STUDIES ON REACTION TIME AND CERTAIN CHARACTERS OF MURRAH BUFFALO BULL SEMEN AND THE EFFECT OF DILUENTS ON THE MOTILITY OF SPERMATOZOA

A THESIS SUBMITTED
TO
MAGADH UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
M. Sc. (Vet)
IN
GYNAECOLOGY, OBSTETRICS AND ARTIFICIAL INSEMINATION

By
C. Sharana Kalmaya
B. V. Sc.

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DECEMBER, 1966.

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I certify that this thesis entitled "Studies on reaction time and certain characters of Murrah buffaloe bull semen and the Effect of Diluents on the Biometry of Spermatozoa ", has been prepared under my supervision by Sri C. SIVARAMA KRISHNAYYA, a candidate for M.Sc. (Vet.) with GYNAECOLOGY, OBSTETRICS AND ARTIFICIAL INSEMINATION as major subject and that it incorporates the results of his own independent study.

1-XII-66

(B.P.S.R.SINGH).

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Author.

LIST OF ABBRAVIATION.

After noon.	...	A.M.
Average.	...	A.V.
Centigrade.	...	$^{o}C.$
Coefficient of variation.	...	C.V.
Cubic centimeter.	...	C.C.
Cubic milli-metre.	...	C.M.M.
Glucose Sodium Bicarbonate Sulphamezathin Yolk.	...	G.B.Y.
Hydrogen ion concentration.	...	pH.
Kilogramme.	...	KGS.
Micron.	...	μ
Skim Milk Yolk.	....	SMY.
Standard deviation.	...	S.D.
Standard Error.	...	S.E.

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C O N T E N T S.

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CHAPTER - I.

I N T R O D U C T I O N.

I N T R O D U C T I O N.

Human being have to depend upon plants and animals for their food products and other necessities. Most useful food products and luxury articles are made available from animal sources. Cattle, buffaloes, sheep, goats, swine, birds and equines are mainly domesticated for these purposes. With the advancing scientific age, improvement in these species of animals is being made through various methods.

India possesses 175.62 millian cattle and 51.137 million buffaloes (1961 census). According to the F.A.O. report in 1958 only 95.4 million was the total buffaloe population allover the world. Amble et al.(1964) have reported milch cows and buffaloes in India 45.5 and 21.9 millions respectively and they produce 13.62 million metric tons of milk, of which 59 % of the bulk is produced by buffaloes. A part from this from the remaining population of buffaloes some are being used as work animals for agricultural purposes. So in India buffalo stands as main source of milk production, having an average annual yield per head of 491 kgs in comparision to 173 kgs. in the case of cows.

Proper breding of live stock is one of the most important ways of bringing about improvement in domesticated animals. Artificial insemination is a modern tool in the hands of breeder for the rapid improvement of live stock as compared to natural breeding.

In recent years Artificial Insemination in animals has gained enough importance for reasons of its obvious advantages over natural mating. Now a days Artificial Insemination is agreed to be the best means for improving the genetic makeup of domesticated animals because this method ensures full utilisation of the highest quality of germ-plasm of the provensires. This type of breeding helps in affecting conception where natural mating is impossible or hazardous. The inheritance of superior quality of breed characters at distant places can be made available easily with advancement in preserving the fertile life of spermatozoa. Thus it is a very fine Zootechnique for exploiting the animal resources for the welfare of humanity and as well as for improvement of live stock.

Anton van Leeuwenhock, and his pupil, Johan Hamm (1677) were the discoverers of the spermatozoa with the help of a magnifying lens. Italian Physiologist Spallanzani (1780) was the first scientist who conducted research on artificial insemination and used dog for this purpose. Ivanoff a Russian sciertist and a pioneer in artificial insemination was the first to undertake artificial insemination in cattle and sheep. Nothing had been worked out until the later part of 19th century. Bringing same dog breeding into light in 1897 W. Heape of England had suggested that "Artificial Insemination" appears to be

easy, conception can be brought,as in normal coitus, to many bitches with a single ejaculate. He claimed that this method could be used to cross dog breeds whose natural breeding is impossible and suggested that this helps in studying genetic and telegonic factors.He also referred the research with mares, the object of which was to over come sterility(Perry,1960).

Professor Hoffman who had recommended supplimentary insemination in mares, had diluted the semen of stallion in cows milk and inseminated. At the same period Sand and Stribolt got successful result in mares in Denmark and reporting this at the Northern live stock conference at Copenhagen (1902) said that the important feature of this practice was the economical use of a valuable stallion and hinted its potentiality for the wide-spread use in improvement of farm animals. After a decade Russians stated that artificial insemination gave better results than natural service. During the same time Ivanoff could succeed in inseminating birds (Perry,1960).

Begining in 1914 G. Amantea, human Physiologist devised artificial vagina for collecting semen of dogs. Keeping the same principle in mind as basic one Russian workers, Milovanov, Filippov and others developed suitable artificial vagina for bulls and other animals

(Perry, 1960). At present mostly the pattern of short artificial vagina is being used (Roberts 1956) . This type ensures the ejaculation of semen directly into the cone or collecting vial . As such the collection of clean and normal ejaculate has become easy and practicable for large scale of mechanical breeding. With the development of artificial insemination, the economic importance of selecting bull of high fertility and the use of ejaculates that can give high fertility rate is appreciated and has prompted the workers to continue the search for an accurate method of assessing the potential fertility of a given sample of semen. As reviewed by Anderson (1945) there is little doubt, that estimation of ejaculate volume, initial motility, spermatozoal concentration, resistance and viability, served to differentiate the infertile or a bull of low fertility ~~bull~~ from that of high fertility (Maule, 1962).

In addition to the ability for transmitting good characters with high fertilising capacity, a bull selected for artificial breeding should also have a good response to the artificial vagina and should be able to give uniform large ejaculates. Much variations are seen in the ejaculates of different bulls and even in the ejaculates of the same bull taken at different intervals. So it is essential to keep a good check on

every sample before releasing for field insemination. It has been suggested that informations about the measurement of certain characteristics can be used to estimate the fertilising capacity of bulls and these practices are being used for selecting bulls for artificial insemination Bishop and Harcock (1955). However there is no single criterion known so far to represent adequately the true quality of semen. As such efforts are being made since last two decades to establish a criteria for evaluation of semen that can give a partial indication of fertilising capacity of semen before it is used for breeding. Uptill now much less work has been done in case of buffaloe semen in comparision to the work done on bull and ram semen. Great variation have been recorded in quality and quantity of semen production between cow bulls and buffaloe bulls. Bhattacharya and Prabhu (1950, 1951, 1952 and 1953).

To have a maximum benifit of an outstanding sire the semen is extended by diluting with semen diluters for bringing conception in a large number of females. To bring perfection of the diluting fluids a day to day improvement of the semen diluter is being carried out from the basic sodium citrate diluter found by Phillips and Lardy (1939). In the year 1955

Almquist et al. working with diluters stated that fresh homagenised milk and skim milk can be used for diluting fluids for bull semen, but they said they could not record significant differences from egg yolk citrate. Kerruish (1956) stated that low cost of the skim milk together with the better results obtained justyfy its wider use as a diluent. Srivastav and Prabhu in the year 1958 stated that glucose sodium bicarbonate diluter was better than egg yolk citrate, autoclaved milk and egg yolk phosphate diluter for buffaloe bull semen . Rathore (1961) stated that the head length and the head breadth of the preserved ram spermatozoa were significantly less than those of unpreserved spermatozoa on studying after 72 hours of preservation but Vandengen and Salisbury (1962) reported that the head of bull spermatozoa changed from an elongated form to a more round form after preservation.

As such it indicates that apart from serving as a diluting fluid and energy supplying medium, the effect of osmotic pressure of the diluting fluid is also there during the preservation period on the sperm cell. This variation of dimentions, on preservation might be a cause for variable results of fertility obtained in different diluters. This is to be investigated with field application.

Murrah breed is one of the best breeds of buffaloes in India. In view of this a preliminary study was undertaken to observe reaction time of the bull and to investigate certain characters of Murrah bull semen and the effect of diluters used to extend the semen, on the morphology of the spermatozoa.

The studies confine on the following aspects:

1. Reaction time.
2. Volume of ejaculate.
3. Colour and consistency.
4. Initial motility.
5. Hydrogenion concentration.(pH).
6. Sperm concentration.
7. Percentage of dead spermatozoa.
8. Morphological abnormalities.
9. Biometrics of spermatozoa.

Much less literature is available on the morphological abnormalities and biometrics of spermatozoa of buffaloe bull. and

10. The effect of Glucose sodium bicarbonate sulphamezathine yolk and skim milk yolk buffers on the biometrics of head length and head breadth at 24, 48 and 72 hours of preservation at $4^{\circ} \pm 1^{\circ}$c temperature.

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CHAPTER - II.

REVIEW OF THE LITERATURE.

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REVIEW OF LITERATURE.

Reaction time taken by the bull depends upon the sexual instinct or libido and the mating capacity of the individual bull. The bulls will be subjective to different factors of external environmental conditions of maintenance apart from the physiological checks induced by the artificial vagina react differently, Donham (1931) Van Der Sluis (1961) . Donham (loc.cit) observed no relationship between promptness in service and semen quality and fertility.

Studying the effect of different levels of protein on the reaction time of buffaloe bulls, Prabhu and Bhaya (1950) stated the reaction time as 52.2,22.3 and 33.3 seconds in normal food, 25 percent less and 50 percent less of protein level of normal food respectively and no statistically significant difference was found in the reaction time between the groups.

Prabhu and Bhattacharya (1951) reported a reaction time of 33.13 and 33.48 seconds for first and second ejaculates in succession for Indian water buffaloes, and reported the difference as non significant. In the same year Prabhu and Guha have observed no significant difference in the reaction time of Kumaumi hill bulls, depending upon the duration of excercise . Prabhu and Bhattacharya (1954) while conducting experiments on local Uttar Pradesh

buffaloe bulls, using them on heat and on non heat teaser cows recorded a reaction time of 24.67 seconds and 26.81 seconds for the first ejaculate on heat and non-heat buffaloe cows and for the second ejaculate in succession it was 23.25 and 26.81 seconds respectively at the intervals of 24-48 hours collections. At the intervals of a week they recorded a reaction time of 40.35 and 34.44 seconds for first ejaculate and 13.13 and 46.59 seconds for second ejaculate respectively and found no significant difference with different teaser cows. In the same period Prabhu and Sharma stated the reaction time for 4 successive ejaculates as 32.69, 80.28, 121.11 and 269.31 seconds for local Utter Pradesh buffaloe bulls at a collection programme of one week intervals. The difference was found to be highly significant from bull to bull and ejaculate to ejaculate. During the same period analysing the records of field centers, Bhattacharya and Prabhu in (1954) in their fourth series of studies for murrah bulls maintained at different centres of the country stated the longest reaction time for one collection as one hour and the shortest as 7 seconds for one particular bull and it varied from 152.27 seconds to 600.15 seconds in different bulls. It varied considerably from bull to bull and from collection to collection. They further stated that an increase in reaction time with increase of artificial service has appeared.

In murrah buffaloe bulls Kushwaha et al.(1955) reported a mean reaction time of 42.38 seconds in autumn as lowest and highest being in spring as 58.93 seconds and in summer and winters it was 53.10 and 48.43 seconds respectively. They found that the reaction time was significantly less in autumn than in spring and summer and concluded that the reaction time is independent of semen quality.

Prabhu (1956) studied factors affecting sex drive and semen production of buffaloe bulls in his second series, with different types of teaser, substituting male for female, different coloured females, using different males, taking collections after a 5 minutes cold water bath for buffaloe bulls and taking collections in the evening times, found significant variation in reaction time when male was substituted for the routine female teaser; buffaloe bulls with lower sex drive showed greater reaction time to the change in the exitability object or time of collection than buffaloe bulls with higher sex drive. Oloufa et al.(1958) recorded in Egyptian buffaloe bulls, a reaction time of 59.20 seconds in summer while studying seasonal variation in reaction time and semen characters with two successive ejaculates with 48.06 seconds as an avarage for the year under study.

