$) was 1346 $\pm$ 458, 872.9 $\pm$ 254 and 995 $\pm$ 65.4 per ml. In Jersey & Ayrshire, the two characters did not differ significantly. Age of the bull did not affect semen characters significantly but motility was highly

significantly correlated (0.60) with 50 to 90 days N.R.(None-return) rate of first insemination.

Nishiyama et al.(1968) studied the seminal characters of one Holstein Friesian bull from 6 to 9 years of age regarding pH,motility,ejaculate volume,methylene blue reduction time,sperm concentration and percentage of abnormal sperms. Year significantly affected all the characters except motility and season affected all except motility and methylene reduction time. The year and season interaction was significant in respect of all characters except ejaculate volume.

Tomar & Kanajia (1970) found that initial motility, sperm density,percentage of abnormal spermatozoa and methylene blue reduction time had significant variation between seasons.

Ansari (1970) studied the semen characteristics in Tharparker bulls and found significant differences amongst bulls in ejaculate volume, initial motility, sperm concentration, percentage of dead and abnormal spermatozoa except pH. He also found no significant seasonal variations in respect of volume, initial motility, percentage of dead and abnormal spermatozoa except pH and sperm concentration.

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CHAPTER III

MATERIALS AND METHODS

MATERIALS AND METHODS

The present study was undertaken on bulls recently brought from the stock of Holstein Friesian Association of America, Brattleboro, Vermount. These bulls were brought to Patna from the Aarey Milk Colony, Bombay on 22.12.70, as these bulls were earlier kept at Bombay. These bulls were maintained at the New Semen Bank, Patna. All the ten bulls brought were included in this study.

The age of these bulls was noted from their registration cards supplied by the Holstein Friesian Association of America to the Bihar Government through the Ministry of Food and Agriculture, Government of India. Their age varied from 14 months to 17 months. These bulls were maintained under the same environment and identical conditions of farm management. They were housed in an asbestos roof byre which had pucca floor and walls. Each animal was placed in a separate stall. The house had a bilateral opening having sufficient ventilation for air and light.

Bulls were put to exercise individually in the morning. They were given cold water-bath in the early hours of the day. During bath, they were brushed and groomed. Preputial hairs were trimmed shorter in order to get clean ejaculate.

HEALTH STATUS : Bulls were vaccinated against H.S.; B.Q.; Anthrax and F.M.D. as per schedule from time to time. As a health check

measure, daily temperature was recorded and maintained for all the bulls. No collection was taken from the bulls showing any symptoms of illness.

: These bulls were previously not trained for Artificial Insemination work; during the period of study, they were trained to ejaculate in artificial vagina.

FEEDING SCHEDULE :- The feeding schedule of the bulls were as follows :-

Bhusa	5 kg.	per bull per day.
Greens	5 kg.	-do-
Wheat bran	1 kg.	-do-
Ground-nut cake	1 kg.	-do-
Maize, Masoor or Gram	1 kg.	-do-
Common salt	2 oz.	-do-
Mineral mixture	1%	of total ration.
Vitablend A D_3 (Glaxo)	1 gm.	per bull per day.

The quality of Bhusa remained reasonably constant throughout the experimental period, but the green fodders were changed according to their availability. Generally, the greens consisted of Jwar, Paragrass, Napier and were made available by the Government Cattle Farm, Patna. Bulls were allowed to drink clean water ad lib.

The experiment continued for six months i.e.

from July to December,1971. This included two seasons,viz. Rainy and Winter. Rainy season was considered to last from July to September while the Winter season ranged from October to December(Tomer et al. 1966).

METEOROLOGICAL DATAS : The meteorological datas were obtained from Agricultural Research Institute,Mithapur,Patna and Central Potato Research Institute,Patna, which are located within a radius of two miles from the place of the study.

TABLE - 3:1

Showing temperature,rainfall and humidity.

Month	Temperature(C)		Total rainfall (in mm.)	Humidity (in percent)
	Max.Av.	Min.Av.		
July'71	31.9	24.6	385.9	79
August'71	30.2	24.5	503.7	78
September'71	31.9	24.4	108.0	77
October'71	29.3	22.1	224.7	73
November'71	27.8	14.4	-	71
December'71	24.4	9.8	-	77

PROCEDURE OF EXPERIMENT : For semen collection one heifer of the same breed (Friesian) was used as dummy. Semen samples were collected

in the artificial vagina. Each bull was allowed one or at times two false mounts before collecting the ejaculate as suggested by Branton et al.(1952). The site and personnel taking collections were kept the same for all bulls during the period of study. Collections were taken in the morning between 8 to 10.30 A.M.,under all possible sterile conditions and the temperature of artificial vagina was maintained at 50° to 55°C. The bulls were tried at various temperature from 45°to 55°C and the particular temperature range for the particular bull was noted.

Breeding behaviour of bulls was studied from the time of approaching the female till the time of ejaculation and dismount.

Semen samples soon after collection were diluted in Egg Yolk Citrate diluter (Salisbury et al.,1948) in the ratio of 1:1. The composition of Egg Yolk Citrate diluter was as follows :-

Crystaline sodium citrate	2.94 gms.
Glass distilled water	100 c.c.

Two parts of the above was mixed with one part of Egg yolk.

To each 100 c.c. of the diluter, thus prepared (a) one lakh unit of pencillin G Sodium and (b) 100 mgs. of Streptomycin Sulphate were added.

Care was taken to bring the temperature of diluter to that of the semen, while diluting the semen. One ml. of diluted

semen from each bull in seperate sterlised test tube was brought to the laboratory in a thermosflask filled up with ice up to 3/4 capacity. The samples brought to the laboratory were kept in a beaker containing water at approximately 32°C for 20 to 30 minutes and were subjected to different tests in the following sequence :-

1. Usually after an hour of collection the motility percentage was determined.
2. After about 2 to 2½ hours of collection, the percentage of dead sperms was estimated.
3. After about three hours of collection the Hydrogen-ion-concentration was noted.
4. After about four hours of collection, the concentration of sperm was obtained.

Altogether after collection it took 5 to 6 hours per day for completing the work on semen picture. All the methods were first standardized and pilot trials made before the start of the experimental tests.

The following three attributes (traits) of the Breeding behaviour and six traits relating to Semen picture were studied :-

1. Reaction time.
2. Reaction towards female.
3. Sex Drive.
4. Colour.

5. Volume.

6. Hydrogen-ion-concentration (pH).

7. Motility percentage.

8. Percentage of dead sperms.

9. Concentration of sperms per ml. of semen.

1. REACTION TIME : Reaction time was noted by means of a stop-watch. The time taken by the bull from his release from the place he was tied till he went to the cow, ejaculated and dismounted was considered the reaction time. The time thus taken was calculated in seconds (Fraser, 1960; Tomer et al.,1966).

2. REACTION TOWARDS FEMALE : In order to study reaction towards females, bulls were tried and grouped under (a) Active (b) Dull and (c) Shy. Bulls that went in full vigour to the cow straight without diverting their attention and showed their full sex interest were placed in 'Active Group'. Bulls that did not go to the cow at first attempt but showed their sex interest were placed in'Dull Group' and bulls showing no sex interest were placed in 'Shy Group'(Mishra et al.,1971).

3. SEX DRIVE : In order to study the sex drive of these bulls, all of them were allowed to mount the heifer and intensity of their libido was noticed. For this purpose each bull was given trials at the same place and at the same time by the same attendants in order to avoid any psychological effect. Intensity of sex drive was classified

as follows :-

Bulls that mounted with full erection of penis and gave full ejaculate were placed in 'Grade A'; those mounting with full erection with no discharge at first attempt were placed in 'Grade B'; those showing sex interest without mounting were placed in 'Grade C' and the bulls having the tendency to run away all the times were placed in 'Grade D' (Mishra et al.,1971).

4. COLOUR : The colour was noted by visual appearance soon after the collection by keeping the collection vial against a white paper and was graded as per procedure laid down by Mahmoud (1952) and was categorised with slight modifications.

A. Creamy white; B. Milky white and C. Thin watery.

5. VOLUME : Volume was noted directly from the graduated collecting conical test tubes just after collection. The volume of an ejaculate was measured up to one-tenth of a milliliter(Kushwaha et al.,1955; Kodagali, 1967; Dessouky & Juma, 1968).

6. HYDROGEN-ION-CONCENTRATION (pH) : pH was measured after about 2 to 3 hours of collection in laboratory with the help of Lovi-Bond Disc comparater using Bromothymol Blue as indicator. In two test tubes previously rinsed with glass distilled water,0.1 ml. of diluted semen was taken. Then 14.9 ml. of glass distilled water was added to each of the test tube; 0.5 ml. of Bromothymol Blue

was added to one of the test tubes and thoroughly mixed. The test tube containing Bromothymol Blue indicator was placed in the right hand hole of the comparator and the other test tube was placed in the left hand side hole. Then the disc was rotated to match the colour. The reading was recorded by colour identification after matching the colour in the disc comparator. The disc comparator ranged from 6.0 to 7.6 pH.

7. MOTILITY PERCENTAGE : The spermatozoan motility was determined by Haemocytometer method (Lasley,1951) which was as follows :-

To maintain the motility of the spermatozoa during motility estimation, the same buffer solution was prepared as was used in the diluent. The composition of buffer solution was :-

Crystalline Sodium Citrate Di-hydrate	2.94 gms.
Glass distilled water	100 ml.

Procedure : The test tubes containing semen samples were taken out of thermosflask and kept in a beaker containing water at room temperature for 20 to 30 minutes to bring the semen at room temperature. Buffer solution was also given the same treatment. The R.B.C. diluting pipette was rinsed with the buffer solution and was thoroughly dried. The semen sample was mixed thoroughly and was sucked up to 0.5 mark of the R.B.C. diluting pipette. The tip of the pipette was then wiped out with a piece of sterlised

filter paper to remove the excess of the sperm adhering to the tip of the pipette as well as to take out the excess semen fluid in the R.B.C. pipette. The semen was then diluted 200 times by sucking buffer solution up to 101 mark of the R.B.C. pipette. The content of the pipette was thoroughly mixed by rotation. A few drops of mixed fluid were discarded and then a drop was placed on the previously focussed counting chamber of Heamocytometer under high power (40 x) objective. A clean coverslip was then placed on the chamber and the number of non-motile and weakly motile spermatozoa in all the 25 big squares were counted and recorded. Care was taken to avoid air bubble being sucked between the chamber and the coverslip. All the spermatozoa that showed movement but not in straight line (a characteristic for progressively motile spermatozoa) were recorded as non-motile. Those having slow movement in smaller circle or head fixed with tail moving, or tail fixed with head moving were recorded as non-motile. Weakly motile or non-motile were grouped in the category of'non-motile' because only two categories were recorded- either motile or non-motile.

The counting chamber was then placed in a petridish. The petridish was then transferred to the freezing chamber of the referigerator for about an hour. After the diluted suspension of the spermatozoa had frozen, the counting chamber was taken out of

the referigerator and then placed in direct sun light for a few minutes to allow the frozen suspension of semen to melt under the coverslip. Then the counting chamber was placed under the fan for rapid drying of the water droplets ever the coverslip. Again, the total number of spermatozoa in the same 25 big squares was counted and recorded. This number included the number of non-motile(weakly motile and non-motile) and motile spermatozoa.

The percentage of motile spermatozoa was calculated as follows :-

$$\frac{\text{No.of non-motile sperms before freezing x 100}}{\text{Total no. of sperms after freezing}} = \text{Percentage of non-motile spermatozoa.}$$

The percentage of motile spermatozoa was calculated by subtracting the percentage of non-motile spermatozoa from 100.

8. PERCENTAGE OF DEAD SPERMATOZOA : The estimation of percentage of dead spermatozoa was done with compound stain as advocated by Swanson et al. (1951). It was prepared as follows :-

Eosin (water soluble)	1 gm.
Nigrosin (B.D.H.)	5 gms.
Sodium citrate di-hydrate	3 gms.
Glass distilled water	100 c.c.