Prabhu and Sharma (1962) found large amount of bull to bull variation in Utter Pradesh buffaloe bulls. Statistical analysis confirmed the earlier findings of no significant variation in semen quality and reaction time. This was attributed to very large bull to bull variations present in the experimental animals. Kale in the same year recorded a reaction time that varied from 6 to 8 minutes.

Misra and Sengupta (1964) while conducting experiments protected a group of murrah bulls from hot winds and radiation of summer sun and the other group of bulls were not protected. In the second group they observed a complete loss of libido which they attributed this to reduced secretion of testosterone. They confirmed this by studying the seminal fructose content, which was 456 mg. per 100 ml. of semen as against 825 mg. per 100 ml. of semen in the first group. They have also showed that the seminal fructose rose to 716 mg. per 100 ml. of semen in the second group after a rest period of three months. Sengard and Sharma in the same year studying characteristics of successive ejaculates of buffaloe bulls recorded a reaction time of 94.9 ± 11.3 seconds for first ejaculate and 143.0 ± 15.1 seconds for the second ejaculate. Viswanath Pillai (1965) recorded a reaction time of 152.5 ± 15.35 seconds and 229.41 ± 40.68 seconds for first and second ejaculates respectively in murrah bulls.

CHARACTERS OF NEAT SEMEN:

Volume of the ejaculate depends mostly upon the secretions of accessary glands. The more the volume of ejaculate the more the animals that can be inseminated. Hence it is imperative to use males that produce large amounts of good quality semen Kumaran (1951). The volume of the ejaculate will help in detecting sterility in bulls partially, which will be low Blom (1950). Further he observed that the volume of ejaculate will be lesser in young bulls, just sexually mature bulls, bulls that are extremly used, in incomplete ejaculates, in practically sterile bulls and in cases of bilateral seminal vesiculitis. There will be a decline in tendency with successive ejaculates in low fertile and strile bulls. Blom (1950), Rollnison (1957). The volume of the semen depends upon the breed and size of the individual also Lagerlaf (1934) Bishop and Hancock (1955).

Veeramaniayyar (1944) reported a volume of 3 to 4.5 millilitres range in murrah buffalo bulls. Reporting the volumes as 2.72 c.c., 1.8 and 1.98 c.c. with different levels of feed i.e. for normal, 25 percent and 50 percent less protein feed levels, Prabhu and Bhaya (1950) for buffaloe bulls stated that statistical analysis did not reveal any significant variations in semen quality in the experiment. Prabhu and Bhattacharya (1952) conducted experiments on Utter Pradesh buffaloe bulls with different physiological state of teaser cow in the first series where collections were taken on alternate days with two successive

ejaculates. In the [illegible] rimert collections were
taken at weekly [illegible] two successive ejaculates
and in the third [illegible] cessive ejaculates at a
weeks interval. The [illegible] irst ejaculates in first
series on heat teaser [illegible] n heat teaser was 1.88 cc.c.
and 1.31 c.c. [illegible] in the second series it
was 3.37 c.c. [illegible] in third series it
was 2.25 c.c. [illegible] ectively. The same
authors reported, for [illegible] er buffaloes, during the
same year, semen [illegible] c. for first ejaculate
and 2.04 c.c. [illegible] taken in a rapid
succession. Average [illegible] ed for Nilibreed was
2.41 ± 0.06 c.c. at [illegible] for murrah as
3.54 ± 0.18 c.c. per [illegible] Patna by Bhattacharya
and Prabhu during [illegible] d that breed
differences were [illegible] ey further quoted
the report of [illegible] (1949) as 1.84 ±
0.99 c.c. on an [illegible] in 1953 they
reported an overall [illegible] 0.20 c.c. in
murrah bulls [illegible] s in India. An
ejaculate volume of [illegible] age with a
range of 1.2 to [illegible] Mahmoud for
Egyptian buffalo [illegible]

Prabhu [illegible]
average 2.14 c.c. [illegible] on an
[illegible] During [illegible] .0 c.c.in

Utter Pradesh buffaloe bulls wher four successive ejaculates were taken at weekly intervals for the first ejaculate. Bhattacharya and Prabhu (1954) reported an average volume of 1.79 ± 0.38 c.c. semen for murrah buffaloe bulls. Kushwaha et al.(1955) reported an average volume of 2.18 c.c. and 2.67 c.c. during their study of 1949-50 ; 1950-51 periods respectively with murrah bulls in different seasons and found no significant variations. Hafez and Darwish (1956) have reported a volume of 3.12 millilitre on an average for the four collections taken in succession at irtervals of 24, 12, 3 and 2 hours with a range of 2.7 millilitre to 4.0 millilitre. Madatov (1956) recorded an average of 3.66 c.c. in U.S.S.R. buffaloe bulls. Sayed and Oloufa (1957) studied the effect of frequency of collection on the semen volume of Egyptian buffaloe bulls and recorded the volume of semen, in once in a week collection and thrice in week collections as 4.88 c.c. and 3.00 c.c.¢., showing significant reduction in semen volume and the same trend was reported in cattle also. An ejaculate of 3.94 c.c. was recorded by Olufa et al.(1958) in summer while studying the seasonal variations in the Egyptian buffaloe bulls with an average of 3.49 c.c. for all seasons. Joshi (1961) gave an average volume of semen for murrah bulls as 3.23 c.c. with a range of 1.6 c.c. to 7.00 c.c. During 1962 Kale has reported

a range of 2.7 c.c. to 4.3 c.c. for buffaloe bulls. It was quoted by Maule (1962) that in Indian buffaloes the volume of semen does not usually exceed 5.0 millilitre and the maximum quantity of 8.2 millilitre of semen was obtained from murrah bull at Indian Veterinary Research Institute, Izatnagar . Carrying out the investigation in September and October 1962 Sengar and Sharma (1964) reported a volume of 1.83 ± 0.42 millilitre of semen for first ejaculate in buffaloe bulls. In the same year Misra and Sengupta, conducted experiments to study the climatic environment and reproductive behaviour of buffaloe bulls and recorded a semen volume of 3.72 ± 0.16 millilitre in summer per ejaculation in one group under with some protection from hot winds and 3.67 ± 1.6 millilitre for the other group which does not have the said protection. Viswanath Pillai (1965) reported 4.74 ± 1.14 millilitre for first collection and Kouser in the same year recorded the volume of semen in murrah bulls as 4.50 ± 0.02 millilitre with a range of 2.0 to 8.0 millilitre.

Colour and consistency of ejaculate has got a special significance to denote the healthyness of the semen. Samples showing abnormal colour and consistency are suggestive of poor quality and should be closely examined to find out the nature of abnormality. In the bull the colour of semen depends upon the

concentration the greater the number of spermatozoa for a giver volume the more the consistency changing from thin to thick consistency with a colour change of watery to creamy. In the case of compleat absence of spermatozoa, the semen as a rule, almost as clear as water. In some odd instances, one finds in bulls that other cells, than spermatozoa may make the semen opaque viz. giant cells in certain cases of degeneration of testicles, big yellow purulent flocculi that may occur due to purulent inflammation of seminal vesicles. In some tough viscous semen may indicate catarrhal conditions in accessary genital glands which may cause infertility Lagerlof (1934, 1942), Blom and Christensen (1965). Roberts (1956) referred Dakins, who indicated that brownish coloured semen in bulls may be observed in orchitis and said that yellow colour seen occationally in bulls semen is due to corotinoid pigment without any significant. A dark red to pink colour may be due to the presence of varying amounts of blood with result of lacerations of the mucous membrane. A light brown colour may be due to presence of feces in the semen.

Mahmoud (1952) estimated the normal colour of buffaloe bull semen as white with a very light tinge of blue. After sexual rest the semen is usually light white at the first collection and deeper white at

(1950). Donham et al.(loc.cit) record five grad
ercentage of motility and stated speci ns exhibiti
ercent motile sperm or above to be normal. The
ility of spermatozoa was explained, Lagerlof (1936),
a necessary physiological phenomenon for fertilisation
spermatozoan which under certain conditions shows an
mpaired motility and need not always be a worthless
ell and vice versa. The motility of spermatozoa serves
as a means for properly distributing spermatozoa throughout
the area of female genital tract in which ovem is found
as a fertilising unit, thus insuring the statistical
criteria of spermatozoa ovum mating Asdell and
Salisbury (1941) . The motility rate given by Herman
and Swanson (1941) was 0 to 5, where 0.5 represent
10 percent motility. Swanson and Herman (1944) stated
that conception increased as motility increased upto
3.0 rating and these after increased motility did not
result in important increase in conception rate. The
index of correlation has also revealed a significant
curriliner correlation between motility rating and
conception. Initial motility was also stated to be
depended upon the activity of spermatozoa as well as
sperm concentration Maule (1962).

Prabhu and Bhaya (1950) recorded, feeding various
levels of protein to buffaloe bulls, the average initial

second collection. He occasionally observed semen having red ting due to laceration of some capillary blood vessels during collection. He also expressed that semen from animals in good health was generally of a thick creamy consistency and with frequent collections and exhaust tests the semen was thin and watery. During hot weather the viscosity of semen decreases. Maule (1962) further quoted that semen from healthy Indian buffaloe bull is opaque milky white or yellowish like that of cattle and the consistency may be thin or thick depending on spermatozoal concentration. Veeramaniayyar (1944), Shukla and Bhattacharya (1949) and Kale (1962) recorded the colour of buffaloe semen as milky and creamy with a consistency of thin and thick. He further stated that creamyness was always associated with thickness. Viswanath Pillai (1965) recorded thick milky and thin milky samples at a ratio of 91.7 and 8.3 percent levels and Kouser during the same year reported the colour of murrah semen as cremy and milky with a consistency of thick and thin.

Initial motility : Reynolds described the normal motility of individual spermatozoa as far back as 1916. Initial motility was found to be one of the most important factor to estimate fertility rate and it was correlated with different tests Donham et al.(1931) Anderson (1937), Herman and Swanson (1941) Lasley and Bogart (1943) Erb et al. and

Blom (1950). Donham et al.(loc.cit) recorded five grades of percentage of motility and stated specimens exhibiting 90 percent motile sperm or above to be normal. The motility of spermatozoa was explained, Lagerlof (1936), as a necessary physiological phenomenon for fertilisation. A spermatozoan which under certain conditions shows an impaired motility and need not always be a worthless cell and vice versa. The motility of spermatozoa serves as a means for properly distributing spermatozoa throughout the area of female genital tract in which ovem is found as a fertilising unit, thus insuring the statistical criteria of spermatozoa ovum mating Asdell and Salisbury (1941) . The motility rate given by Herman and Swanson (1941) was 0 to 5, where 0.5 represent 10 percent motility. Swanson and Herman (1944) stated that conception increased as motility increased upto 3.0 rating and these after increased motility did not result in important increase in conception rate. The index of correlation has also revealed a significant curriliner correlation between motility rating and conception. Initial motility was also stated to be depended upon the activity of spermatozoa as well as sperm concentration Maule (1962).

Prabhu and Bhaya (1950) recorded, feeding various levels of protein to buffaloe bulls, the average initial

motility, with 50 percent less and with 25 percent less protein with a control group ; as 3.80 ± 0.44 ; 3.00 ± 0.28 and 3.68 ± 0.28 respectively. Prabhu and Bhattacharya (1951) studying comparison of first and second ejaculates in Indian water buffaloe found an initial motility of 3.07 and 3.32 respectively. The same authors when conducted experiments with local Utter Pradesh buffaloe bulls with teasers on heat and not on heat cows recorded a motility rating of 3.37 and 2.98 for first ejaculates and 2.44 and 2.34 for the second ejaculate of the same collection. Prabhu and Sharma (1954) studying the comparative effect of 4 successive ejaculates recorded an initial motility of 2.69; 2.31, 1.89 and 1.01 respectively for successive ejaculates of the buffaloe bull. Studying the seasonal variation in semen characters Kushwaha et al.(1955) recorded an initial motility of 3.57 and 3.74 during 1949-50 and subsequent year being best in spring 4.15; 3.75 in summer, 3.40 in winter and 3.34 in autumn. Sayed and Oloufa studying the effect of frequency of collection on Egyptian buffaloe bulls, stated the motility as 84.71 percent and 55.95 percent with collections of once in a week and thrice in a week in 1957. Hafez and Darwish (1957) reported an initial motility of 7.5 for fresh semen in buffaloes and observe dropping down of motility after third ejaculate. Oloufa et al.(1958) recorded a motility of 75.37 percent in summer

with an average of 64.02 for the year under study i.e. 3.5 and 3.0 respectively. Tomar and Desai (1958-59) while studying preservation of buffaloe semen rated motility in ten categories from 0 to + 5 every 0.5 = 10 percent motile spermatozoa. Kale (1962) stated an initial motility of +4.0 to 4.5 for buffaloe bull semen. During 1964 Misra and Sengupta recorded a motility of 1.58 ± 0.09 in summer with buffaloe bull having wind protection and 0.87 ± 0.30 without protection from hot winds while Sengar and Sharma recorded 1.83 initial motility in the same year for the first ejaculate in their study. Kouser (1965) observed a motility of 4 ± 0.5 with a range of 3 to 4.5. Viswanath Pillai in the same year noted a motility of (++) according to Bloms classification.

Hydrogenion concentration (pH) is used to express the acidity or alkalinity and is expressed as negative logarithm of hydrogenion concentration. The changes in the pH of semen is caused by the metabolic activity of the spermatozoa. The study of this character is important because any diluter used should be approximately of the same pH as that of semen, helping in keeping quality of semen extended . Studying the bovine semen Davis et al. (1940) grouped the semen samples according to the concentration of sperm per mm^3 showing higher the concentration the lower the pH from 1 to 999,000 mm^3 , the pH value was 6.98 and from 1,000,000 to 1,999,000 mm^3

the mean pH was 6.74. No difference in rate of decline when stored at different levels. pH was found to have negative correlation with sperm density Schneerson(1936) Davis et al.(1940) Swanson and Herman (1941) Anderson (1942) stated high pH was associated with epididymitis, small testis with either few or no spermatozoa and in sterile bulls the pH of successive ejaculates tend to alkalinity. In 1944 he stated that pH had shown a negative correlation with concentration, volume of semen and motility, and when ejaculate had an initial pH of 7.11 or higher showed poor or no motility on collection with low concentration. Blom (1950) stated higher pH was observed in extream utilisation, incompleat ejaculate, pathological conditions of testis or glandular vesicularis, hence he stated that routine examination will help in diagnosis. Rollinson (1951) stated increased pH was associated with poor fertility,and it varied directly with volume in fertile bulls and vice versa in infertile bulls. Jean Blain (1953) stated that sperm viability was affected when pH was below six and above seven but under eight with a better viability at 6.5 and 7.0 pH. Initial alkalinity especially in bulls and rams, is often accompanied with low fertility either with low concentration or absence of spermatozoa Manu (1954). Veeramaniayyar (1944) recorded a pH of 5.6 to 6.4 in buffaloe bull semen. Prabhu and Bhaya (1950)

while studying semen characters with normal level, 25 and 50 percent less of protein in diet had recorded a pH of 6.80; 6.81 and 6.79 respectively. While studying the effect of successive collections Prabhu and Bhattacharya (1951) recorded a pH of 6.79 and 6.82 for first and second ejaculates. The same authors (1954) had recorded a pH of 6.78 and 6.71 in once in a week collections made on, on heat and or no heat cows in buffaloe bulls. Prabhu and Sharma (1954) has compared four ejaculates taken in rapid succession from water buffaloe, at weekly intervals, pH for the first ejaculate was 6.81 with a range of 6.6 to 7.2 and found highly significant between bulls and ejaculates. During the year 1949-50 and 50-51 Kushwaha et al.(1955) recorded in buffaloe bull semen a pH of 6.6 to 6.9 during first year and 6.5 to 6.9 during second year; in different months, with an average of 6.68 and 6.73 and was significant between bulls and months at one percent level. Sayed and Oloufa (1957) had recorded a pH of 6.8 and 6.89 in collections made once in a week and thrice in a week with the semen of Egyptian buffaloe bulls. Oloufa et al.(1958) recorded a pH of 6.82 summer with an average for all seasons as 6.88. The pH in summer was less than all seasons. Tomar and Desai (1958-59) recorded a pH of 6.8 to 6.9 while studying the preservation of semen, in different diluters for buffaloe bull semen. Kale (1962) got a pH range of

6.4 to 6.7 while studying preservation of buffaloe bull semen. Sengar and Sharma (1964) had recorded a pH of 6.57 ± 0.05 for first ejaculate while studying the successive ejaculates of buffaloe bull semen. Viswanath Pillai (1965) had recorded a pH of 6.43 ± 0.13 and 6.59 ± 0.08 for first and second ejaculates and during the same year Kouser had observed a pH of 6.7 ± 0.002 with a range of 6.3 to 7.00.

Spermatozoan concentration: Study of spermatozoal concentration is very important , particularly when semen is extended in a higher dilutions for Artificial insemination work, to determine the optimum dilution. It is felt that sperm concentration is relatively lower in buffaloe bull than in the cow bull (Perry 1960). Salisbury et al.(1934) has employed a standard equipment, haemocytometer and counted fifteen large squares out of 25 large squares and calculated the concentration. Belding (1935) stated that errors due to sampling, dilution could be over come by bulk dilution method and this gave consistent results than the dilution made in R.B.C. pipette in haemocytometer count of spermatozoa. On investigation it was agreed that sperm concentration was reduced in case of reduced fertility and sterility, Anderson (1937),Lasley and Bogart (1943), Haq (1949), Blom (1950), Rollinson (1951),Bishop et al. and Cummings (1954). Concentration is stated to be

related to initial motility Erb et al.(1950). The concentration was also stated to be affected in epididymitis, degeneration of testis; vesiculitis and testicular abnormalities Lagerlof (1934) cited by Anderson (1945),Lagerlof (1936), Anderson (1937,1942), Haq (1949), which Blom (1950) has confirmed on experimenting. Raouf (1965) stated that concentration increases as the bull matures with age .

The concentration of spermatoza in buffaloe semen was estimated to vary between 631 to 1034 millions per millilitre Shukla and Bhattacharya (1951) studied sperm concentration in Indian water buffaloe and recorded 1075.92 millions /c.c for first ejaculate as compared to second ejaculate of 970.79 millions /c.c. which was not significant. Studying about semen characters of Egyptian buffaloes Mahmoud (1952)reported 210 to 2000 millions /c.c. Prabhu and Sharma (1954) observed an average of 635,1109,787 and 815 millions/c.c. for four successive ejaculates taken in rapid succession for local Utter Pradesh buffaloes. The lowest recorded was 328 millions /c.c. and the highest being 1376 millions /c.c. between bulls and ejaculate variation was high; all differed with second ejaculate. Prabhu and Bhattacharya (1954) studying the effect of collection of semen on heat and on non heat teaser cow recorded a concentration of 622 millions/c.c. for

first ejaculate and 723 million /c.c. for second ejaculate during weekly interval collections. Studying seasonal variations Kushwaha et al.(1955) recorded a sperm concentration of 938.66 millions /c.c. and 973.04 millions/c.c. on an average for Murrah buffaloe bulls during 1949-50 and 1950-51 and in summer as 1034.39 /millions/c.c. which was the highest. In local Utter Pradesh buffaloes Prabhu (1956) had reported a sperm concentration of 810 and 947 millions /c.c. for first and second ejaculates. Hafez and Darwish (1956) did not observe any alteration in sperm concentration for successive ejaculates taken at intervals of 24,12,8 and 2 hours, the average concentration being 645 x 10^3 c.mm. In Russia Madatov (1956) recorded an average sperm concentration of 980 millions /c.c. of buffaloe semen. Sayed and Oloufa (1957) stated in buffaloes a concentration of 1,208,000/c.mm in Egyptian buffaloes while studying the effect of frequency of collection in once in a week collections and 786000/c.mm in thrice in a week collection. Studying the seasonal variation in semen quality Roy et al. (1958) stated an overall average concentration of 1264.5 millions /c.c. and Roy (1958) reported a sperm concentration of 1295.7 ± 124.7 in winter; 1563.2 ± 191.6 in spring; 1277.4 ± 117.6 in summer and 1040.3 ± 114.7 millions per millilitre in rainy season. Oloufa et al.(1958) studying seasonal

variation in Egyptian buffaloe bulls recorded a sperm concentration of 1036.62 x 10^3/c.mm. in summer with an over all average of 935.13 x 10^3 /c.mm. for the year. During the year 1964 Sengar and Sharma reported a sperm concentration of 1074. 7x10^6 /millilitre for first ejaculate. Misra and Sengupta in the same year recorded a concentration of 1527 ± 100 x 10^6/millilitre and 1104 ± 352 x 10^6/ millilitre in summer in the two groups of buffaloe bulls protected from hot winds and in non protected group respectively. Viswanath Pillai (1965) had observed with murrah buffaloe bulls, a sperm concentration of 1110.3 ± 154.69 millions/c.c. and Kouser, in the same year, recorded 1254 ± 6 millions per millilitre with a range of 670 to 1960 millions spermatozoa per millilitre.

Percentage of dead spermatozoa: Physical and chemical activity of the semen depends upon the proportion of live and dead spermatozoa, the motility and dead and live percentage go hand in hand while evaluating semen sample . The discovery of Lasley et.al in 1942 offered a new method for semen quality assay. They used eosin and opal blue to differentiate live and dead spermatozoa in isotonic phosphate buffer showing dead spermatozoa taking stain with eosin, differentiating the live, unstained spermatozoa against the back ground of opal blue. Below 50 percent of live spermatozoa was stated

to affect fertility Lasley and Bogout (1943) and a lenier relationship was found with motility, which was directly related to fertility Lasley (1944) Erb et.al.(1950),and Cupps et al.(1953). Eric Blom (1950) studied a stain with 5 percent eosin and 10 percent Nigrosin, that differentiated live spermatozoa clearly on a brownish violet background from dead spermatozoa that were red. Hancock (1951) stated that the proportion of stained (dead) spermatozoa increased with the temperature difference between semen and stain. At the same time Mayer et al.(1951) used fast-green instead of opal blue, giving a homogenous green background, that is rest full to the eyes, and found superior to opal blue. He got the best values at pH 6.8 to 7.8 .Below 5.8 pH agglutination of sperm occured and above 8.5 the head and tail of many sperms were separated. In the same year Swanson and Beardone explained the benifits of the eosin-Nigrosin stain, that can be used even with egg yolk mixed diluters and said ever the morphological characters can also be studied clearly. The same authors studied different strengthes of eosin-Nigrosin, with eosin fast green as control and summarised that one percent eosine and five percent Nigrosin at pH of 6.4 to 8.5 is most suitable stain and reported that variying proportions of semen stain (1:1 to 1:20) did not effect the percentage of dead.

Prabhu and Bhattacharya (1951) gave an overall percentage of dead spermatozoa for first ejaculate as

22.64 compared to 17.2 percent for second ejaculate in Indian water buffaloe; the difference being statistically significant at one percent level. The same authors in the year 1954 recorded a percentage of dead sperm with semen collected on heat and on non heat buffaloe teaser cows as 37.46 and 25.81 percent and 34.98 and 21.9 percent for the first and second ejaculates respectively and differences were nonsignificant. Hafez and Darwish (1956) observed that successive ejaculates did not effect the percentage of live spermatozoa in semen collected at 24,12,8 and 2 hours interval. Sayed and Oloufa (1957) reported 91.18 percent and 72.19 percent live sperms for buffaloe bulls with semen collected at intervals of once in a week and reported thrice in a week showed a sigrificant decrease live spermatozoa. Roy (1958) observed a percentage of live sperm 69.0 ± 3.0 in winter; 62.2 ± 3.9 inspiring; 63.2 ± 4.6 in summer, 79.2 ± 2.3 in rainy Reason and 82.7 ± 1.8 in autumn.

Naidu (1960) recorded an average of unstained spermatozoa as 90.18 percent in buffaloe bull semen. At the same time Prabhu and Bhaya recorded a dead spermatozoa of 22.9; 21.9 and 19.3 percent with normal feed and 25 percent and 50 percent in feed having less protein respectivly . Kale (1962) recorded live spermatozoa varying from 75.0 to 82.0 percent while studying preservation of buffaloes semen with milk diluters.