This solution was kept on a water-bath for 30 minutes, cooled, filtered and then kept in a referigerator.

To a large drop of this stain, a small drop of thoroughly mixed semen was added on a glass slide so that the ratio between the semen and stain was 1:2 (Swanson et al.,1951). These two were thoroughly mixed by gentle blow of air through a pipette. Within a minute, thin smears were drawn on two clean grease-free slides. The smears were dried in air and examined under oil immersion objective. The dead spermatozoa were seen red due to eosin stain and the background was voilet. Those taking partial stain anteriorly or posteriorly were considered dead, as majority of workers are of the view that partially stained spermatozoa were on the way to death (Perry,1960; Maule,1962). Assesment of the dead spermatozoa was made by counting two hundred spermatozoa in the field taking diagonally across the slide (Asdell,1955).

9. CONCENTRATION OF SPERMS PER Ml. OF SEMEN : Sperm concentration was determined by Haemocytometer. The R.B.C. diluting pipette and Neubauer counting chamber were used for the same. The diluting fluid for evaluation of sperm concentration was used as advocated by George (1952). It was prepared as follows :-

Sodium chloride	3 gms.
Glass distilled water	100 c.c.

To the above solution a few crystals of eosin (water soluble) was added. This solution was prepared fresh and used for a week only.

The test tube containing semen sample was rotated between the two palms with a quick motion for thorough mixing. Then the semen was sucked up to 0.5 mark of the R.B.C. pipette. The tip of the pipette was wiped off carefully with a piece of sterlised filter paper and then the above solution (Physiological saline solution with eosin) was sucked up to 101 mark in the pipette. The diluted semen was thoroughly mixed by rotating the pipette.

The previously cleaned, dried counting chamber was focussed under high power objective (40 X). A clean coverslip was placed on the chamber . Then,after discarding a few drops of diluted semen from the R.B.C. pipette, the counting chamber was charged by touching the side end of the coverslip with the tip of the pipette. Care was taken against accumulation of excess diluted seminal fluid in the chamber. Overflowing was also avoided. The charged Haemocytometer was left for 3 to 4 minutes to allow the spermatozoa to settle down. The field was then examined under high power (40 X) objective of the microscope. The number of spermatozoa in five big squares,i.e. the four corners and one in the middle was counted . The total number of the sperms was then multiplied by the dilution rate.

The total number of sperms in one ml. was then calculated as follows :-

The volume of counting chamber was 1 mm. x 1 mm. x 0.1 mm.=0.1Cu.mm.
Let the number of sperms present in 5 big squares be 'n'.

∴ n x 5 was the number of sperms present in = 0.1Cu.mm.of diluted semen.

∴ n x 5 x 10 " " " = 1Cu.mm.of diluted semen.

∴ n x 5 x 10 x 200 " " = 1 Cu.mm.of semen sample.
(n x 10000000 or n x 10^6).

Because the dilution of the neat semen was 1 : 1 the number of sperms so obtained was multiplied by dilution rate, i.e. 2 . Thus the number of sperms per ml. of semen was calculated.

DESIGN OF EXPERIMENT :

A. Sexual behaviour :- All the ten available bulls were used on different dates decided randomly, for the study of the breeding behaviour. Schedule of trials as well as collections were so drawn up that each bull was allowed two trials in a week and the gap between the consecutive trials were two to three days.

For each bull the number of observations were as follows :-

Number of observations per bull per week ... 2

Number of observations per bull per month ... 2 x 4 = 8

Number of observations per bull per season ... 2 x 4 x 3 = 24 per trait.

Due to certain unavoidable circumstances the actual number of observations was less than originally planned.

Due to non-servicing of two bulls-Bull nos. 50 & 59 had to be dropped from the orbit of the experiment. The number of observation bull wise actually obtained in the two seasons was as follows :-

TABLE 3:2

Showing actual number of observations.

Bull No. (Tattoo No.)	Number of observations	
	Rainy season	Winter season
42	15	15
43	15	15
44	16	15
46	14	15
49	14	15
54	15	15
57	7	15
98	16	15
Total -	112	120 = 232

B.Semen Picture :- The plan of study was similar to that in the case of Breeding behaviour. A few bulls failed to ejaculate even being physically fit. Some samples showed no motility and as such they were discarded and not considered for study. The collections obtained from each bull in each season were as follows:-

TABLE 3:3

Showing actual number of collections on which seminal picture was considered.

Bull No. (Tattoo No.)	Number of observations	
	Rainy season	Winter season
42	9	13
43	12	14
44	14	13
46	13	13
49	13	15
54	13	15
57	7	14
98	16	15
Total -	97	112 = 209

The number of observations on each bull was not equal as a few bulls failed to ejaculate even after several mounts. A few of them gave watery or dirty ejaculate having no motility. Less number of observations was due to the fact that almost all the bulls were suffering from Piroplasmosis (in July) and F.M.D.(in September). Collections and trails for observations on breeding behaviour and semen picture were not taken till the 28th October,1971.

PROCEDURE OF STATISTICAL ANALYSIS :

Observations in percentages,i.e. for dead spermatozoa

and motility were transformed by Sin $^{-1}$(p) transformation before analysis by using Bliss table (Snedecor,1967). For statistical analysis 0.1 was put in place of Zero-value. The mean values for volume,Reaction time, pH were determined from non-transformed data. The data on concentration of spermatozoa were subjected to logarithmic transformation for statistical analysis (Castle, 1969). After the analysis and interpretation the observations were retransformed to their respective original values.

Critical difference test was resorted to in case of significant variance ratio value, to know the significant difference between any two particular treatments. They are as follows :-

C.D. = $t \times \sqrt{S\left(\frac{1}{r_1} + \frac{1}{r_2}\right)}$ where, C.D. = Critical difference.

t = t-value at 5% and 1% level for error d.f.

S = mean S.S. of error.

r_1 & r_2 = Number of replicates.

Other statistical values like Mean,S.E.,C.V. % were calculated as per Snedecor (1967).

STATISTICAL TESTS : Effects of different variables on different attributes of semen picture were tested by the application of Analysis of Variance. Chi-square test was applied in traits- Sex drive, Reaction towards female and colour of semen (Snedecor,1967).

Correlation study was undertaken in respect of each of the traits-Volume,pH,Individual motility percentage and concentra-

-tion of sperms with reaction

was used :-

$$r = \frac{\text{Covariance b}}{\sqrt{(\text{Variance o}}}$$

Where r = Correlation

i.e. X and Y.

Tables, photogra

in the present study for dra

* *

S

-tion of sperms with reaction time. For this the following formula was used :-

$$r = \frac{\text{Covariance between X \& Y}}{\sqrt{(\text{Variance of X})\,(\text{Variance of Y})}}$$

Where r = Correlation coefficient between two traits i.e. X and Y.

Tables, photographs and graphs have been incorporated in the present study for drawing out valid inferences and comparisons.

* * *

CHAPTER IV

RESULTS

RESULTS

REACTION TIME :-

The mean reaction time based on 209 observations on 8 Holstein Friesian bulls was estimated to be 125.11 ± 4.74 seconds with 54.81% of co-efficient of variation (Table 4:1). From above noted table it was evident that the maximum reaction time was demonstrated by bull no. 44 (160.18 seconds) whereas the minimum was shown by bull no.49 (96.25 seconds). The difference in reaction time among the different bulls turned out to be statistically significant (Table 4:2). The results have also been represented graphically (Graph No.1).

The differences between the average reaction time of bull no.42 and 44; 43 and 46; 43 and 49; 44 and 46; 44 and 49; 44 and 54; 46 and 57; 46 and 98; 49 and 57; 49 and 98; 54 and 57, and 54 and 98 were found to be significants; whereas the other differences among other bulls were found to be non-significant(Table

In the rainy and winter seasons, the mean reaction time was 166.52 ± 6.72 and 89.24 ± 4.45 seconds with co-efficient of variation 39.76% and 52.83% respectively (Table 4:4 & Graph No.3) Seasons were also found to significantly affect the reaction time of bulls (Table 4:5).

REACTION TOWARDS FEMALE :-

Reaction of the bulls towards female was studied in

eight Holstein Friesian bulls and were categorised into three classes as described in Chapter III. Out of 232 trials conducted on eight bulls they were found to be active in 93.1% and dull in 6.9% of trials. None of the bulls showed shy character at any of these trials. Paucity of data did not allow the testing of differences between bulls as regards this trait.

Chi-square test revealed that seasons did not seem to affect the reaction towards females (Table No. 4:6).

SEX DRIVE :-

The total number of observations recorded on eight bulls during the study were 232. Sex drive was differentiated into four catergories - A,B,C and D as described in the last chapter: 72.4%; 19.4%; 6.9% and 1.3% of the observation were found to be in A,B,C & D groups respectively. Due to less number of observations in some Cells, Chi-square test could not be undertaken.

Sex drive seemed to be influanced by seasons in Holstein Friesian bulls as evidenced by Chi-square test (Table 4:7).

COLOUR :-

Semen from different collections of eight Holstein Friesian bulls were categorised in three classes according to colour and consistancy viz- Creamy white, Milky white and Watery thin.

The colour of semen was found to have no association with season as the Chi-square value was not significant (Table 4:8).

VOLUME :-

The overall mean volume of ejaculate in Holstein Friesian bulls was found to be 2.74 ± 0.07 ml. with a co-efficient of variation of 37.22%. The maximum average volume of semen was provided by bull no.98 (3.31 ± 0.16 ml.) whereas the bull no.43 donated the poorest volume of semen ejaculate (2.36 ± 0.20 ml.), (Ref. Table 4:9 & Graph No.1). Analysis of variance revealed that bulls significantly influanced the volume of ejaculate (Table 4:10). None of the pair combinations showed significant difference in volume of seminal ejaculate except the following pairs of bulls - Bull nos. 42 & 44; 42 & 98; 43 & 44; 43 & 98; 44 & 49; 44 & 54; 44 & 57; 49 & 98; 54 & 98 and 57 and 98 (Table 4:11).

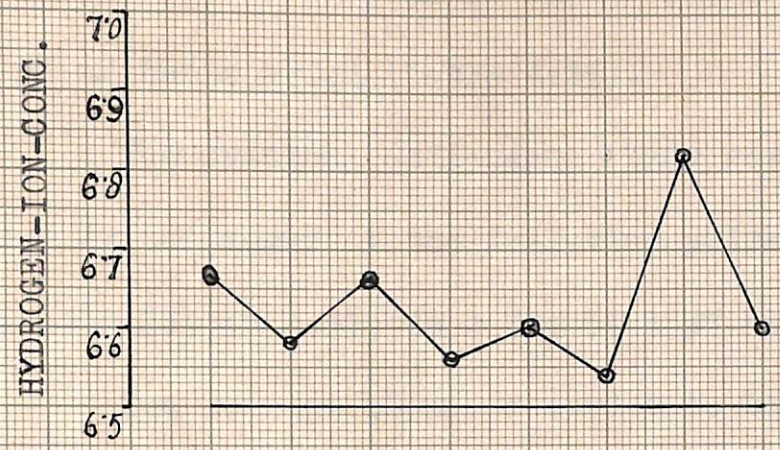
The mean semen volume in rainy and winter seasons was found to be 2.55 ± 0.10 and 2.90 ± 0.028 ml.with 40.78% and 33.10% of co-efficient of variation respectively (Table 4:12 & Graph No.3). Seasonal variations in volume of semen ejaculated by bulls turned out to be significant (Table 4:13).

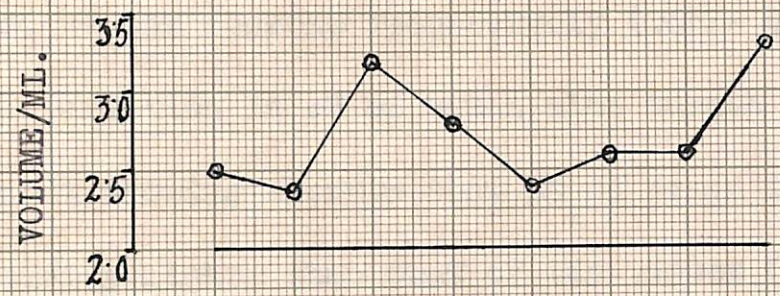
HYDROGEN-ION-CONCENTRATION (pH) :-

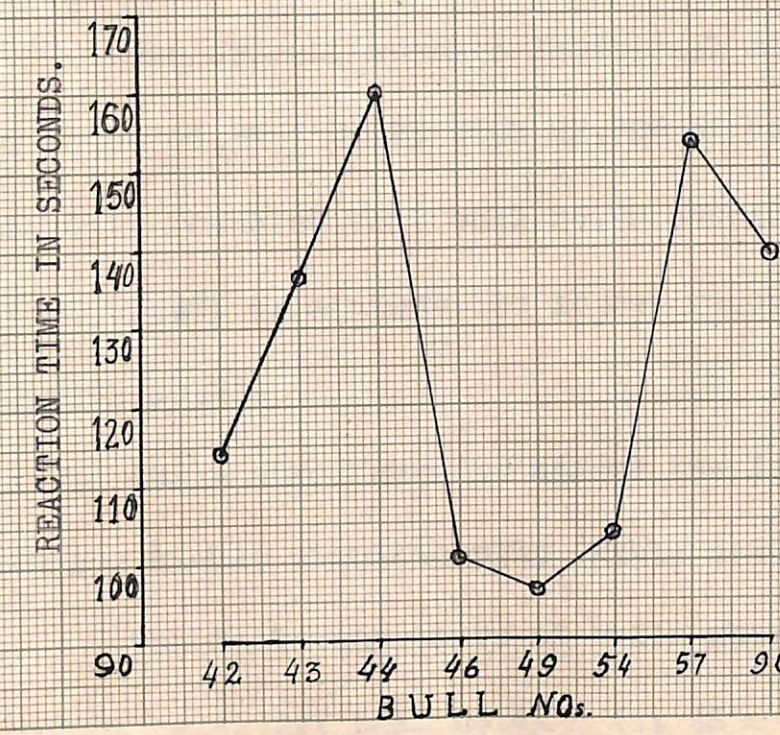
The overall average pH of semen was obtained as 6.62 ± 0.017 with a co-efficient of variation of 3.62%. The bull-wise mean pH alongwith their S.E. and C.V.% revealed that bull no.57 showed the maximum pH whereas the minimum average pH of semen was recorded

GRAPH No.1

Showing average reaction time, ejaculate volume and hydrogen-ion-concentration of different bulls.







in case of bull no.54, the range being 6.54- 6.82 (Table 4:14 and Graph no.1). Bulls were found to differ significantly as regards the average pH of their semen (Table 4:15).

The critical difference study revealed significant pH difference in the semen of the following pairs of bulls- 42 & 57; 43 & 57; 44 & 57; 46 & 57; 49 & 57; 54 & 57 and 57 and 98(Table 4:16).

The average pH was recorded to be 6.66 ± 0.025 and 6.58 ± 0.022 with co-efficient of variation 3.75 and 3.64% in the rainy and winter seasons respectively (Table 4:17 & Graph No.3). Seasonal variation in pH of semen also turned out to be statistically significant showing thereby that pH of semen in rainy season was significantly higher than that of the winter (Table 4:18).

INDIVIDUAL MOTILITY PERCENTAGE :-

The overall average Individual motility percentage was recorded to be 66.38 ± 0.025 with 22.78% of co-efficient of variation. Table no. 4:19 revealed that bull no.98 showed the highest individual motility of 79.47% as against the lowest recorded for bull no.57, 46.86% (Graph No.2).

Bulls were found to affect the individual motility of semen samples highly significantly (Table 4:20).

The following pairs of bulls showed significant difference in individual motility: bull no.42 & 44; 42 & 46; 42 & 49; 42 & 54;

42 & 98; 43 & 46; 43 & 49; 43 & 54; 43 & 57; 43 & 98; 44 & 54; 44 & 57; 44 & 98; 46 & 57; 49 & 57; 49 & 98; 54 & 57 and 57 and 98 (Table 4:21), whereas all the rest combinations turned out to be non-significant.

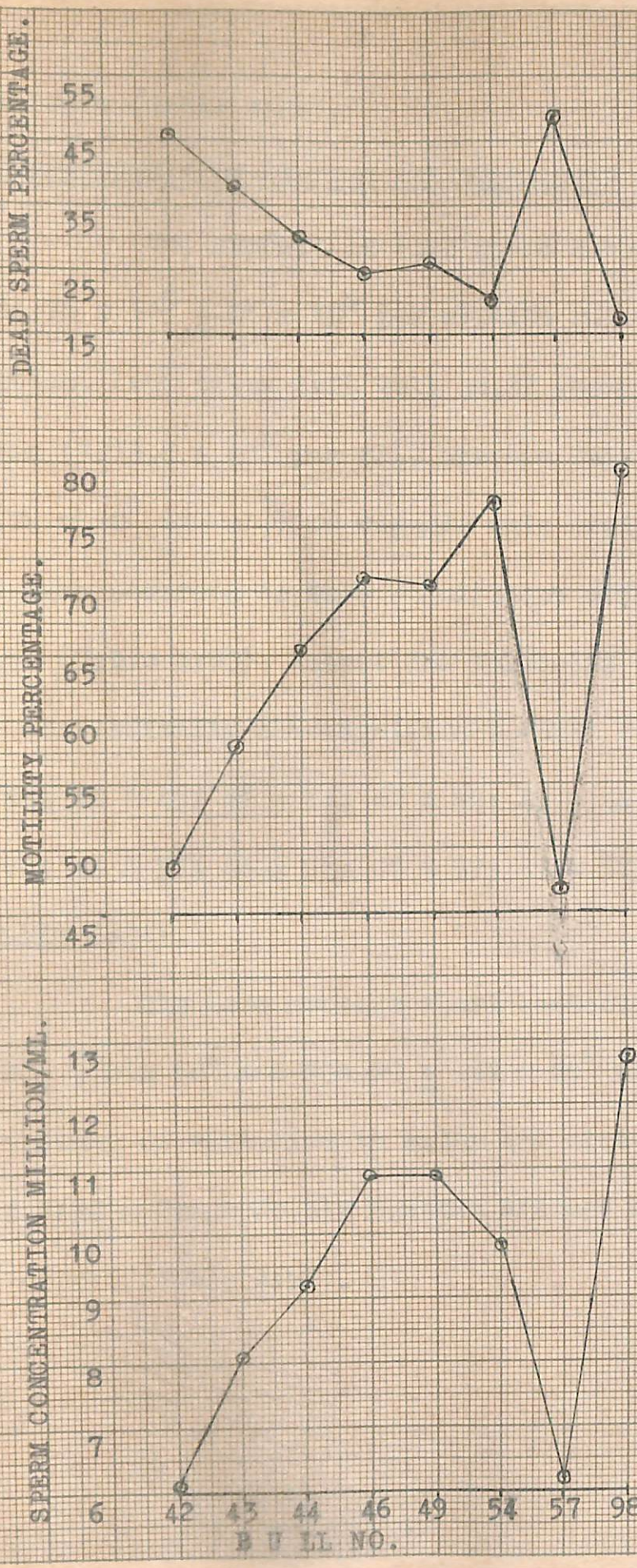
Average individual motility percent in rainy and winter seasons were 67.16 ± 0.047 and 65.68 ± 0.042 with 22.47% and 23.10% of co-efficient of variation respectively (Table 4:22 & Graph No.3). Non-significant effect of seasons on the trait in question was recorded (Table 4:23).

PERCENTAGE OF DEAD SPERMATOZOA :-

The overall average percentage of dead spermatozoa in the semen samples was estimated to be 29.68 ± 0.023% with 38.56% of co-efficient of variation (Table 4:24 & Graph No.2). Between bulls variation for this trait was highly significant (Table 4:25).

Critical difference test showed significant difference in bull nos. 42 & 44; 42 & 46; 42 & 49; 42 & 54; 42 & 98; 43 & 46; 43 & 49; 43 & 54; 43 & 98; 44 & 54; 44 & 57; 44 & 98; 46 & 57; 49 & 57; 54 & 57 and 57 and 98; in other pair combinations the differences were statistically non-significant (Table 4:26).

In rainy season, the mean percent of dead spermatozoa was 29.47 ± 0.051, whereas in the winter seasons it was 29:86 ± 0.043 with 39.20% and 38.15% of co-efficient of variation (Table 4:27 & Graph No.3). Seasons had shown no effect on the dead percentage of



GRAPH No. 2

Showing average sperm concentration (million/ml.), motility percentage and percentage of dead spermatozoa bull-wise.

spermatozoa (Table 4:28).

SPERM CONCENTRATION MILLION PER MILLILITER OF SEMEN :-

The overall average sperm concentration was calculated as 924.70 x 10^6 ±.01 x 10^6 per ml.with 2.21% of co-efficient of variation. From table 4:29 bull no.98 showed highest sperm concentration (1273.5 x 10^6 per ml.) whereas bull no.42 was the poorest (613.76 x 10^6 per ml.) as regards this trait (Table 4:29 & Graph No.2). Bulls were observed to have significant effect on the sperm concentration (Table 4:30). Except the following pairs rest were found to be statistically non-significant -Bull Nos. 42 & 43; 42 & 44; 42 & 46; 42 & 49; 42 & 54; 42 & 98; 43 & 46; 43 & 49; 43 & 57; 43 & 98; 44 & 57; 44 & 98; 46 & 57; 49 & 57; 54 & 98 and 57 and 98 (Table 4:31).

The mean average sperm concentration was recorded as 889.20 ± .01x 10^6 and 954.99 ± .01 x 10^6 with 2.49% and 1.94% co-efficient of variation in rainy and winter seasons respectively (Table 4:32 & Graph No.3). Seasons did not seem to influance the trait at all (Table 4:33).

TABLE 4:1

Showing average reaction time of different Holstein Friesian Bulls with S.E.& C.V. %.

Bull No.	No.of obs.	Mean (in Second)	± S.E. (in Second)	C.V. %
42	22	114.31	11.40	46.80
43	26	136.73	12.97	48.34
44	27	160.18	20.86	67.67
46	26	100.38	9.49	48.21
49	28	96.25	10.68	58.70
54	28	103.57	10.19	52.04
57	21	153.38	12.69	37.87
98	31	139.58	10.22	40.76
Overall	209	125.11	4.74	54.81

TABLE 4:2

Analysis of variance showing the effect of bulls on reaction time.

Sources of variation	d.f.	S.S.	M.S.	Value of F.
Between bulls	7	114771.71	16395.95	3.81 **
Within bulls	201	863620.76	4296.62	
Total	208	978392.47		

** Denotes significant at 1% level.

TABLE 4:3

Showing critical differences amongst different bulls in respect of reaction time.