Viswanath Pillai (1965) recorded in Murrah bulls percentage of dead spermatozoa as 21.07 ± 0.97 and 13.67 ± 0.84 in first and second ejaculates. Kouser in the same year recorded a dead sperm percentage of 10.8 ± 0.06 with a range 4 to 19 percent in Murrah bulls. Saxena in the same year recorded an unstained spermatozoal percentage in buffaloe semen as 88 ± 1.

Morphological abnormalities : Any deviation from normal shape and size of spermatozoa is stated to be abnormal. Broman has detected pathological spermatozoa as far back as in 1900 in human semen and Williams (1920) Largerlof (1934) investigated thoroughly in bulls with diminished or arrested fertility. Lagerlof had shown by experiments, that pathological spermatozoa is connected with disturbances in spermato-genesis due to changes in epithelium of seminiferons tubules, may be due to deficient development of testicles, but commonest cause was stated to be degeneration or inflammatory process in the testicular Parenchyma which coincided with the findings of Lagerlof (1936) Anderson (1937) Haq (1939) Blom (1950). A good semen semple, was stated should have below 16 to 20 percent of abnormal spermatozoa Williams and Savage (1925), Monech (1933), Hancock and Rollinson Haq (1949) Crespo (1956), Munro (1961) where as Foote and Bronton, Swanson and Herman and Laing reported 30-35 percent did not affect fertility (Roberts, 1956). Blom (1950) recorded a primary abnormal spermatozoa of 4.6 percent in fertile bulls, 17.06 to 24.2

percent in problem bulls, with an average of 21 percent in sterile bulls and stated no bull was found with more than 15 to 20 percent primary abnormalities, unless this finding was accompanied with reduced fertility or pathological condition. Cupps et al.(1953) noted 19 to 43.7 percent in low fertility bulls and attributed to partly to the deffi-ciency of follicular stimulating hormone.

Shukla and Bhattacharya (1949) recorded a total abnormalities of spermatozoa ranging from 5 to 11 percent with a mean of 7.7 ± 0.1 percent in murrah buffaloes. Prabhu and Bhaya during the year 1950 recorded an abnormal percentage of 5.09; 4.18 and 4.99 percent in normally fed, 25 percent less and 50 percent less protein fed buffaloe bulls respectively. Taking ejaculates at weekly intervals Prabhu and Bhattacharya (1951) recorded an abnormal percen-tage of 5.14 for first and 4.16 for second ejaculates in buffaloe bulls. Prabhu and Sharma (1952) had estimated abnormal spermatozoa of buffaloe semen by counting 300 spermatozoa. The same authors (1953) reported a total abnormalities of 3 to 11 percent in buffaloe bulls.

Prabhu and Bhattacharya (1954) studied the percen-tage of abnormal spermatozoa with Indian water buffaloes, collecting semen on teaser cows, on heat and not on heat, at intervals of 24-48 hours and week days and recorded a percentage of abnormal spermatozoa for first ejaculations as 11.3 percent and 14.00 percent at 24-48 hours intervals on the two types of dummies and 10.44 percent and 10.58

percent at intervals of 7 days . For the second ejaculate, in succession made, they recorded 11.69 and 11.75 percentage and 9.06 and 10.39 percent for 24-48 hours and 7 days collection intervals; the difference found was not significant. Kushwaha et al.(1955) while studying the seasonal variations in semen characters of murrah bulls studied percentage of abnormalities in two successive years as 6.62 and 7.8 during 1949-50, 1950-51 being 5.88 percent in spring, 7.39 percent in summer, 7.61 percent in Autumn and 7.95 percent in winter. Hafez and Darwish (1956) noted an abnormal spermatozoa of 21.1 percent in Egyptian buffaloe bulls with a range of 15 to 22 percent, when collections were made at 24,12,8 and 2 hours intervals with four successive ejaculates. The percentage of abnormal spermatozoa was affected highly with successive ejaculates irrespective of intervals. Madatov (1956) found the abnormal spermatozoal percentage in Russian buffaloe bulls as three percent with a range of 1 to 11.5 percent.

Sayed and Oloufa (1957) studying the effect of frequency of collection in comparision of cattle and buffaloes stated the percentage of abnormal as 5.06 and 4.71 percent in ejaculates at weekly intervals and 7.66 and 11.19 percent in thrice in a week ejaculations which are statistically highly significant. Oloufa et al.(1958) recorded a live percentage of 85.83 and 83.41 during summer and over all average for the year in Egyptian

buffaloe bulls respectively. Roy (1958) recorded in murrah buffaloe bulls, maintained at Mathura a total abnormalities of 17.2 ± 1.2 percent varying with different seasons; 10.1 ± 1.1 percent in rainy season, 12.4 ± 2.9 percent in autumn; 15.2 ± 2.2 percent in summer 21.1 ± 2.5 percent in spring and 23.6 ± 2.1 percent in winter. Lowest was recorded in rainy season and highest in winter season. Naidu (1960) recorded an average of sperm abnormalities as primary and secondary with 2.6 ± 0.29 and 4.46 ± 0.07 percent respectively in murrah buffaloe bulls.

Sengar and Sharma (1964) recorded spermatozoal live percentage of 83.6 ± 1.3 in first ejaculate, while Misra and Sengupta during the same year recorded, in buffaloes under hot wind, protected and non protected groups in summer a total sperm of 1527 ± 100/ millilitre 10^6 and 1104 ± 352 /millilitre x 10^6 with a live sperm of 1399 ± 105.00/ millilitre x 10^6, and 767 ± 393 /millilitre x 10^6. Saxena (1965) recorded the abnormal sperm percentage of 9 ± 0.3 in Indian buffaloe bulls. Viswanath Pillai (1965) recorded an abnormal percentage of spermatozoa as 9.54 ± 1.62 percent for first ejaculate and 8.67 ± 1.48 percentage for second ejaculate.

Biometrics of spermatozoa: Withe the good number of studies made with semen characteristics of bull no single reliable test could be postulated for the selection of bulls for

their fertility . Savage, Williams and Fowlers (1927) studied the frequency distributions of head length of bull sperm and found that the distribution was normal for bulls of normal fertility and only slightly skewed for bulls of low fertility. Macgregor (1941) described the spermatozoa of buffaloe bull as more rectangular than bull sperm and stated that the pale stained portion of the head is narrower. Mukherjee and Bhattacharya (1949) had measured the head length, head breadth and tail length with ocular screw micrometer, included only the maximum length and maximum breadth of sperm head and found 6.832 ± 0.373 μ as head length, 4.010 ± 0.051 μ as head breadth and 52.248 ± 0.254 μ as tail length. Mahmoud (1952) made a comperative study on the morphology of spermatozoa of Egyptian buffaloes and native bulls and comparison is given in table A. Comparing the spermatozoa of buffaloe and bull spermatozoa Guha et al.(1959) recorded the measurements of the buffaloe spermatozoa on anaverage 8.0 x 5.0 ; 12.5 μ and 54.6 μ for buffaloe bull as compared to bull sperm head, middle piece and tail 10.0 x 5.6 μ 13.9 μ and 60.3 μ.

T A B L E A.

Spermatozoal measurements (Mahmoud 1952)

Parts of sperm	Buffaloe bull.			Bull		
	Av.	S.D.	C.V.	Av.	S.D.	C.V.
Head length in /u	7.436	0.442	5.9	9.126	1.326	14.5
Head breadth (anterior)	4.264	0.520	12.3	4.732	0.494	10.4
Head breadth (posterior)	3.172	0.442	14.0	2.730	0.520	19.4
Neck.	0.442	0.208	47.1	0.650	0.338	51.4
Mid piece length.	11.648	0.936	7.9	12.558	0.624	4.9
Breadth of mid	1.092	0.286	26 .2	1.066	0.236	26.8
Length of tail.	42.882	3.042	7 .1	46.28	6.84	13.2 0.32
Ratio of = =Head breadth anterior / =Head breadth posterior	1.34	0. 33		24.8	1.79	1.7

Naidu (1960) had recorded a head length and breadth with murrah bull spermatozoa as 0.771 and 0.511 micrometer units respectively in fresh semen and he also studied the effect of 4 diluters separately and found the head length as slightly lesser than that of fresh semen. No significant difference could be noticed and the mean head length and head breadth recorded were 7.125 ± 0.01875/u and 4.7664 ± 0.0093 /u . Venkataswamy and Vedanayagum (1962) studied biometrics of cattle and buffaloes and results were tabulated as follows.

THE BIOMETRICS OF NORMAL SPERMATOZOA OF DIFFERENT BREEDS.

Species &&	Number of Animals	Number of Spermes	Head (in μ) Length	Head (in μ) Breadth	Mid Piece length in μ	Tail length in μ.
Sindhi	8	512	9.17	5.12	13.92	47.98
Hallikar	3	240	9.51	5.36	14.98	49.07
Ongole	2	200	8.25	4.63	13.95	41.32
Gir	2	235	9.40	5.36	14.63	48.90
Murrah buffaloe bull.	7	535	7.40 ±1.07	4.48 ±1.29	12.41 ± 0.85	43.61 ± 5.56

when the bulls are grouped according to the age, the cells of the older bulls (above 10 years) were found to be significantly longer in head, mid-piece, tail length except those of head breadth of young bulls.

TABLE SHOWING THE EFFECT OF AGE OF BULLS ON THE BIOMETRICS OF THEIR SPERMATOZOA.

Age of bulls	No. of bulls	Head length in μ	Head breadth in μ	Mid piece length μ	Tail length μ
10 years and above.	7	9.38	5.04	14.47	46.95
Below 10 years.	3	9.03	4.99	13.88	45.81

Disproportionate long cells were noted in one 15 year old murrah bull and also in that of two sindhi bulls aged 12 and 13 years but non in young bulls and is approximately one and a half times than the normal cells. The percentage of cells vary 1 to 5. This seems to be associated with a

questionable fertility. The species and the breeds significantly differ with reference to biometrics of gametes.

Rathore (1965) studied the effect of egg yolk citrate and egg yolk glycine diluters on morphology of ram spermatozoa after 72 hours of preservation using eosin and nigrosin stain. The head length and head breadth of the unstained spermotozoa were significantly less than those before preservation, given as below.

	Head length (in μ)	Head breadth (in μ)
Fresh spermatozoa	9.4795	6.1621
In egg yolk citrate.	9.4263	6.1405
In egg yolk glycine.	9.4377	6.1096

Saxena (1965) studied the effect of four diluters on the morphology of buffaloe bull spermatozoa at 72.120 and 168 hours of preservation and found no significant difference in head length and head breadth of the spermatozoa. The head length and head breadth in fresh semen were $7.6822 \pm 0.0729\ \mu$ and $4.8219 \pm 0.0187\ \mu$. He had studied the same effects on Hariana bull and ram spermatozoa and no significant difference could be observed. Kumar (1966) studied the effect of four dilutors at intervals of zero hours, 48, 96 and 144 hours and recorded a variability of head length and head breadth in preserved semen up to 144 hours but there was no significant difference. Joshi (1966) studied on ram semen

the effect of diluents in four series. The differences in the measurements of head length and head breadth of spermatozoa at zero hours, 48, 96 and 144 hours were not found to be significant.

Extension and preservation of semen: As far back as 1803 spallanzani, was first scientist to observe that freezing stallion semen in snow or winter cold did not necessarily kill the "spermatic vermiculi" but held them in motionless state until exposed to heat and postulated that sperm life can be prolonged by cooling. At the end of 19th century Professor Haffman of stuttgart had recommended supplementary insemination after natural mating and he was the first scientist to dilute stallion semen in cows milk and do intra uterine insemination. The prime importance of using diluter is to ensure breeding of good number of females with the semen from high potential sires and to enable the control of venereal diseases. As such the dilution fluid should have the properties of maintaining the life of spermatozoa for a longer time with considerably the same fertilising capacity, protecting from cold shock, supplying nutrient to spermatozoa with same isotonicity of the seminal plasma having a good buffering capacity to nullyfy the metabolic end products of spermatozoa. The components of the diluter should also be of easily available type free from bacterial and infectious organisms. The commonly used diluents at present

are, the earliest found egg yolk phosphate buffer, egg yolk citrate, egg yolk glucose bicorbonate and milk diluters with egg yolk. Egg is being used inevitably in diluters.