Sl. No.	Bull Nos.		Differences in average	Critical Differences At 1% level	Critical Differences At 5% level
1	42	43	22.42 N.S.	-	37.2008
2	42	44	45.87 *	48.5556	36.8872
3	42	46	13.93 N.S.	-	37.2008
4	42	49	18.06 N.S.	-	36.5932
5	42	54	10.74 N.S.	-	36.5932
6	42	57	39.07 N.S.	-	39.1804
7	42	98	25.27 N.S.	-	35.8092
8	43	44	23.45 N.S.	-	35.2996
9	43	46	36.35 *	46.8786	35.6132
10	43	49	40.48 *	46.0530	34.9860
11	43	54	33.16 N.S.	-	34.9860
12	43	57	16.65 N.S.	-	37.6908
13	43	98	2.85 N.S.	-	34.1628
14	44	46	59.80 **	46.4658	35.2996
15	44	49	63.93 **	45.6144	34.6528
16	44	54	56.61 **	45.6144	34.6528
17	44	57	6.80 N.S.	-	37.3772
18	44	98	20.60 N.S.	-	33.8100
19	46	49	4.13 N.S.	-	34.9860
20	46	54	3.19 N.S.	-	34.9860
21	46	57	53.00 **	49.6134	37.6908
22	46	98	39.20 *	44.9694	34.1628
23	49	54	7.32 N.S.	-	34.3196
24	49	57	57.13 **	48.8136	37.0832
25	49	98	43.33 *	44.0664	33.4768
26	54	57	49.81 **	48.8136	37.0832
27	54	98	36.01 *	44.0664	33.4768
28	57	98	13.80 N.S.	-	36.2992

N.S. Denotes non-significance ; ** Denotes significance at 1% level;

* Denotes significance at 5% level.

TABLE 4:4

Showing season-wise average reaction time.

Seasons	No.of obs.	Mean (seconds)	± S.E. (seconds)	C.V.%
Rainy	97	166.52	6.72	39.76
Winter	112	89.24	4.45	52.83
Overall	209	125.11	4.74	54.81

TABLE 4:5

Analysis of variance to see the effect of seasons on reaction time.

Sources of variation	d.f.	S.S.	M.S.	Value of F
Between seasons	1	310477.99	310477.99	96.19 **
Within seasons	207	668114.69	3227.60	
Total	208	978392.68		

N.B. ** Denotes significance at 1% level.

TABLE 4:6

Showing seasonal effect on reaction towards female.

Seasons	Active	Dull	Total	Chi-square value
Rainy	102	10	112	1.39 N.S.
Winter	114	6	120	
Total	216	16	232	

N.S. Denotes non-significance.

TABLE 4:7

Showing effect of season on Sex drive.

Seasons	A	B	C & D	Total	Chi-square value
Rainy	68	31	13	112	14.83 **
Winter	100	14	6	120	
Total	168	45	19	232	

** Denotes significance at 1% level.

TABLE 4:8

Showing seasonal effect on colour of ejaculate.

Seasons	Creamy white	Milky white	Watery thin	Total	Chi-square value.
Rainy	57	23	20	97	4.09 N.S.
Winter	71	30	11	112	
Total	125	53	31	209	

N.S. Denotes non-significance.

TABLE 4:9

Showing bull-wise average volume of ejaculate with S.E. & C.V. %.

Bull No.	No. of Obs.	Mean (in ml.)	+ S.E. (in ml.)	C.V. %
42	22	2.49	0.24	45.38
43	26	2.36	0.20	43.64
44	27	3.18	0.21	34.59
46	26	2.84	0.17	32.04
49	28	2.37	0.17	38.81
54	28	2.60	0.15	31.15
57	21	2.59	0.21	37.06
98	31	3.31	0.16	28.18
Overall	209	2.74	0.07	37.22

TABLE 4:10

Analysis of variance showing the effect of bulls in respect of volume of ejaculate (ml.).

Sources of variation	d.f.	S.S.	M.S.	F.
Between bulls	7	25.56	3.65	3.84 **
Within bulls	201	191.95	0.95	
Total	208	217.51		

** Denotes significance at 1% level.

TABLE 4:11

Showing critical differences amongst bulls in respect of ejaculate volume.

Sl.No.	Bull Nos.		Difference ofAvs. of ejaculate volume	Critical differences At 1% level	At 5% level
1	42	43	0.13 N.S.	-	0.5488
2	42	44	0.69 *	0.6966	0.5292
3	42	46	0.35 N.S.	-	0.5288
4	42	49	0.12 N.S.	-	0.5292
5	42	54	0.11 N.S.	-	0.5292
6	42	57	0.10 N.S.	-	0.5684
7	42	98	0.82 **	0.6966	0.5292
8	43	44	0.82 **	0.6708	0.5096
9	43	46	0.48 N.S.	-	0.5292
10	43	49	0.01 N.S.	-	0.5096
11	43	54	0.24 N.S.	-	0.5096
12	43	57	0.23 N.S.	-	0.5488
13	43	98	0.95 **	0.6450	0.4900
14	44	46	0.34 N.S.	-	0.5096
15	44	49	0.81 **	0.6708	0.5096
16	44	54	0.58 *	0.6708	0.5096
17	44	57	0.59 *	0.7224	0.5488
18	44	98	0.13 N.S.	-	0.4900
19	46	49	0.47 N.S.	-	0.5096
20	46	54	0.24 N.S.	-	0.5096
21	46	57	0.25 N.S.	-	0.5488
22	46	98	0.47 N.S.	-	0.4900
23	49	54	0.23 N.S.	-	0.5096
24	49	57	0.22 N.S.	-	0.5488
25	49	98	0.94 **	0.6450	0.4900
26	54	57	0.01 N.S.	-	0.5488
27	54	98	0.71 **	0.6450	0.4900
28	57	98	0.72 **	0.6966	0.5292

N.S. Denotes non-significance; ** Denotes significance at 1% level; * Denotes significance at 5% level.

TABLE 4:12

Showing season-wise average volume of ejaculate(ml.) with S.E.& C.V.%.

Seasons	No.of Obs.	Mean (in ml.)	± S.E. (in ml.)	C.V. %
Rainy	97	2.55	0.10	40.78
Winter	112	2.90	0.028	33.10
Overall	209	2.74	0.07	37.22

TABLE 4:13

Analysis of variance to see the effect of seasons on volume of ejaculate.

Sources of variation	d.f.	S.S.	M.S.	Value of F.
Between season	1	6.51	6.51	6.44 *
Within season	207	211.00	1.01	
Total	208	217.51		

* Denotes significance at 5% level.

TABLE 4:14

Showing bull-wise average pH of semen sample with S.E.& C.V.%.

Bull No.	No. of Obs.	Mean	± S.E.	C.V. %
42	22	6.67	0.055	3.74
43	26	6.58	0.054	4.10
44	27	6.66	0.047	3.60
46	26	6.56	0.045	3.50
49	28	6.60	0.039	3.18
54	28	6.54	0.044	3.51
57	21	6.82	0.055	3.66
98	31	6.60	0.038	3.18
Overall	209	6.62	0.017	3.62

TABLE 4:15

Analysis of variance to see the effect of bulls in respect of pH of semen.

Sources of variance	d.f.	S.S.	M.S.	Value of F.
Between bulls	7	1.30	0.1857	3.26 **
Within bulls	201	11.65	0.0569	
Total	208	12.95		

** Denotes significance at 1% level.

TABLE 4:16

Showing critical differences amongst different bulls in respect of pH.

Sl.No.	Bull Nos.		Differences in Avs. pH value	Critical differences At 1% level	Critical differences At 5% level
1	42	43	0.09 N.S.	-	0.13524
2	42	44	0.01 N.S.	-	0.13328
3	42	46	0.11 N.S.	-	0.13524
4	42	49	0.07 N.S.	-	0.13132
5	42	54	0.13 N.S.	-	0.13132
6	42	57	0.15 *	0.185	0.14112
7	42	98	0.07 N.S.	-	0.15680
8	43	44	0.08 N.S.	-	0.1274
9	43	46	0.02 N.S.	-	0.12936
10	43	49	0.02 N.S.	-	0.12544
11	43	54	0.04 N.S.	-	0.12544
12	43	57	0.24 **	0.17802	0.13524
13	43	98	0.02 N.S.	-	0.12348
14	44	46	0.10 N.S.	-	0.1274
15	44	49	0.06 N.S.	-	0.12544
16	44	54	0.12 N.S.	-	0.12544
17	44	57	0.16 *	0.18318	0.13916
18	44	98	0.06 N.S.	-	0.12152
19	46	49	0.04 N.S.	-	0.12544
20	46	54	0.02 N.S.	-	0.12544
21	46	57	0.26 **	0.17802	0.13524
22	46	98	0.04 N.S.	-	0.12348
23	49	54	0.06 N.S.	-	0.12348
24	49	57	0.22 **	0.17552	0.13336
25	49	98	0.00 N.S.	-	0.12152
26	54	57	0.28 **	0.17552	0.13336
27	54	98	0.06 N.S.	-	0.12152
28	57	98	0.22 **	0.17286	0.13132

N.S. Denotes non-significance; ** Denotes significance at 1% level;

* Denotes significance at 5% level.

TABLE 4:17

Showing season-wise average pH value with S.E.& C.V. %.

Seasons	No.of Obs.	Mean	± S.E.	C.V. %
Rainy	97	6.66	0.025	3.75
Winter	112	6.58	0.022	3.64
Overall	209	6.62	0.017	3.62

TABLE 4:18

Analysis of variance showing seasonal effect on pH of semen.

Sources of variation	d.f.	S.S.	M.S.	Value of F.
Between season	1	0.32	0.32	5.24 *
Within season	207	12.63	0.061	
Total	208	12.95		

* Denotes significance at 5% level.

TABLE 4:19

Showing bull-wise average percentage of individual motility with S.E.& C.V.%.

Bull No.	No. of Obs.	Mean	± S.E.	C.V.%
42	22	48.75	0.256	30.80
43	26	57.96	0.236	28.72
44	27	65.34	0.196	24.49
46	26	71.68	0.059	12.27
49	28	70.46	0.038	10.44
54	28	77.00	0.076	13.62
57	21	46.86	0.318	43.29
98	31	79.47	0.014	6.07
Overall	209	66.38	0.025	22.78

TABLE 4:20

Analysis of variance to see the effect of bulls on percentage of motile spermatozoa.

Sources of variation	d.f.	S.S.	M.S.	Value of F.
Between bulls	7	9670.3194	1381.4742	12.41 **
Within bulls	201	22362.8422	111.2579	
Total	208	32033.1616		

** Denotes significance at 1% level.

TABLE 4:21

Showing critical differences amongst different bulls in respect of individual motility percentage.

Sl. No.	Bull Nos.		Differences of mean	Critical differences At 1% level	Critical differences At 5% level
1	42	43	5.30 N.S.	-	5.9976
2	42	44	9.65 **	7.8174	5.9388
3	42	46	13.57 **	7.8948	5.9976
4	42	49	12.80 **	7.7658	5.8996
5	42	54	17.06 **	7.7658	5.8996
6	42	57	1.07 N.S.	-	6.3112
7	42	98	18.78 **	7.5852	5.7624
8	43	44	4.35 N.S.	-	5.6840
9	43	46	8.27 **	7.5594	5.7428
10	43	49	7.50 **	7.4304	5.6448
11	43	54	11.76 **	7.4304	5.6448
12	43	57	6.37 *	7.9980	6.0760
13	43	98	13.48 **	7.2498	5.5076
14	44	46	3.92 N.S.	-	5.6840
15	44	49	3.15 N.S.	-	5.5860
16	44	54	7.41 **	7.3530	5.5860
17	44	57	10.72 **	7.9206	6.0172
18	44	98	9.13 **	7.1724	5.4488
19	46	49	0.77 N.S.	-	5.6448
20	46	54	3.49 N.S.	-	5.6448
21	46	57	14.64 **	7.9980	6.0760
22	46	98	5.21 N.S.	-	5.5076
23	49	54	4.26 N.S.	-	5.5272
24	49	57	13.87 **	7.8690	5.9780
25	49	98	5.98 *	7.0950	5.3900
26	54	57	18.13 **	7.8690	5.9780
27	54	98	1.72 N.S.	-	5.3900
28	57	98	19.85 **	7.6884	5.8408

N.S. Denotes non-significance; ** Denotes significance at 1% level; * Denotes significance at 5% level.

TABLE 4:22

Showing season-wise average of individual motility of sperms with S.E. & C.V.%.