Semen extenders: The diluters commonly used for buffaloe bull semen at present are a glucose sodium bicarbonate sulphamezethin Yolk (GBY) and Baffaloe skim milk yolk(SMY) extenders. The glucose sodium bicarbonate sulphamezathir yolk (GBY) was proved to be better and it provides, the readily available energy in the shape of glucose, having a good buffering capacity in combination with sodium bicarbonate, supplimented by bacteriostatic action of sulphamezathin. This diluter was proved to be a better diluter for extension and preservation of buffaloe bull semen with good fertility rates over other diluters. Kamps-chmidt (1951),Gokhale, Srivastav and Prabhu (1958), Naidu (1960) Mahajan and Sharma (1962), Saxena (1965), and Kumar (1966). The milk reported can be used as a semen diluent for buffaloe bull semen by Veeramaniayyar (1944). The skim milk with egg yolk was proved to be a better diluter of present day diluters for semen preservation. It is also easily and cheaply available one. The milk of the same species, of which the semen to be extended was proved to be better than that of other species. The milk of mare was proved better for stallion's semen than cows milk. Mihilov (1949),Michajilov (1950), and the later author further reported that lactose content of milk on

heating splits into glucose and galactose which acts as ready made nutrient to spermatozoa preserved and he further stated that cows milk for bulls semen and mares milk for stallin semen were good. The spermicidal factors present in the milk were reported to have been destroyed by heat treatment at 92° to 95°c for 10 minutes by Thacker et al Almquist (1954), Simunic (1950). Buffaloe milk was recorded to have almost the same pH 6.4 to 6.8 like its semen. Bhimeswara Rao and Dastar (1955) Joskowski (1956) recorded better survival of spermatozoa and better fertility results in skim milk with 10 percent egg yolk even at high dilutions. Buffaloe milk was found to be superior than cows milk to extend buffaloe semen Oloufa and Sayed (1956). Milk yolk diluter was stated to be better diluter than whole milk and reconstituted milk Joshi (1961), Kale (1962) had proved that milk diluters were in general better for preserving buffaloe semen. Kouser (1965) had also proved that skim milk yolk have given better results.

Importense of egg yolk sulphonamides and Antibiotics in semen extenders : The egg yolk added to the diluters was proved to be having :(a) Substances like phospholipides and lipoprotein complexes which were proved to be good resistance and storage factors along its most important character for checking the cold shock to the spermatozoa, while preserving at low temperatures, (b) It is also proved

that substances present, will help in buffering the metabolic end products of spermatozoa, giving necessary metabolites, will act as enhancers and inhibitors of respiration of spermatozoa. The metabolites present in egg are said to have the sparing effect on fructose utilisation and prolonges the keeping quality of semen. These were proved by Phillips and Lardy (1939), Tosic and Walton (1946), Swanson (1948), Stewart et al. (1950), Kampschmidt et al. (1950, 1953), Almquist (1951), Vantierhaven et al. (1952), Srivastav et al. Kok (1953), Black shaw (1954) and Bialy and Black shaw (1957).

The sulphonamides and Antibiotics were also used (1) to act as bacteriostatic and bactericidal factors for gram positive, gram negative and vibriofotus organisms, during preservation of semen at low temperatures. (2) These were also proved to be non toxic to spermatozoal survival at optimal levels with a wide range and prolong the life of spermatozoa (3) These substances were also proved to have a capacity that depresses, the fructose utilisation and total reduceble substances and also derpresses the sperm matabolism, prolonging the livability of spermatozoa in the extenders, at low temperatures with slight decrease of their concentration. These were proved by Knodt and Salisbury (1946), Almquist et al Foote and Salisbury (1947), Almquist (1949, 1951), Adler and Rasebeach Cecilbranton and Prather, Eugenia orthey and Gilman (1953), Dilliello et al. (1957) and Sharma et al. (1962.

CHAPTER -III.

MATERIAL AND METHODS.

MATERIALS AND METHODS.

The studies were made with Murrah buffaloe bulls maintained at Semen Bank ,Patna. Five Murrah bulls were selected atrandom for this experiment. Their age varied from six to ten years. These bulls were maintained under the same identical conditions of management. They were given excercise in the early hours in the morning. Concentrates were supplied twice daily at the rate of 1.5 kilograms in the morning and the same quantity in the evening. The ingredients of concentrate constituted wheat bran, ground nut oil cake, salt and mineral mixture. The bulls were fed with 15.5 kiolograms of green fodder and 5.5 kilograms of hay. The bulls were given water at libidum and bath twice a day and morning bath was given about one hour earlier before the schedule of semen collection programme.

The experiment period lasted from may 1966 to July 1966.

An anoestrous buffaloe cow was used as dummy for collection of semen from bulls. Every bull was allowed one false mount before taking the first ejaculate for this study. 30 semen collections, from each bull six collections, were studied, using artificial vagina. Collections were taken twice in a week from each bull in the morning hours from 8.30 A.M. to 11.00 A.M. under identical conditions. A separate clean artificial

vagina with 40-45° c temperature with an insulating bag to the semen collection vial was used for collecting semen. Only first ejaculate of the semen was used for the study. The semen soon after collection was taken into the laboratory and kept in a water bath at 32°c while all the tests were conducted. The technique followed was standardised for examination of the required semen characters, before proceeding with actual observations. The following characters along with reaction time were studied :

Reaction time which is the time taken by the bull to mount and give ejaculate, from the release of the bull to the teaser, was measured in seconds with the aid of a stop watch as studied by Mukherjee and Bhattacharya (1951)

CHARACTERS OF NEAT SEMEN:

Volume of the ejaculate was noted directly from the graduated collection vial up to one tenth of millilitre Kushwaha et al.(1955).

Colour and consistency was noted by visual appearance soon after the collection by keeping the collection vial against a white paper and was graded as per the study of Mahmoud (1952).

a) Thick Creamy.
b) Thick Milky.
c) Milky.
d) Thin Milky.
e) Opaque
f) Watery.

Initial motility after noting the volume, colour and consistency initial motility with individual motility was studied under microscope. A clean glass slide, with a composite sample of small semen drop, was taken and warmed up at the back of the palm. The drop was spread and viewed under microscope under low power objective for wave motion and at the edges to ascertain an approximate percentage of live spermatozoa that are progressively motile. This was done before the edges got dried. From these observations the motility rating was made in the following way from 0 to 5 (0.5 representing 10 percent livability.) Herman and Swanson (1941).

Grade	Percentage of live sperm.	Movements
0	No motile spermatozoa	No movement.
0.5 to 1.0	10 to 20 percent.motile spermatozoa	No waves, movements mostly weak and oscillatory.
1.0 to 1.5	20 to 30 percent motile spermatozoa .	With vigorous and rapid motion without wave motion.
1.5 to 2.0	30 to 40 percent motile spermatozoa.	-do-
2.0 to 2.5	40 to 50 percent motile spermatozoa.	Wave formation with slight whorls which move slowly accross the field.
2.5 to 3.0	50 to 60 percent motile spermatozoa.	-do-

Continued next page.

Grade	Percentage of live sperm.	Movements
3.0 to 3.5	60 to 70 percent motile spermatozoa.	With swirling motion, rapid and vigorous movement, waves and whorls or eddies form and change with great rapidity.
3.5 to 4.0	70 to 80 percent motile spermatozoa.	-do-
4.0 to 4.5	80 to 90 percent motile spermatozoa.	With movements of churning of the swirls and eddies are extreamly rapid and can be compared with the tides in sea.
4.5 to 5.0	90 to 100 percent motile spermatozoa.	-do-

Power of Hydrogerion (pH) was measured immediately after the semen collection with the help of B.D.H. capillary set. A small drop of neat semen was taken in a small watch glass and added equal quantity of Bromothymol blue indicator with a pH range of 6.2 to 7.6; mixed well, and was taken in the capillary and compared to the different matches of the capillary set of the same indicator and pH was noted Mukherjee and Bhattacharya (1951).

Sperm concentration was determined with the help of a haemocytometer slide. A counting fluid, with 2 percent sodium chloride and 1 percent eosin, of 0.9 millilitre was taken in one clean glass test tube and in other test tube 9 millilitre was taken with the help of a pipette Smith and Mayer (1955). The semen vial kept in water bath was taken and rotated between the palms

gently for uniform distribution of the spermatozoa. 0.1 millilitre of the neat semen was taken in a micropipette and added to the test tube which cortained 9.9 millilitre courting fluid making the concentration of semen to 1:100. The test tube with diluted semen was rotated between palms to ensure even mixing of neat semen added and one millilitre of this diluted fluid was taken and added to 9 millilitre of the diluting fluid kept in another test tube making a final dilution of 1:1000. Thus these tubes were brought to College laboratory and estimated for sperm concentration. The tube having 1:1000 dilution was rotated to mix the diluted semen evently and the haemocytameter adjusted with the counting chamber under focus, was charged with a drop of the well mixed sample with help of a Pasteur pipette and allowed the fluid under cover glass to settle the spermatozoa for 10 minutes. Spermatozoal count was done with corner big squares to avoid clumsyness of the central division. All the 4 corners with 16 large squares were counted to avoid error and took the mean for 16 large squares within the triple lines. A zero added to the derived figure gives the concentration of the spermatozoa in millions per millilitre.

Percentage of dead spermatozoa was estimated by staining the semen sample with one percent eosin and five percent nigrosin compound stain, prepared in three percent sodium

citrate (analar) solution, immediately after semen collection and taking smears on clean slides, Swanson and Beardon (1951). A drop of neat semen was taken on a clean slide and three or four drops of stain, kept in water bath, was added and mixed by blowing air with a pipette, instead of a glass rod, which the author felt that it may help in minimising the artefacts.

The smear was prepared in the following manner :-

A slide held with thumb and the middle finger of right hand was touched with its lower edge to the semen and stain mixer and the smear was prepared by pushing the same at 45° angle edge, on the slide held in the left hand between index finger and thumb, applying a little pressure with index finger of right hand on the slide that is pushed. Then the smear was dried in air, keeping in slant. This proceedure carries the stained semen with the slide, minimising the crushing of spermatozoa. Assesment of the dead spermatozoa were made by counting 300 spermatozoa (Rollinson 1951) atrandomly selected fields under oil immersion. The slides were mounted with a cover glass with D.P. (GURR). The dead sperms were stained with eosin, and live spermatozoa were unstained, with the homogenous violet-brownish background of nigrosin.

Morphological abnormalities the smears taken for live and dead count were used for studying abnormalities, according

to Swanson and Beardon (1951) and Hancock (1947). The morphological characters were studied as per Bloms (1950) classification under different heads.

a) Primary abnormalities.

b) Secondary abnormalities.

Under (a) primary abnormalities :pyriform head, tapering head, narrow head, dwarf head, giant head, short thick head, other abnormal heads, abnormally stained heads, loose abnormal heads, sundry abortive forms, normal heads with highly coiled tail,double forms, abaxially attached middle piece and middle piece defects, were grouped. Under middle piece defects the author included short middle piece, swollen middle piece, and abortive middle piece.

Secondary abnormalities: loose normal heads, spermatozoa with proximal protoplasmic droplets spermatozoa with distal droplets,bent tails and detached galea-capitis were grouped. The slides were read under binocular microscope with oil immersion with a magnification of about 1200 times. 300 sperms were typed under different heads.

<u>Biometric measurements of spermatozoa</u> the smears were used for this also. For each sample of semen five slides of uniform thick smears were used for this and from each smear six unstained straight spermatozoa were selected

atrandom and measured by using an ocular micrometer with 10^x eye piece under oil immersion with a magnification of 1000 times with blue light filter. The head length, head breadth and tail lengths were measured ; 30 spermatozoa were measured for each ejaculate in the fresh semen sample of each bull; total of 180 spermatozoa were measured for each bull, for six ejaculates taken. A total of 900 spermatozoa were measured from five bulls for this purpose.