Seasons	No.of Obs.	Mean	± S.E.	C.V. %
Rainy	97	67.16	0.047	22.47
Winter	112	65.68	0.042	23.10
Overall	209	66.36	0.025	22.78

TABLE 4:23

Analysis of variance to see seasonal effect on individual motility of spermatozoa.

Source of variation	d.f.	S.S.	M.S.	Value of F.
Between season	1	42.5519	42.5519	< 1 N.S.
Within season	207	31990.6097	154.5440	
Total	208	32033.1616		

N.S. Denotes non-significance.

TABLE 4:24

Showing bull-wise mean percentage of dead spermatozoa with S.E.& C.V.%

Bull No.	No.of Obs.	Mean	± S.E.	C.V.%
42	22	46.42	0.268	32.43
43	26	37.85	0.272	40.37
44	27	30.25	0.201	40.12
46	26	23.90	0.064	25.35
49	28	25.78	0.046	21.36
54	28	20.48	0.097	35.30
57	21	48.50	0.322	33.77
98	31	17.70	0.020	18.24
Overall	209	29.68	0.023	38.56

TABLE 4:25

Analysis of variance to see the effects of bulls on percentage of dead spermatozoa.

Sources of variation	d.f.	S.S.	M.S.	Value of F.
Between bulls	7	9046.3206	1292.3315	10.53 **
Within bulls	201	24661.6101	122.6945	
Total	209	33707.9307		

** Denotes significance at 1% level.

TABLE 4:26

Showing critical differences amongst bulls in respect of percentage of dead spermatozoa.

Sl. No.	Bull Nos.		Differences of mean	Critical differences At 1% level	Critical differences At 5% level
1	42	43	4.98 N.S.	-	6.2720
2	42	44	9.58 **	8.2044	6.2328
3	42	46	13.68 **	8.2044	6.2328
4	42	49	12.43 **	8.1270	6.1740
5	42	54	16.04 **	8.1270	6.1740
6	42	57	1.19 N.S.	-	6.6052
7	42	98	18.07 **	7.9464	6.0368
8	43	44	4.60 N.S.	-	5.9584
9	43	46	8.70 **	7.9206	6.0172
10	43	49	7.45 *	7.7658	5.8996
11	43	54	11.06 **	7.7658	5.8996
12	43	57	6.17 N.S.	-	6.3504
13	43	98	13.09 **	7.5852	5.7624
14	44	46	4.10 N.S.	-	5.9584
15	44	49	2.85 N.S.	-	5.8408
16	44	54	6.46 *	7.6884	5.8408
17	44	57	10.77 **	8.3076	6.3112
18	44	98	8.49 **	7.5078	5.7036
19	46	49	1.25 N.S.	-	5.8996
20	46	54	2.36 N.S.	-	5.8996
21	46	57	14.87 **	8.3592	6.3504
22	46	98	4.39 N.S.	-	5.7624
23	49	54	3.61 N.S.	-	5.8016
24	49	57	13.62 **	8.3202	6.2524
25	49	98	5.64 N.S.	-	5.6448
26	54	57	17.23 **	8.2302	6.2524
27	54	98	2.03 N.S.	-	5.6448
28	57	98	19.26 **	8.0754	6.1348

N.S. Denotes non-significance; ** Denotes significance at 1% level; * Denotes significance at 5% level.

TABLE 4:27

Showing season-wise average dead percentage of sperms in Holstein-Friesian semen with S.E. & C.V. %.

Seasons	No.of Obs.	Mean	± S.E.	C.V. %
Rainy	97	29.47	0.051	39.20
Winter	112	29.86	0.043	38.15
Overall	209	29.68	0.023	38.56

TABLE 4:28

Analysis of variance to see the seasonal effects on percentage of dead spermatozoa.

Sources of variation	d.f.	S.S.	M.S.	Value of F.
Between season	1	3.1503	3.1503	< 1 N.S.
Within season	207	33704.7804	162.8250	
Total	208	33707.9307		

N.S. Denotes non-significance.

TABLE 4:29

Showing bull-wise average concentration of spermatozoa in semen sample (million per ml.) with S.E. & C.V. %.

Bull No.	No.of Obs.	Mean	± S.E.	C.V. %
42	22	613.76	0.011041	2.29
43	26	812.83	0.010940	2.25
44	27	918.33	0.010990	2.37
46	26	1091.40	0.010641	1.52
49	28	1091.40	0.010447	1.13
54	28	984.01	0.010617	1.54
57	21	622.30	0.011169	2.50
98	31	1273.50	0.010593	1.55
Overall	209	924.70	0.010304	2.21

TABLE 4:30

Analysis of variance to see the effects of bulls on sperm concentration (million per ml.)

Sources of variation	d.f.	S.S.	M.S.	Value of F.
Between bulls	7	2.303915	0.329130	11.14 **
Within bulls	201	5.948535	0.029544	
Total	208	8.252450		

** Denotes significance at 1% level.

TABLE 4:31

Showing critical differences amongst different bulls in respect of concentration of sperms.

Sl. No.	Bull Nos.		Differences of mean	Critical differences At 1% level	Critical differences At 5% level
1	42	43	0.122 *	0.12642	0.09604
2	42	44	0.175 **	0.12642	0.09604
3	42	46	0.250 **	0.12642	0.09604
4	42	49	0.250 **	0.12642	0.09604
5	42	54	0.205 **	0.12384	0.09408
6	42	57	0.006 N.S.	-	0.10192
7	42	98	0.316 **	0.12126	0.09212
8	43	44	0.053 N.S.	-	0.09212
9	43	46	0.128 **	0.12126	0.09212
10	43	49	0.128 **	0.11868	0.09016
11	43	54	0.083 N.S.	-	0.09016
12	43	57	0.116 *	0.129	0.098
13	43	98	0.195 **	0.1161	0.0882
14	44	46	0.075 N.S.	-	0.09212
15	44	49	0.075 N.S.	-	0.09016
16	44	54	0.030 N.S.	-	0.09016
17	44	57	0.169 **	0.129	0.098
18	44	98	0.142 **	0.1161	0.0882
19	46	49	0.000 N.S.	-	0.09016
20	46	54	0.045 N.S.	-	0.09016
21	46	57	0.224 **	0.129	0.098
22	46	98	0.067 N.S.	-	0.0882
23	49	54	0.045 N.S.	-	0.0882
24	49	57	0.244 **	0.12642	0.09604
25	49	98	0.067 N.S.	-	0.08624
26	54	57	0.199 **	0.12642	0.09604
27	54	98	0.112 *	0.11352	0.08624
28	57	98	0.311 **	0.12384	0.09408

N.S. Denotes non-significance; ** Denotes significance at 1% level; * Denotes significance at 5% level.

TABLE 4:32

Showing season-wise average concentration of sperms (million per ml.) with S.E. & C.V.%.

Seasons	No.of Obs.	Mean	± S.E.	C.V. %
Rainy	97	889.20	0.010162	2.49
Winter	112	954.99	0.010375	1.94
Overall	209	924.70	0.010304	2.21

TABLE 4:33

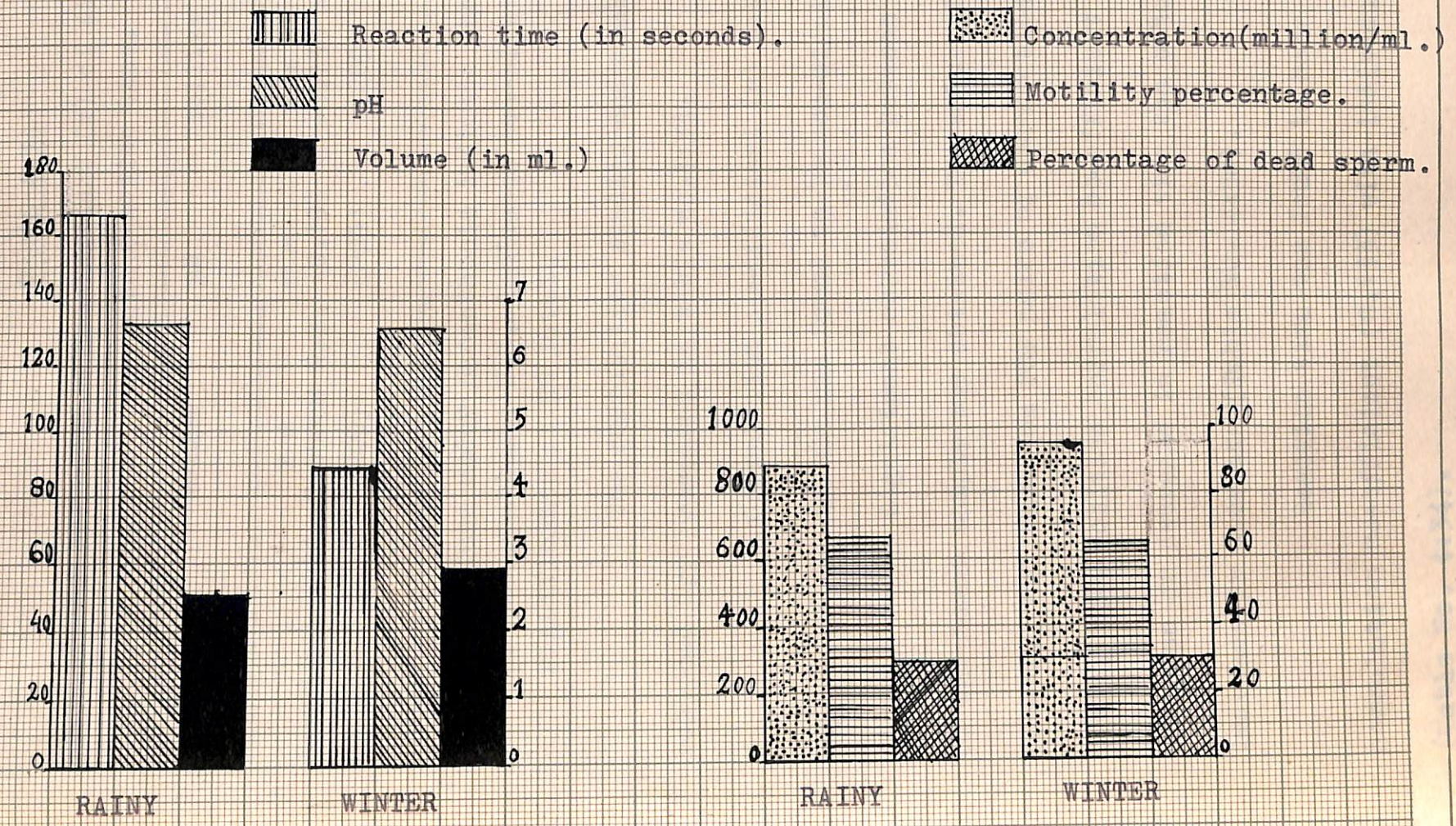
Analysis of variance to see the effects of seasons on concentration of sperms (million per ml.).

Sources of variation	d.f.	S.S.	M.S.	Value of F.
Between seasons	1	0.051778	0.051778	1.30 N.S.
Within season	207	8.200672	0.039616	
Total	208	8.252450		

N.S. Denotes non-significance.

GRAPH No.3

Showing seasonal variations in Reaction time (in seconds), pH, Volume (in ml.), Concentration (million per ml.), Motility percentage & Percentage of dead sperm.



CORRELATION STUDIES :-

TABLE 4:34

Showing the correlation co-efficient (r) of different semen characters with reaction time.

Characters	Volume	pH	Motility %	Concentration (million per ml.)
Reaction time	r = -0.00058 N.S. (209)	r = +0.199 * (209)	r = -0.133 N.S. (209)	r = -0.020 N.S. (209)

N.B.-The figure under paranthesis indicate the number of pairs of observations; N.S.-indicates non-significant; * - indicates significance at 5% level.

The correlation studies undertaken and the findings recorded are presented in table no.4:34.