Mensuration: To measure the head length posterior edge of the head was taken as tangent at straight angles to the main axis of the micrometer and the anterior position of the head was located and the divisions on the ocular micrometer were noted. In the same way head breadth was also measured. The measurements were taken at the maximum possible dimensions of the head. The length of the tail was determined by placing the micrometer parallel to the tail and number of divisions were noted. The occular divisions were converted into microns (/u) and worked out the means of biometrics of the spermatozoa (each ocular division = 0.137 divisions of stage micrometer or 1.375/u).

The second part of the experiment, mainly comprises the study to see the effect of diluters on the dimensions of the spermatozoal head. The split samples of the same semen sample were preserved in glucose sodium bicorbonate sulphamezathin egg yolk diluter and

Skim milk egg yolk diluter. Slides were prepared at every 24 hours interval up to 72 hours of preservation under refrigeration condition at 4° ± 1°c temperature. Biometries of the unstained (live) spermatozoal heads were determined as in fresh semen at every 24,48 and 72 hours of preservation. A set of 10 slides, five for each diluted semen sample were prepared for each interval. From each slide again six unstained spermatozoa were selected atrandom and measurement of the head length and head breadth were taken. Total of 900 spermatozoa were measured for each interval, for the semen sample preserved in each diluter, for the five bulls, of six collections for each bull. The over all total spermatozoa measured in each diluter for the three intervals were 2700 totaling to 5400 in both the diluters. Hence the grand total of the spermatozoa measured in the fresh semen samples and as well in the preserved samples were 6300.

The diluters used for preservation of semen were :

(a) Glucose-sodium bicarbonate-Sulphamezathin (1.c.1) diluter used was prepared with

1) 5 percent dextrose solution (Analar)
2) 1.3 percent sodium bicarbonate solution(Analar)
3) 2 percent sulphamezathin (1.c.1) solution

Those three solutions were prepared in double glass distilled water and autoclaved at 15 lbs pressure

for 15 minutes and cooled. All the solutions were filtered before autoclaving. The so prepared solutions were mixed in a ratio of 4:1:1 respectively and tested for pH. It was varying from 6.6 to 6.8 which was required for. Then the diluter was preserved in refrigerator while in use. This was the technique followed by Gokhale in 1958. Every day, when required, the diluter was prepared by mixing 20 percent fresh egg yolk with 500 I.U. penicillin and 500 /µg of streptomycine sulphate.

(b)
Skim milk diluter was prepared from buffalo skim milk obtained daily from Patna Milk Supply Scheme. This was heated in a sterilised flask upto 92° to 95° temperature for ten minutes in a water bath. Cooled and cream layer at the top, if any, was avoided by decanting and used as a buffer. For this also 500 i.u of penicillin and 500 /µg of streptomycine was added and kept in refrigerator until further use Almquist et al.(1954). When diluent was to be prepared ten percent fresh egg yolk was added and throughly mixed Joshi (1961). Both diluters were made ready for use. The dilution rate was made uniform throughout the study as 1:10. The diluted sample of semen was examined under microscope immediately after dilution and at every 24,48 and 72 hour interval of preservation for the life of spermatozoa before preparing the stained smears for biometrical measurements.

Sterilisation:-

All the glass ware and the other equipment used in the laboratory were washed and sterilised by the corventional methods. The solutions were prepared with the double distilled water.

Statistical Analysis:- was done according to Snedocor (1961).

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CHAPTER -IV.

EXPERIMENT AND RESULT.

E X P E R I M E N T

The reaction time ... ction time calculated for
semen samples were examined ... able (1) and represented
... gnificant values. Graph No.1.

(1) Volume of the ejaculate.
(2) Colour and consistency. ... mean volume was worked out to be
(3) Initial motility. ... 2 and graph 1) with a 63.50
(4) Hydrogen ion concentration. ... lation. The range was 8.8 milli-
(5) Sperm concentration. ... s) given in (Table 2). Analysis
(6) Percentage of dead sperm ... re was no significant variation
(7) Morphological abnormalities ... lections. The analysis of
(8) Biometrics of sperms ... The bullwise average volumes
(9) The effect of Glucose ...
mezathine yolk and skimmilk ... ollowing catogaries could be
of head length and head ... ercentages.
preservation at 4° ± 1° ...

The average ... RCENTAGE OF THE COLOUR AND
together with their ... RRAH BULL SEMEN .
and coefficient of variation ...
estimates are given in table ...

%	Percentage.
	23.33
	33.33
	30.00
	13.33

Reaction time: The average ... erved to be 56.66 percent with
be 152.60 ± 27.17 seconds ... ercent and thin consistency of
of variation. The range ... aculates studied.
given in table 2. The ...
collection was observed ...
data to 'F' test. The ... tivity of spermatozoa was noted
... d graph 1) with a coefficient of

EXPERIMENT AND RESULT.

The reaction time of the bulls was noted and the semen samples were examined for the following characters:

(1) Volume of the ejaculate.

(2) Colour and consistency.

(3) Initial motility.

(4) Hydrogen ion concentration (pH)

(5) Sperm concentration.

(6) Percentage of dead spermatozoa.

(7) Morphological abnormalities.

(8) Biometrics of spermatozoa.

(9) The effect of Glucose sodium bicarbonate sulphamezathine yolk and skim milk yolk buffers on the biometrics of head length and head breadth at 24, 48 and 72 hours of preservation at $4^{\circ} \pm 1^{\circ}$c temperature.

The average measures of all the characters together with their standard deviation, standard error and coefficient of variation were worked out. These estimates are given in tables 1 to 15.

Reaction time: The average reaction time was recorded to be 152.60 ± 27.17 seconds with 97.40 percent of coefficient of variation. The range was 670 seconds (15 to 685 seconds) given in table 2. The insignificant effect of bulls and collection was observed on reaction time by subjecting the data to 'F' test. The same has been entered in Table 4.

The details of the average reaction time calculated for different bulls are shown in table (1) and represented graphically showing the non significant values. Graph No.1.

Volume of the ejaculate : The mean volume was worked out to be 3.27 ± 0.38 millilitres (Table 2 and graph 1) with a 63.50 percent of coefficient of variation. The range was 8.8 millilitres (0.7 to 9.5 millilitres) given in (Table 2). Analysis of variance revealed that there was no significant variation between bulls and between collections. The analysis of variance is given in table 4. The bullwise average volumes were shown in table 1.

Colour and consistency: The following catogaries could be observed which are given in percentages.

TABLE SHOWING THE PERCENTAGE OF THE COLOUR AND CONSISTENCY OF MURRAH BULL SEMEN .

S.No.	Name of the character.	Percentage.
1.	Thick creamy	23.33
2.	Thick Milky.	33.33
3.	Milky	30.00
4.	Thin milky	13.33

The thick consistency was observed to be 56.66 percent with medium consistency of 30.00 percent and thin consistency of 13.33 percent of the total ejaculates studied.

Initial motility: The mean activity of spermatozoa was noted to be 3.98 ± 0.116 (Table 2 and graph 1) with a coefficiert of

variation of 15.98 percent. The range was 2.00 (2.5 to 4.5) as shown in table 2. The analysis of variance given in table 4 shows no significant variation between bulls and between collections.

Hydrogen ion concentration (pH):The mean pH was recorded to be 7.07 ± 0.03 (Table 2 and graph 1) with a coefficient of variation of 2.27 percent with a range of 0.6 (6.8 to 7.4). The analysis of variance was run to see the effect of bull and collection and the "F" test indicated that non of them was significant (Table 4).

Sperm concentration: The mean sperm concentration was calculated as 1071.73 ±70.96 millions per millilitre (Table 2 and graph 1) with a coefficient of variation of 36.36 percent. The range was found to be 1571 millions per millilitre (409 to 1980). The data analysed for F test did not show any significant effect of bull and collection (Table 4.) The concentration varied with consistency and colour as shown in the table.

TABLE SHOWING THE VARIATION IN CONCENTRATION OF SPERMATOZOA WITH COLOUR AND CONSISTENCY OF MURRAH BULL SEMEN (IN MILLIONS).

Sl.No.	Name of character	Measures in millions	
		Minimum	Maximum
1.	Thick Creamy.	1390	1988
2.	Thick Milky.	1095	1380
3.	Milky.	560	1000
4.	Thin milky	409	560

PART - VII.

REFERENCES.

GRAPH -1.

KEY	SCALE.
Reaction time.	1 cm = 50 seconds.
Volume (Volume of the ejaculate)	" = One millilitre.
pH (Hydrogen ion concentration)	" = One unit.
Motility (Initial motility)	" = One unit.
Concentration (Sperm concentration)	" = 2.00 millions.
Dead Percentage(Percentage of dead sperm).	" = 6 percent.
Morphological abnormalities.	" = 10 percent.