Correlation co-efficient (r) between reaction time and ejaculate volume was found to be non-significant (r= -0.00058).

Significant correlation between reaction time and pH of the semen sample was obtained. The value of the correlation co-efficient based on 209 pairs of observation turned out to be 0.199. This positive correlation co-efficient indicates that with increase or decrease in reaction time the other trait would also increase or decrease (Table No. 4:34).

Reaction time was found to have non-significant negative correlation co-efficient (0.133) with individual motility percentage (Table No. 4:34).

The correlation co-efficient between reaction time and concentration turned out to be -0.020. This was found to be statistically non-significant (Table No. 4:34).

* * *

CHAPTER V

DISCUSSION

D I S C U S S I O N

REACTION TIME :-

The average reaction time based on 209 observations on 8 Holstein Friesian bulls was found to be 125.11 ± 4.74 seconds whereas Fraser (1960) recorded the average reaction time to be 12.5 minutes but he had also noted that 50% of the bulls served within two minutes. In Jersey breed the reaction time was found to vary from 30-60 seconds (Bhatia,1960). Most of Indian breeds had longer reaction time. Bhattacharya & Prabhu (1955 b) recorded average reaction time in Shahiwal to be 267.87 seconds. In Tharparker,different workers had recorded differences in reaction time (Sinha,1964;Ansari, 1970 & Mishra et al.,1971).

The results of the present study reveal highly significant variations between bulls as shown in Table 4:1 and 4:2. This is in agreement with the findings of Mukherjee & Bhattacharya (1952), Sinha (1964) and Ansari (1970). This variation may be due to the sexual instinct of the bulls (Donham et al. 1931). Hafs et al.(1959) observed that reaction time depends on the intervals between two collections. It had been observed that reaction time increased gradually with increase in the frequency of collection (Tomar,1970).

Significant seasonal variation as found in this study (Table 4:5 & Graph 3), is in consonance with the findings of Mukherjee & Bhattacharya (1952), Singh & Prabhu (1963) and Bhatnagar&Lohia(1961).

This variation might be due to the change in different climatic variables like: Temperature, Managemental conditions and Physical status of the bulls. Apart from this, a good number of variables might be responsible for the variations in this trait. While comparing the reaction time of these bulls with those of the Indian breeds, it appears that the exotic breed under the study is quicker in service. A bull with shorter reaction time might be of great value for the artificial insemination centres as much time, manpower and money are wasted on slow mounting bulls.

REACTION TOWARDS FEMALE :-

The present study revealed that out of 232 observations on 8 bulls 93.1% were active and 6.9% were dull. No bull was found to be shy at any stage. It has been also observed that seasons did not affect this trait (Table 4:6). In available literature on the Holstein Friesian bulls, such study could not be traced. However, study on 55 Tharparker bulls showed 78.2% active, 16.4% dull and 5.4% shy (Mishra et al., 1971) only. This character seems to be influanced by age and other hormonal effects of the bulls. It also indicates the serving ability of the bull. Bane (1967) advocated the measures of scale to study the sex-libido and categorised it into six classes. A good bull must have good sex-libido. In different bulls, sex-libido was found to be different (Miliecvic et al., 1968 ; Ansari, 1970).

High sex-libido is one of the most important factor for breeding bulls because nearly all the semen characteristics are found to be related to the degree of sex-libido of a particular bull. Although the genetic make-up of these bulls is unknown, the differences in this trait may be due to their genetic differences as the environmental factors were kept uniform for these bulls. Informations from such study would be useful in using these exotic bulls for Cross-breeding programme in the State.

Friesian bulls in U.S.A. are, as a rule, very masculine in type with pronounced development of secondary sexual characteristics. Usually they have excellant service ability, but at the same time many of them are very ferocious and dangerous. In Netherland and Sweden the Friesian bulls are in general, of more feminine type and docile but are not so good in their service ability.

SEX - DRIVE :-

The present study revealed significant seasonal variation in the sex drive of Holstein Friesian bulls on the basis of 232 observation (Table 4:7). This finding is in agreement with the findings of Trautwein et al.(1958) & Miliecvic et al.(1968).From the available literature it could not be acertained whether there was non-significant variation in this trait.

It is believed that the quality of ejaculate depends upon the extent of pre collection stimulation (Hellstrom, 1947 ;

Collins et al.,1951; Branton et al.,1952 & Crombach et al.,1956). It has been claimed that in a more exited bull there was more contraction of the epididymis (Parsutin,1956). Quick-serving bulls will be helpful in implementation of breeding programme properly. Hence, in selecting bulls, sex-drive should receive weight.

COLOUR :-

The colour of the semen in the present study was found to vary from creamy White to watery thin and it was in agreement with the description detailed by Mukherjee and Bhattacharya (1952); Shukla and Bhattacharya (1952); Brochat (1952)& Kodagali(1963) but it differs from the reports of Maule (1962). The Chi-square test showed non-significant difference between seasons (Table 4:8) which is contrary to the findings of Kodagali (1963) and Ansari (1970).

It is therefore, contended that probably the bulls right from their birth lived under the different environmental conditions. Hence the season and climate had no marked effect on the colour of the semen of exotic bulls. Colour of the semen is reported to depend upon the density and the flavin content of the semen. Further study on these bulls might throw more light on this aspect.

VOLUME :-

The mean volume of the ejaculate was estimated to be 2.74 ± 0.07 ml. which is slightly better than the value obtained

by Van Denmark (1956), which was 2.34 ml. for the first year after puberty. Lagerlof (1936) and McKenzie (1939) listed an average ejaculate of 3 ml. It differs from the finding of Erb et al.(1942); Nadaraja (1968); Dessouky & Juma (1968); Rao (1950); Herman and Ragsdale (1939); Herman & Swanson (1941). The comparatively low volume in comparison to these workers might be due to age factor as conceded by Williams (1932); Anderson (1940) and Baker et al.(1955b) in addition to some inherent physiological factors.

There is great variation in bull-wise average volume of semen obtained (Table 4:9 & Graph 1). The finding gets support from Erb et al.(1942); Anderson (1945); Mukherjee & Bhattacharya(195 Bhattacharya & Prabhu (1952); Hafs et al.(1958); Sinha & Prasad(1966 Tomar et al.(1966); Kodagali (1967) and Ansari (1970) but the presen findings are different from those of Kushwaha et al.(1955) and Nadaraja (1968).

Seasonal variation in ejaculate volume (Table 4:12 & 4:13 and Graph 3) obtained in the present study corroborate the findings of Erb et al.(1942); Phillips et al.(1943); Swanson and Herman (1944) and Hafs et al.(1958), but is contrary to the results of Vlachos & Karogiannidis (1956); Hafez & Bonadonna (1958);Kodagali (1963); Dessouky & Juma (1968) and Ansari (1970).

Volume of the ejaculate has great impact on breeding programme. With higher volume of ejaculate larger number of cows

could be served and less number of bulls would be required. It has been reported that Holstein Friesian had a larger volume of ejaculate than other exotic ones (Anderson,1941). As the bulls used in this study were in the age group of 14 to 17 months, so lower volume of ejaculates were obtained. But the ejaculate volume may probably be higher with advance of age. Further investigations on these bulls might lead to better understanding of their adaptability under the Indian conditions, as far as the characters studied are concerned.

HYDROGEN -ION-CONCENTRATION :-

Average pH of semen was found to be 6.62 ± 0.017 (Table 4:14), which is within the range of the normal value of 6.5 to 6.9 (Anderson,1942; Romijin,1948 b;and Dessouky & Juma,1968). But this value is slightly greater than that reported by Rao (1960).

Individual variations among the 8 Holstein Friesian bulls had been obtained (Table 4:14 & 4:15 and Graph 1). These agree with the findings of Rao (1950); Tomar et al.(1966). Anderson(1945& 1952) showed that between bulls there were highly significant differences in pH change of semen samples after incubation.

The mean pH was found to be 6.66 ± 0.025 and 6.58 ± 0.022 in rainy and winter seasons respectively and significant differences between seasons had been observed (Table 4:17 & 4:18 and Graph 3). It agreed with the findings of Swanson and Herman (1944); Mukherjee & Bhattacharya (1952); Kushwaha et al.(1955); Oloufa and

Sayed,(1956); Tomar et al.(1966) but differed from the reports of Dessouky and Juma (1968) and Miliecvic et al.(1968).

The pH of the bull semen had generally been towards acidic side (Devis and Williams,1939). The change in the pH of semen is caused by the metabolic activity of the spermatozoa. Thus more acidic semen is always associated with higher number of spermatozoa.

INDIVIDUAL MOTILITY :-

The average motility percentage was recorded to be 66.36 ± 0.025 which agrees with the finding of Lasley (1951) who recorded 64.1% motility and also with those of Hafs et al.(1958) but it differed from the findings of Donham et al.(1931); Anderson (1938); Herman & Swanson (1941); Lasley & Bogart (1943); Erb et al. (1950) and Kodagali (1963). This might be due to the breed differences and the other variables.

Variation between bulls had been found to be significant (Table 4:19 & 4:20 and Graph 2) which tallies with the findings of Phillipso et al.(1943); Anderson (1945); Thomson (1950); Hafs et al. (1958); Tomar et al.(1966) but differed from those of Mercier and Salisbury (1946).

The statistical analysis showed that the seasons have no significant effect (Table 4:22 & 4:23 and Graph 3) on the motility of spermatozoa. This is in agreement with the finding of Erb et al. (1942); Phillips et al.(1943); Dessouky & Juma (1968) and Ansari(1970)

but differs from that of Anderson (1945); Mercier & Salisbury (1946); Mukherjee & Bhattacharya (1947); Mies Filho & De Paulo Graca (1950); Kodagali (1963); Sinha & Prasad (1966). Thus it can be stated that differences in breed, individual genetic make-up of bulls and the environmental factors have great bearing on the initial motility of spermatozoa. It also indicates that if semen is collected under ideal and sterile conditions seasonal effect would be greatly minimised.

PERCENTAGE OF DEAD SPERMATOZOA :-

The mean percentage of dead spermatozoa in the present study was recorded to be 29.68 ± 0.023 which is higher than the value reported by Saxena (1965); Lasley (1944) and Ansari (1970) in different Indian and exotic breeds. Climatic changes, age of the bulls and managerial conditions in which the bulls are being maintained might be the factors responsible for it.

Significant differences between bulls (Table 4:24 & 4:25 and Graph 3) were observed which is in accordance with the findings of Erb et al.(1942); Phillips et al.(1943); Mercier and Salisbury (1946); Thomson (1950); Tomar et al.(1966) and Ansari(1970).

Seasons had been found to have no effect on percentage of dead spermatozoa which is in accordance with the findings of Sinha et al.(1966) and Ansari (1970) but it is not in agreement with the findings of Erb et al. (1942); Phillips et al.(1943); Tomar et al.(1966).

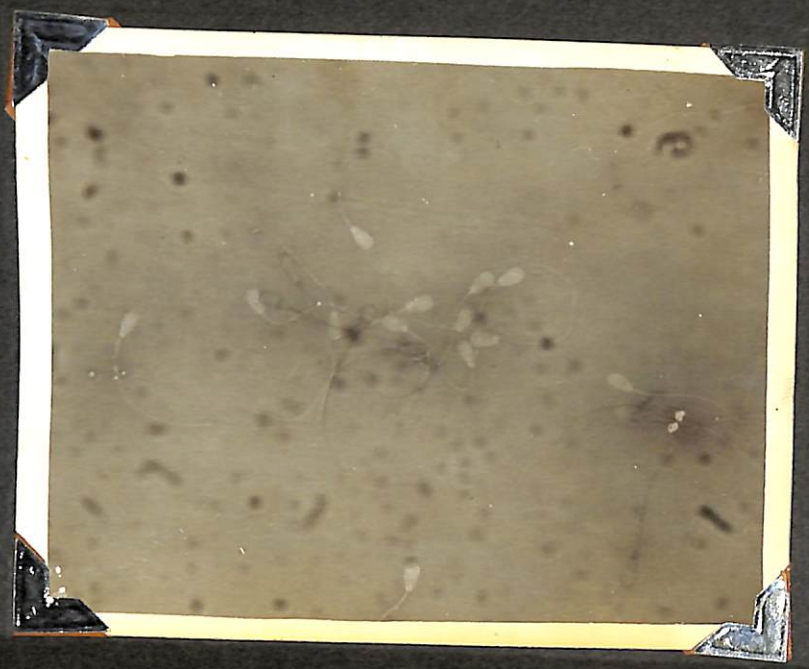


Fig. 3 Good semen sample showing low number of dead spermatozoa.

Fig. 4 Bad semen sample showing large number of dead spermatozoa.

This variation might be attributed to the variations in feeding standard and managerial conditions of the farm (Stojnov et al.,1967). Another reason which may be assigned is that the present study covered a period of only six months,which is rather a shorter time. Bulls under the present study were found satisfactory for A.I. work.

SPERM CONCENTRATION MILLION PER ML. OF SEMEN :-

The average concentration of sperm per ml. of semen based on 209 observations was 924.7 ± 0.01 million,which is well within the range of 831 to 1737 million per ml. This is in agreement with the findings of Herman and Regsdale (1939); Comstock & Green (1939); Shukla & Bhattacharya (1949); Nadaraja (1968).

Highly significant differences in this trait was noted between the bulls (Table 4:29 & 4:30 and Graph No.2). This is in accordance with those of Lagerlof (1934); Phillips et al. (1943); Swanson & Herman (1944) and Anderson (1945).

No significant seasonal variation on the trait in question was noted. This disagrees with the findings of Erb et al. (1942); Phillips et al.(1943); Mukherjee & Bhattacharya (1952); Schindler (1954) and Dmitriev (1965).

The variation of the present findings with those of other workers might be due to lesser number of observations, the

Fig. 1 Good semen sample showing high concentration of sperms.

Fig. 2 Bad semen sample showing low concentration of sperms.

Fig. 2 2nd semen sample showing low concentration of sperms.

less marked variation in the climatic conditions of these two seasons and the constancy of managerial conditions under which these bulls had been kept.

The artificial insemination is one of the most important tools for the breeder to bring about genetic improvement in the livestocks. This method also helps spread of superior germplasm throug the maximum use of carefully selected sires. The concentration of the ejaculate had a bearing on the number of cows to be inseminated and hence it is of great value. It had been advocated by several workers that one insemination should consists of 5 to 10 million spermatozoa. In the present finding an average of 924 million sperms per ml. of semen had been recorded. Thus, it is expected that with the use of extender nearly 90 to 100 cows can be inseminated with one ml. of such semen. As the bulls were of younger age, there is scope for further increase in the concentration and other semen characters which may be of greater value than what it has at present.

CORRELATION STUDY :-

The correlation between reaction time and other semen attributes like; volume,pH,motility and concentration were calculated but except the correlation co-efficient between reaction time and pH, the same for other combinations (reaction time correlated with other seminal characters), turned out to be non-significant. In literature on this aspect could be traced. Hence no comparison is

possible.

Since the present study incorporates only a limited number of observations, further studies with greater bulk of data appear necessary before establishing any authenticity.

* * *

CHAPTER VI

SUMMARY

SUMMARY

Under the present study 8 Holstein Friesian bulls of age group 14-17 months, stationed at New Semen Bank, Patna, were taken up during the two seasons- the rainy and winter in respect of breeding behaviour and semen picture. The work was undertaken keeping in view the introduction of exotic germ-plasm in this country, mainly through A.I. and limited informations available on these points under the Indian conditions.

The breeding behaviour was studied on 232 observations whereas 209 observations of the semen picture were recorded.

The mean values of different attributes of breeding behaviour and semen picture under study are given below :-

Reaction time - 125.11 ± 4.75 seconds with 54.81 C.V.%.

Reaction towards female - Active-93.1%; Dull-6.9%; Shy- nil.

Sex-drive - A-72.4%; B-19.4%; C- 6.9%; D- 1.3%.

Volume - 2.74 ± 0.07 ml. with 37.2 C.V.%.

Colour - It ranges from creamy white to watery thin.

Hydrogen-ion-concentration(pH)- 6.62 ± 0.017 with 3.62 C.V.%.

Individual motility percentage - 66.36 ± 0.025 with 22.78 C.V.%.

Percentage of dead spermatozoa - 29.68 ± 0.023 with 38.56 C.V.%.

Sperm concentration million per ml.- 924.7 ± 0.01 with 2.21 C.V.%.

Significant variation in all the traits were noted

because of differences in bulls.

Except reaction towards female, colour, individual motility, percentage of dead spermatozoa and sperm concentration, significant seasonal variations were noted in all other traits.

Correlation study of reaction time with volume, pH, individual motility and sperm concentration revealed that except reaction time and pH, no significant correlation with reaction time was found with any other trait. All these correlations were negative in nature and non-significant.

Although the observations made and informations and inferences derived from this study are likely to be of practical value under the existing breeding programme, much work embodying greater bulk of data is required to obtain a reliable picture on these aspects.

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REFERENCES CITED

REFERENCES CITED

Abdel- Raouf, M. (1965) Nord.Vet.Med., 17:318.

Almquist,J.O.,Amann,R.P.& Hale,E.B. (1963) J. Dairy Sc., 46:1176.

Anderson,J. (1938) Ann.Report Vet.Dept.,Nairobi, Kenya Colony,85. cited by Kumaran,J.D.S.(1951).

Anderson,J. (1940) Vet.J., 96:18. cited by Rao,C.K.(1950).

Anderson,J. (1941) Vet.Rec., 53:197.

Anderson,J. (1942) Vet.Rec., 54:317.

Anderson,J. (1945) J.Agric.Sc., 35:184. (A.B.A.,13, No.199).

Anderson,J. (1952) J.Agric.Sc., 42:172. (A.B.A.,20,No.1615).

Ansari,M.R. (1970) Studies on seasonal variations in Sex-libido,Reaction time & other Semen characteristics of Tharparker bulls. M.Sc.(Vet.) Thesis, M.U.

Asdell,S.A. (1955) Cattle fertility & Sterility (1st Ed.). Little,Brown & Co., Toronto.

Baker,F.N.,Van Demark, N.L.& Salisbury,G.W. (1955a) J.Anim.Sc., 14:746.

Baker,F.N.,Van Demark, N.L.& Salisbury, G.W. (1955b) J.Dairy Sc., 38:1000.

Bane,A.	(1954)	Acta.Agric.Scand., 4:95. (A.B.A.,22, No.1413).
Bane,A.	(1967)	Cited by Ansari,M.R.(1970).
Barbulescu,I.et al.	(1965)	Lucr.Stiint.Inst.Cerc. Zooteh., 22:419. (A.B.A.,35,No.307).
Basirov,E.B.	(1960)	Zivotnovodstvo, 22(2):74. (A.B.A.,28,No.1294).
Bhatia,H.M.	(1960)	Animal breeding Co-ordination of A.H. Research in India, I.C.A.R., Delhi.
Bhattacharya,P & Prabhu,S.S.	(1952)	Indian J.Vet.Sc., 22:163.
Bhattacharya,P & Prabhu,S.S.	(1955b)	Indian J.Vet.Sc., 25:277.
Bhatnagar,V.K.& Lohia,K.L.	(1961)	Indian J.Vet.Sc., 31:325.
Bishop,M.W.H.& Hancock,J.L.	(1955)	Vet.Rec., 67:363.
Blome,E.	(1942)	Medlemsbl.danske Dyrlaege-foren, 1942:81. (A.B.A.,12:197 pp.).
Boyd,L.J.	(1957)	Diss.Abstr.,17:205. (A.B.A.,26,No.749).
Branton,C.,D'Arensbourg,G.& Johnston,J.E.	(1952)	J.Dairy Sc., 35:801.
Branton,C.,Kellgren,H.C. & Patrick,T.E.	(1953a)	J.Dairy Sc., 36:1301.
Brochat,M.	(1952)	C.R.Soc.Biol.(Paris)146:556. (A.B.A.,21, No.210).
Brown,M.A.	(1959)	Sthwest.Vet.,13:49. (A.B.A.,28,No.1908).

Castle,F. (1969) Five-figure logarithmic an other Tables. Macmillan, St.Martin's Press,New York

Chominat,R. & Agache,J. (1967) Elevage Insem.,No.98:3. (A.B.A.,35,No.3554).

Comstock,R.E.& Green,W.W. (1939) Proc.Amer.Soc.Anim.Prod.,3 Ann.Meet.,213.(A.B.A.,8No.

Conn, E. (1946) Vet.Rec., 58:610.

Collins,W.J.,Bratton,R.W.& Henderson,C.R. (1951) J.Dairy Sc., 34:224.

Crombach,J.J.M.L.,de Rover, W. & de Groot,B. (1956) Pap.3rd Int.Congr.Anim. Reprod.(Camb.),Sect.3,80-8 (A.B.A.,24 No.1627).

Crombach,J.J.M.L. (1961) Z.Tierz.Zucht.Biol.,75:331 (A.B.A.,30, No.967).

Dessouky,F.& Juma,K.H. (1968) J.Agric.Sc.Camb.,71:37.

Devis,H.P.& Williams,N.K. (1939) Am.Soc.Animal Prod.Proc., 32:232. Cited by Rao,C.K.(1950).

Dmitriev,V.B. (1965) Sb.Nauch.Trud.Pushkin. Nauchnoisseled.Lab.Razv. Sel'-Khoz.Zhivol.,No.11:17 (A.B.A.,35,No.315).

Donham.C.R.,Simms,B.T.& Shaw, J.N. (1931) J.Amer.Vet.Med.Ass.,78:665

Erb,R.E.,Andrews,F.N.& Hilton,J.H. (1942) J.Dairy Sc.,25:815.

Erb,R.E.,Ehlers,M.H.,Mikota, L.& Schwarz,E.	(1950)	Tech.Bull.St.Coll.Wash., No. 2:52. (A.B.A.,20,No.1090).
Fraser,A.F.	(1957)	Brit.J.Anim.Behav.,5:110.
Fraser, A.F.	(1960)	Cornell Vet.,50:126.
George, O.T.	(1952)	Cited by Ansari,M.R.,(1970).
Hafez,E.S.E.	(1962)	Reproduction in Farm animals. Lae & Febigr,Philadelphia,U.S.A.
Hafez,E.S.E.,Schein,M.W.& Ewbank,R.	(1969)	Behaviour of Domestic Animal. Bailliere.Tindall & Cassell,Ltd.
Hafez,E.S.E.& Bonadonna,T.	(1958)	Zootec.e.Vet.,13:281. (A.B.A.,27,No.218).
Hafez,E.S.E.& Bonadonna,T.	(1959)	Sthwest,Vet., 12:107. (A.B.A.,29,No.815).
Hafs,H.D.,Bratton,R.W.,Henderson, C.R. & Foote,R.H.	(1958)	J.Dairy Sc.,41:96.
Hafs,H.D.,Hoyt,R.S. & Bratton,R.W.	(1959)	J.Dairy Sc., 42:626.
Hale,E.B.	(1966)	J.Anim.Sc., 25:36.
Hellstrom,P.	(1947)	Lantmannen(stockh.),31:198. (A.B.A.,15,No.177).
Herman,H.A.& Regsdale,A.C.	(1939)	Univ.Missou.Agr.Exp.Stat. Bull. 407. (Cited by Kumaran J.D.S.,1951).
Homan,H.A.& Swanson,E.W.	(1941)	J.Dairy Sc., 24:321.
Hiroe,K.,Tomizuka,T.,Hanada, A.& Masaki,J.	(1965)	Bull.Nat.Inst.Animal Ind.,