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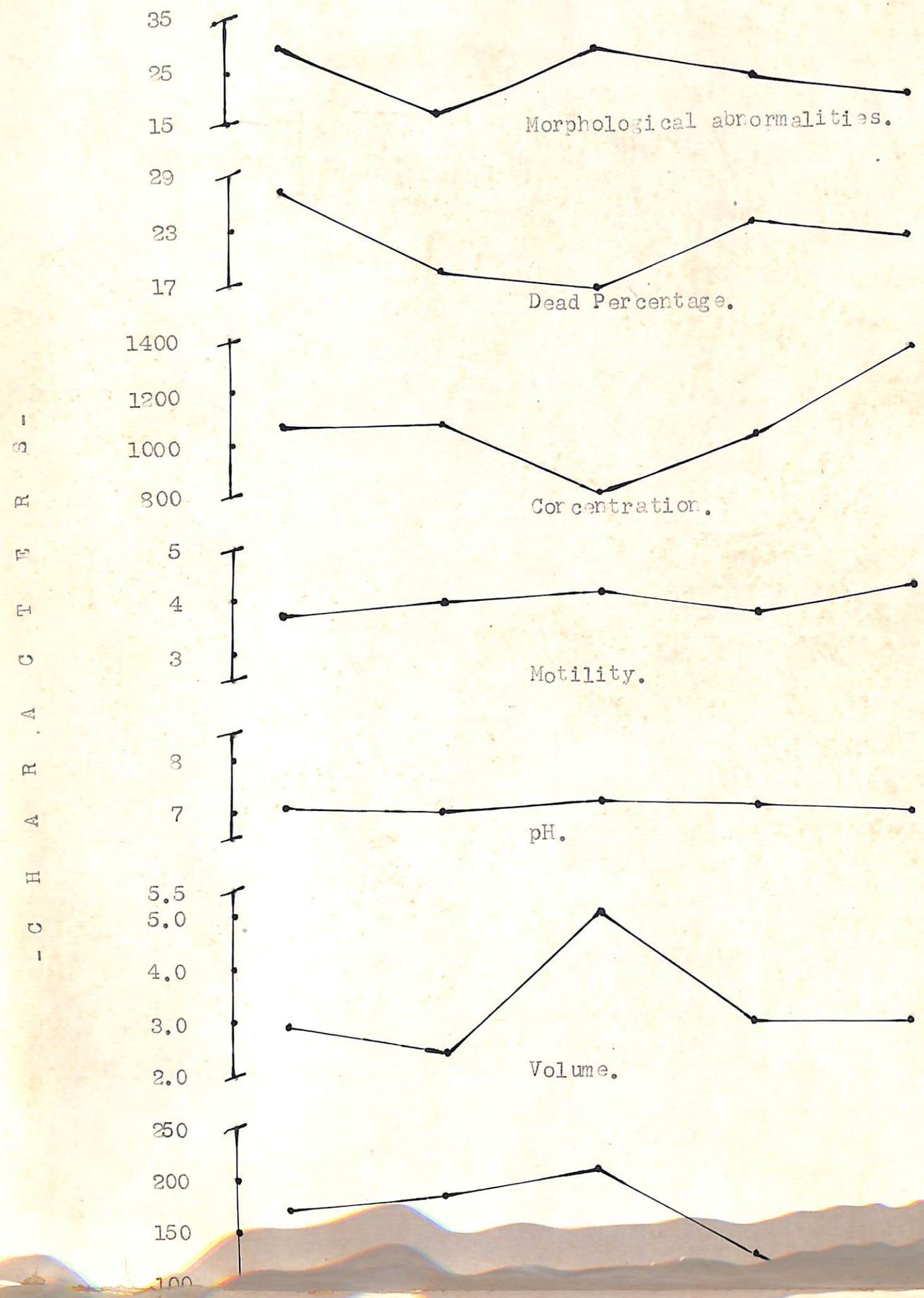
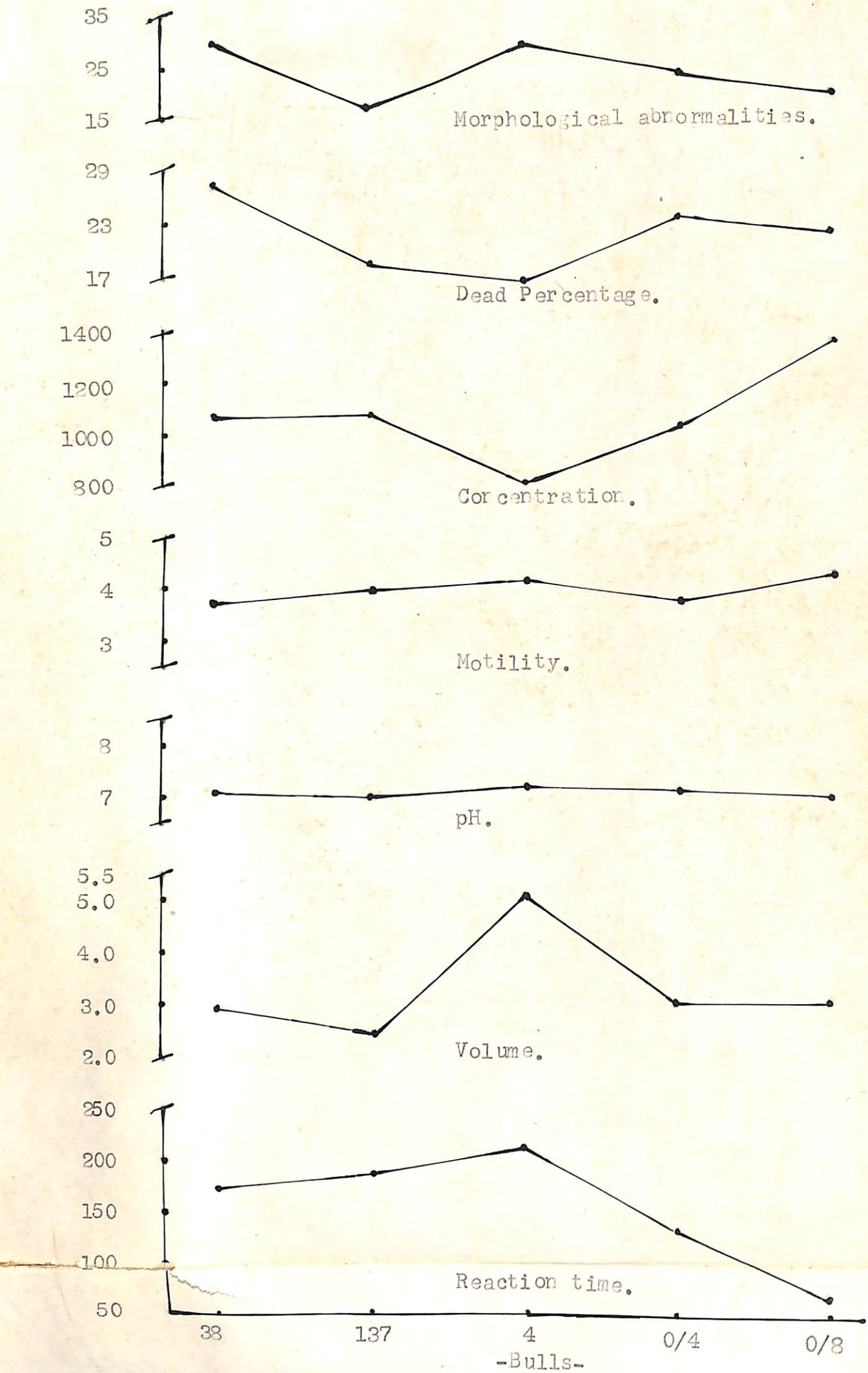
GRAPH- 1.


- C H A R A C T E R S -
35
25
15
Morphological abnormalities.
29
23
17
Dead Percentage.
1400
1200
1000
800
Concentration.
5
4
3
Motility.
8
7
pH.
5.5
5.0
4.0
3.0
2.0
Volume.
250
200
150
100

GRAPH- 1.



GRAPH SHOWING AVERAGES OF DIFFERENT SEMEN CHARACTERS.

Percentage of dead spermatozoa: The mean percentage of dead spermatozoa estimated from eosin nigrosin stained smears by courting 300 spermatozoa was 21.78 ± 1.37 percent(Table 2 and graph 1) with a coefficient of variation of 42.44 percent. The range recorded was 46.00 percent (3.00 to 54.00).The data were subjected to "F" test (Table 4) by running analysis of variance and no significant effect of bull and collection was detected.

Morphological abnormalities: These were estimated by analysing 300 spermatozoa according to the classification of Blom (1950). The average abnormalities were worked out to be 24.427 percent (Table 1 and graph 1). Out of which (a) Primary abnormalities were 13.452 percent with a range of 29.333 percent (6.0 to 35.333) percent and (b) secondary abnormalities were 10.975 percent with a range of 21.00 percent (1.667 to 22.667)percent (Table 2). The details of percentages worked out Bull wise was shown in table (1) and the details of percentages of every abnormality is shown in table 3. Among the primary abnormalities 7. 139 percent were of head abnormalities, upto 4.181 percentage were of midpied abnormalities and 2.132 percent were of tail abnormalities and double forms. Some of the abnormalities are shown in microphotographs.

Biometrics of spermatozoa :-

(a) Neat semen: The mean head length, head breadth, tail length including middle piece and total length of unstained

PHOTOMICROGRAPHS.

PLATE - 1.

NORMAL SPERMATOZOA.

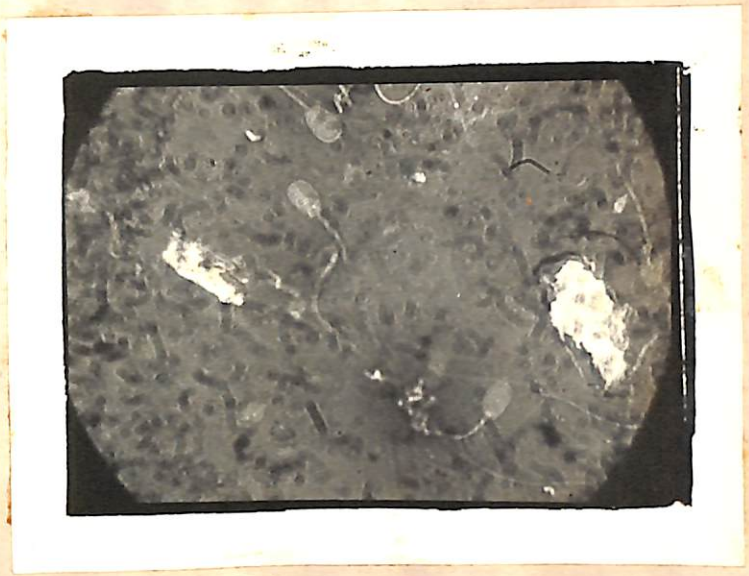
PLATE -2.

SPERMATOZOA "PYRIFORM HEAD "

PLATE -3.

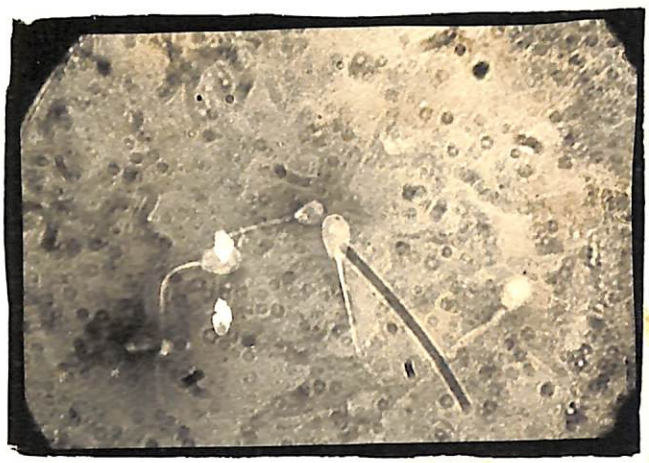
SPERMATOZOA " PYRIFORM HEAD WITH FILIFORM TAIL"

PLATE -4.



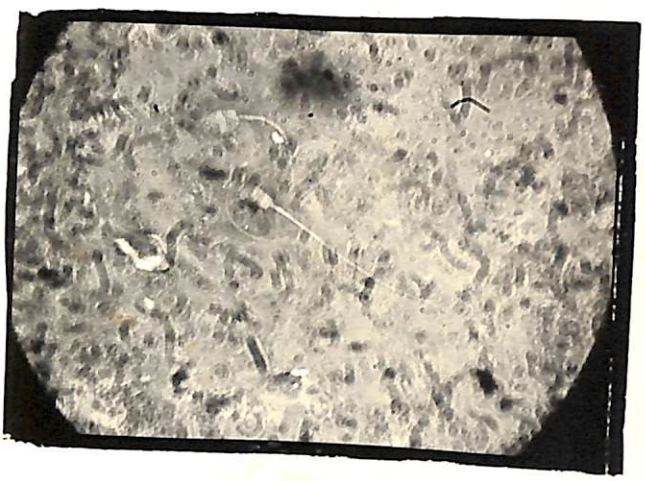
SPERMATOZOA " NARROW TAPERING HEAD "

PLATE -5.



SPERMATOZOA " DWARF TAPERING HEAD "

PLATE -6.



SPERMATOZOA " NARROW HEAD"

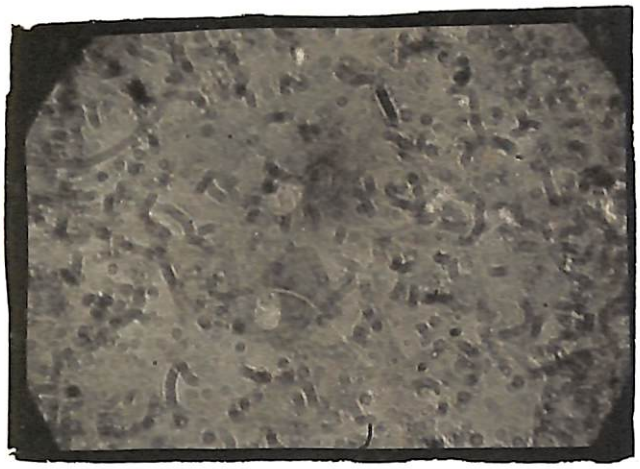
PLATE -7.

SPERMATOZOA " GIANT HEAD, AND DETACHED GALEA CAPITIS".

PLATE -8.

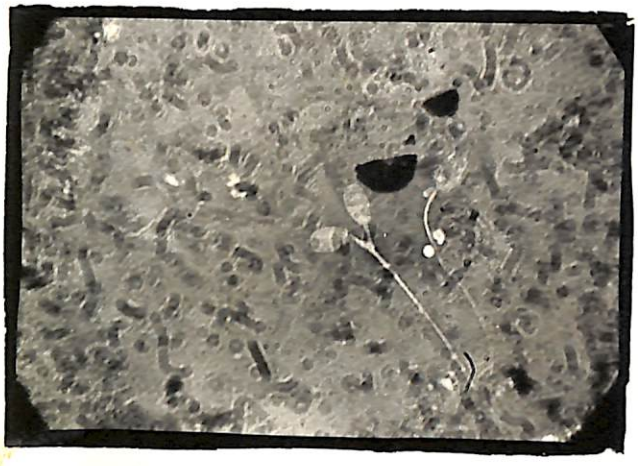
SPERMATOZOA " OVAL HEAD"

PLATE -9



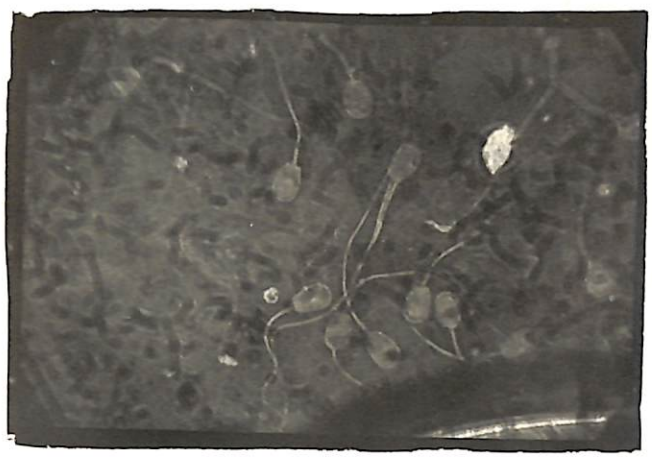
SPERMATOZOA "BROKEN NECK AND COILD TAIL".