		Japan. Summarin of Reports, 1965.No.8:1. (Nutr.Abst.,36,No.1465).
Hooker,C.W.	(1944)	Am.J.Anat.,74:1.
Hultnas,C.A.	(1967)	7th F.A.O.Swiss.Intern.P.G. Training Course on Anim. Reprod.,Royal Vet.College, Stockholm. cited by Ansari,M.R.(1970).
Ionova,A.G.	(1968)	Dokl.Timiryazev.Sel.Khoz. Akad.,Mosk.,No.135:23. (A.B.A.,37,No.1461).
Ishei,S.& Okamoto,S.	(1953)	Biol.Abst.,1955,29B,28678. (A.B.A.,24,No.150).
Johnston,J.E.& Branton,C.	(1953)	J.Dairy Sc., 36:934.
Kalev,G.& Venkov,T.	(1959)	Zivotn(Sofija) 1:101. (A.B.A.,30,No.1024).
Kerruish,B.M.	(1955)	Brit.J.Anim.Behav. 3:125.
Kozlo,V.E.	(1967)	Sb.Nauch.Rab.Vses.Ord.Trud. Krasn.Znam.Nauchnoissled. Inst.Zhivot.No.5:24. (A.B.A.,37,No.389).
Kodagali,S.B.	(1963)	Ind.J.Vet.Sc., 33:26.
Kodagali,S.B.	(1967)	Ind.J.Vet.Sc.,44:773.
Kushwaha,N.S.,Mukherjee, D.P.& Bhattacharya,P.	(1955)	Indian.J.Vet.Sc.,25:317.
Lagerlof,N.	(1934)	Acta.path. et microbiol. Scand.,Suppl. XIX.Cited by Kumaran,J.D.S.(1951).

Lagerlof,N.	(1936)	Vet.Rec., 10:1936.
Lasley,J.F.,Easley,G.T.& McKenzie,F.F.	(1942)	Anat.Rec.,82:167. (A.B.A.,11, No.171).
Lasley,J.F.	(1944)	J.Anim.Sc., 3:433.
Lasley,J.F.& Bogart,R.	(1943)	Res.Bull.Mo.Agric.Exp.Sta., No. 376:56. (A.B.A.,12, No.197).
Lasley,J.F.	(1951)	J.Anim.Sc., 10:211.
Leidl,W.& Biegert,W.	(1967)	Berl.Munch.Tierarztl. Wschr., 80:345. (A.B.A.,37,No.299).
Lepard,O.L.,Shurat,C.E.& Foster,A.	(1941)	J.Dairy Sc., 24:509.
Lewis,R.C.	(1948)	J.Anim.Sc., 7:514.
Madden,F.W.,Herman,H.A.& Berousek,E.R.	(1947)	Res.Bull.Mo.Agric.Exp.Sta., No.407:15. (A.B.A.,16,No.1392).
Mahmoud,I.N.	(1952)	Bull.Fac.Agric.Found.Univ. Cairo., 5:16. (A.B.A.,21,No.1262).
Marandici,A.& Lopazan,E.	(1960)	Lucr.Sti.Inst.Cerc.Zooteh. (Bucurestic) 18:189. (A.B.A.,29,No.2101).
Maule,J.P.	(1962)	The semen of Animals & A.I., Commonwealth Agric.Bureaux Fernham Royal,Bucks.,England.

McKenzia,F.F. (1939) The cattleman,26:53. Cited by Kumaran,J.D.S.(1951).

Mercier,E.& Salisbury,G.W. (1946) Cornell Vet., 36:301. (A.B.A.,11,No.322).

Miasnicov,I. (1966) Revta.Zootech.Med.Vet.,16. (A.B.A.,35,No.336).

Mies Filho,A.& De PauloGraca,V.(1950) Publ.Inst.Zootec. (Rio de J.), 9:13. (A.B.A.,20,No.192).

Miliecvic,D.,Varadin,M.& Borjancic,S. (1968) Congr.Reprod.Insem.Artif. Paris.,1968,Resume.72. (A.B.A.,37,No.63).

Mishra,H.R.,Prasad,R.J.,Singh, S.D.& Biswas,S.C. (1971) A study on breeding behaviour & some economic characters of Tharparker bull under progeny Testing Programme (Unpublished).

Mukherjee,D.P.& Bhattacharya,P.(1947) Abst.Proc.Ind.Sc.Cong., Part 3:215. Cited by Ansari,M.R.(1970).

Mukherjee,D.P.& Bhattacharya,P.(1952) Ind.J.Vet.Sc., 22:73.

Nadaraja,R. (1968) Cylon Vet.J.,Vol.,XV:118. (A.B.A.,36,No.3599).

Nishiyama,H.,Ogaw,K.,Nakanishi, Y.& Oyamada,T. (1968) Bull.Fac.Agri.Kagoshima Univ., 18:157. (A.B.A.,36,No.597).

Oloufa,M.M.& Sayed,A.A.	(1956)	Vet.Med.J.Cairo Univ.,3:163. Cited by Oloufa et al.(1959), Ind.J.Dairy Sc.,12:17.
Okamoto,S.,Ishei,S.& Mukai	(1962)	Bull.Kyushu Agric.Exp.Sta., 7:409.(A.B.A.,32,No.1037).
Parsutin,G.V.	(1956)	Plenary Pap.3rd int.Congr. Anim.Reprod.(Camb.),1956:45. Cited by Maule,J.P.(1962).
Perry,E.J.	(1960)	A.I.of Farm animals,1st Indian Ed.1965.Oxford & I.B.H.Publishing C.,Calcutta.
Phillips,R.W.& Andrews,F.N.	(1936)	Massachusetts Agric.Exp.Sta. Bull,, 33. Cited by Salisbury & Van - Demark (1961).
Phillips,R.W.,Knapp,B.,Eaton, O.N.& Heemstra,L.C.	(1943)	Amer.J.Vet.Res. 4:115.
Prabhu,S.S.& Guha,S.	(1951)	Ind.J.Vet.Sc.,22:93.
Rao,C.K.& Hart,G.H.	(1948)	Amer.J.Vet.Res., 9:286.
Rao,C.K.	(1950)	Indian J.Dairy Sc.,3:75.
Raps,G.& Cannon,C.Y.	(1947)	J.Dairy Sc. 30:933.
Rimoldi,A.& Brigatti,C.	(1941)	Fecond.Artif.(Milano)No.8. (A.B.A.,13,No.88).
Romijin,C.	(1948b)	Tijdschr.Diergeneesk.,73:7136. (A.B.A.,16,No.228).
Rosenhalm,E.& Mackle,N.	(1967)	Zuchthygiene 2:19. (A.B.A.,35,No.2509).
Rossner,M.	(1955)	Z.Tierz.Zucht Biol.,65:77. (A.B.A.,23,No.,1181).

Salisbury,G.W.	(1944)	J.Dairy Sc.,27:551.
Salisbury,G.W.,Knodt,C.B.& Bratton,R.W.	(1948)	J.Anim.Sc.,7:283.
Sarthou-Moutengou,J.	(1950)	Centres Riv.Zootec.,24:105. (A.B.A.,19,No.343).
Saxena,V.B.	(1965)	M.V.Sc.Thesis,Agra Univ. Cited by Ansari,M.R.(1970).
Schein,M.W.& Hale,E.B.	(1965)	'Sex & Behaviour' F.A.Beach (Edit.) New York. Cited by Hafez,E.S.E.(1962).
Schmidt,K.	(1954)	Mh.Vet.Med., 9:349. (A.B.A.,23,No.1718).
Schmidt,K.Strassburg,H.& Koriath,G.	(1956)	Tierzucht, 10:7. (A.B.A.,25,No.724).
Schindler,H.	(1954)	Bull.Res.Coun.Isreal.,4:184. (A.B.A.,23,No.679).
Sciuchetti,A.	(1938)	Z.Tierz, 41:161. (A.B.A.,7:121 pp.).
Shukla,D.D.	(1956)	Ind.Vet.J., 33:3.
Shukla,D.D.& Bhattacharya,P.	(1949)	Ind.J.Vet.Sc.,19:161.
Shukla,D.D.& Bhattacharya,P.	(1952)	Ind.J.Vet.Sc., 22:93& 179.
Singh,G.S.& Prabhu,S.S.	(1963)	Ind.J.Vet.Sc., 33:29.
Sinha,H.S.	(1964)	'A critical studies of semen evaluation of the three A.I. centres of Bihar'.M.Sc.(Vet.) A.H.,Thesis,M.U.
Sinha,H.S.& Prasad,R.B.	(1966)	Ind.J.Dairy Sc.19:135.

Sinha,R.C.,Gupta,B.P.S.& Roy,A.	(1966)	Ind.J.Dairy Sc.,19:18.
Smith,G.	(1951)	Proc.Brit.Soc.Anim.Prod. 13th & 14th Meet. 1950. (A.B.A., 19,No.1715).
Smith,S.E.& Asdell,S.A.	(1940)	Cornell Vet., 30:499.
Snedecor,W.G.& Cochran,W.G.	(1967)	'Statistical Methods' Oxford & I.B.H.Publishing Co.,17, Park Street,Calcutta-16.
Stojanov,T.,Pavlov,D.& Raicev,D.	(1967)	Zhivotnovudstvo,21(4):28. (A.B.A.,35,No.612).
Stone,E.J.,Johnston,J.E. & Mixner,J.P.	(1950)	J.Dairy Sc., 33:442.
Sumner,S.L.,Ancalmo,R.& Warnick,A.C.	(1968)	J.Anim.Sc., 27:1197.
Swanson,E.W.& Herman,H.A.	(1944)	J.Dairy Sc., 27:303.
Thomson,D.M.	(1950)	Vet.Rec., 62:480. (A.B.A.,18,No.1407).
Tomar,N.S.,Mishra,B.S. & Johari,C.B.	(1966)	Ind.J.Dairy Sc.,19:87.
Tomar,N.S.	(1970)	'A.I.& Reproduction of cattle & Buffaloes',Saroj Prakasan, 646 Katra,Allahabad-2.
Tomar,S.S.& Kanajia,A.S.	(1970)	J.Res.Punjab Agri.Univ.,7:541.
Trautwein,K.,Bauer,H.& Fluhr,F.	(1958)	Zuchthyg.FortPfstor Besam Haustiere,2:217. (A.B.A.,28,No.640).
Tripathi,V.N.& Prabhu,S.S.	(1966)	Ind.J.Dairy Sc., 19:207.

VanDemark,N.L. (1956) Plenary Pap.3rd inst.Congr. Anim.Reprod.(Camb.)1956:80. (A.B.A.,25,No.728).

Verevkina,T.P.& Smirnov-Ugrjumou,D.V. (1938) Probl.Zivolno,3:178. (A.B.A.,8:35pp.).

Vlachos,K.& Karogiannidis,A. (1956) Bull.Physiol.Path.Report.Arif Insem.Thessalonika, 1:101. (A.B.A.,37,No.,444).

Weatherby,E.J.,Reece,R.P.& Bartlett,J.W. (1940) Proc.Amer.Soc.Anim.Prod. 33rd Ann.Meet.224. (A.B.A.,10;100 pp.).

Weatherby,E.J.,Reece,R.P.& Bartlett,J.W. (1942) J.Anim.Sc., 1:80.

Williams,W.L. (1932) Vet.Med.27:288. Cited by Rao,C.K.(1950).

Wiltbank,J.N.,Rowden,W.W.& Ingalls,J.E. (1965) Res.Bull.Neb.Agric.Exp.Stn. No. 224:19p. (A.B.A.,35,No. 304).

Zelfel,S. (1964) Kuhn-Archiv, 77:241. (A.B.A.,33, No.328).

Zuliani,T. (1957) Zootec e vet.,12:209. (A.B.A.,25,No.1864).

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