PLATE -10.



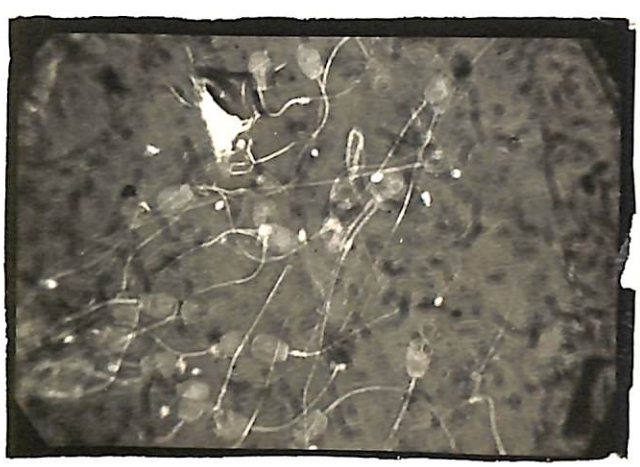
SPERMATOZOA "DOUBLE HEAD".

PLATE -11.



SPERMATOZOA " DOUBLE MIDDLE PIECE ABAXIALLY ATTACHED".

PLATE -12.



SPERMATOZOA "DOUBLE TAIL".

PLATE -13.

SPERMATOZOA "DOUBLE MIDDLE PIECE WITH OVER TAPED DOUBLE HEAD.

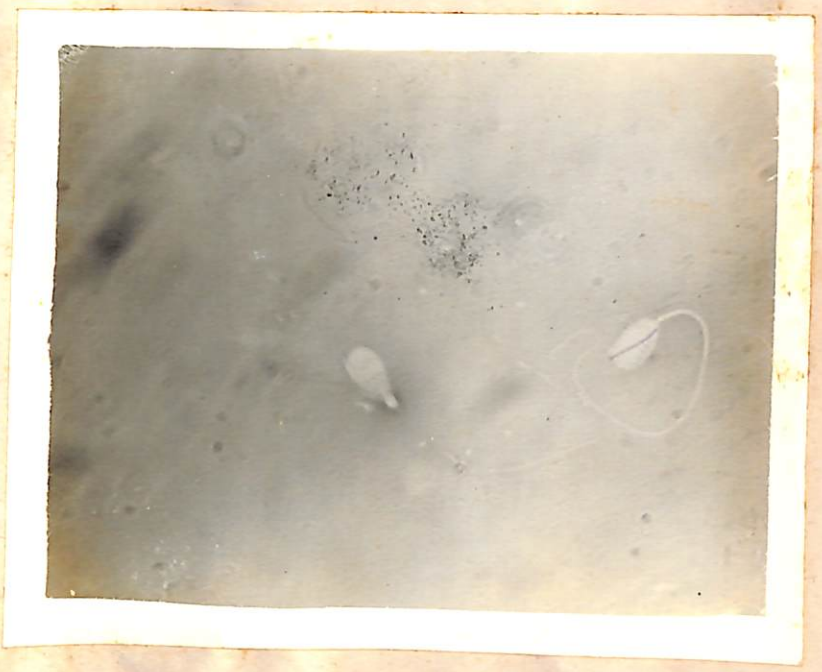
PLATE - 14.

SPERMATOZOA "ABAXIALLY ATTACHED MIDDLE PIECE "

PLATE -15

SPERMATOZOA "SHORT MIDDLE PIECE"

PLATE -16



SPERMATOZOA "LONG MIDDLE PIECE SWOLLEN AT THE END".

PLATE -17.

SPERMATOZOA "ABORTIVE MIDDLE PIECE".

PLATE-18.

SPERMATOZOA " PROTOPLASMIC DROPLET AND BENT TAILS"

spermatozoa were calculated to be 6.994 ± 0.015 , 4.443 ± 0.019, 54.173 ± 0.102 and 61.173 ± 0.207 microns (μ) respectively (Table 2). The size of spermatozoa of different bulls are shown in Table 5 with a coefficient of variation of 0.214 ; 0.432, 0.190 and 0.340 percent respectively. The range for head length, head breadth tail length and total length was 5.500 to 9.625; 2.750 to 5.500 ; 41.250 to 63.250 and 48.125 to 71.500 microns (Table 2) respectively. The data were subjected to "F" test and found that there is no effect of bull and collection on head length, head breadth but tail length was found to be influenced by bull which was statistically significant at even 1 percent level (Table 6).

EFFECT OF DILUENTS

At 24 hours of Preservation: The mean head length and head breadth of spermatozoa preserved in glucose sodium bicarbonate egg yolk diluter were calculated to be 6.835 ± 0.009 and 4.085 ± 0.013 microns respectively, where as in skim milk egg yolk diluter were 6.817 ± 0.100 and 4.174 ± 0.140 microns respectively with a coefficient of variation 0.14 and 0.32 percent in the former diluter and 1.50 and 3.33 percent in the next diluter respectively. (Table 7). The mean head lengths and head breadths for individual bulls are given in Table 9 and 10. The data were analysed and "F" test was run, which revealed no significant variation in head length between bulls but a significant variation in head length was seen at one percent level between diluters. This test does not show any significant interaction between bulls and

diluters. (Table 8). With regards to the head breadth the "F" test revealed a significant variation at one percent level between bulls and between diluters and had show interaction also at the same level. (Table 8).

(c)
At 48 hours preservation: The mean head length and head breadth calculated for the spermatozoa preserved in glucose sodium bicorbonate egg yolk and skim milk egg yolk were 6.805 ± 0.010 and 4.065 ± 0.010 microns (μ) in former diluter and 6.831 ± 0.100 and 4.103 ± 0.140 microns (μ) in the later diluter respectively. With a coefficient of variation of 0.15 , 0.24 ; 1.50 and 3.4 percent respectively (Table 7). The mean head length, head breadth with coefficient of variation were shown for individual bulls in table 9 and 11. The data were analysed and the "F" test revealed a significant variation in head length between bulls and between diluters at one percent level without any interaction (Table 8). The "F" test for head breadth revealed a significant variation at one percent level between bulls, betweer diluters and inter action between bulls and diluters Table (8).

(d)
At 72 hours preservation: The mean head length, and head breadth for the spermatozoa preserved in glucose sodium-bicarbonate yolk and skim milk yolk diluter were 6.799 ± 0.011 and 4.021 ± 0.014 microns (μ) in former diluter and 6.853 ± 0.077 and 4.030 ± 0.144 microns (μ) in the later

diluter with a coefficient of variation of 0.016 ; 0.035; 1.12 and 3.53 percent respectively (Table 7). The respective mean head lengths and head breadths with coefficient of variation for individual bulls are shown in tables 9 and 12. The analysis of variance run for the data revealed in "F" test a significant variation with regards to head length and head breadth at one percent level between bulls and between diluter with interaction only for head breadth at one percent level (Table 8).

The data were analysed without taking the preservation time interval into consideration the "F" test revealed significant variations in head length and head breadth at one percent levels between bulls and diluters with interaction (Table 13).

For the analysis of variance carried without taking the diluter into consideration the head length and head breadth had shown a significant variation at one percent level between bulls and between intervals, but interaction was observed only for head breadth at five percent level (Table 14).

In the same way the "F" test carried out for diluter and time interval, without taking the bull into consideration revealed significant variation in head length and head breadth at one percent level between diluters. Only for head breadth between intervals and with interaction for head length at 5 percent level. (Table 15).

The Bar diagram revealed a significant difference of head length and head breadth between unpreserved and preserved spermatozoa at every interval of preservation i.e. 24,48 and 72 hours of preservation in the Glucose sodium-bicarbonate sulphamezathin yolk(GBY) and skim milk yolk (SMY) diluter. At 24 and 48 hours preservation no significant differences in head length between the two diluters GBY and SMY was found, but at 72 hours preservation the head length of spermatozoa preserved in glucose sodium bicarbonate sulphamezathin yolk was lesser than the head length of spermatozoa preserved in skim milk yolk diluter. And significant difference of head breadth between the two diluters was noted at all the intervals of preservation i.e. 24,48 and 72 hours preservation. The head breadth of spermatozoa preserved in glucose sodium bicarbonate sulphamezathin yolk diluter was always lesser than the head breadth of spermatozoa in skim milk yolk at all the intervals of preservation i.e. 24,48 and 72 hours.

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CHAPTER -V.

D I S C U S S I O N.

D I S C U S S I O N.

The reaction time and some of the semen characteristics studied for murrah buffaloe bulls at Patna have been discussed with the findings of different authors.

The reaction time : The mean reaction time recorded for the first ejaculate in buffaloe bulls of Semen Bank at Patna was 152.6 ± 27.17 seconds. Present finding is quite in agreement with the finding of Viswanath Pillai (1965). He has recorded 152.5 ± 5.35 seconds for the first ejaculate at Bombay. Prabhu and Sharma (1954) and Sengar and Sharma (1965) have also recorded similar findings in different ejaculates taken in succession. But Kushwaha et al.(1955) and Oloufa et al.(1958) have shown lesser reaction time in different breeds of buffaloe. As suggested by Oloufa et al this difference in reaction time may be due to difference in genetic make up of individual bull as well as difference in managerial practices and also due to different environment to which animals are subjected. Further Misra and Sengupta (1964) have reasoned that loss of libido is also due to decrease in secretion of testosterone. In present findings the reaction time is a bit high probably only because of stress of collection in hot summer and excessive use of bulls. Prabhu (1956) and Russin workers have suggested that to get lesser

reaction time the environment of collection should be changed and also heat cows should be substituted in place of dummy cow for arousing sexual vigour.

The analysis of variance was run to see the effect of bull and ejaculate on the length of reaction time and it appeared insignificant. This is also tallying with the openion of Prabhu and Bhattacharya (1950, 1951 and 1954) in buffaloe bulls and with the findings of Prabhu and Guha (1951) in bulls.

The graph (1) shows an inverse relationship between concentration and linear relationship with percentage of abnormal spermatozoa.

CHARACTERS OF RAW SEMEN.

Volume of the ejaculate : The mean volume of the ejaculate recorded was 3.27 ± 0.38 millilitres which almost agrees with the silimar work carried out by Veernamaniayyar (1944) and Joshi (1961) recording 3 to 4.5 c.c. and 3.2 c.c. in Murrah buffaloes respectively and was also in agreement with the findings of Prabhu and Bhattacharya (1952) with Utter Pradesh buffaloe bulls and Hafez and Darwish (1956) with Egyptian buffaloe bulls. But the result did not tally with the results of Bhattacharya and Prabhu (1953 and 1954) who recorded 2.85 ± 0.20 c.c. and 1.79 ± 0.38 c.c. respectively in Murrah buffaloes stationed at different places of our

country and also with Kushwaha et al.(1955). Kale (1962), but this was lesser than the findings of Bhattacharya and Prabhu (1952) (3.54 ± 0.13 c.c.) and similar was the findings of Viswanath Pillai (1965) and Kouser (1965) with Murrah bulls. Madatov (1956) with U.S.S.R. buffaloes, Sayed and Oloufa (1957), Oloufa et al.(1958) with Egyptian buffaloes, Misra and Sengar and Sengar and Sharma (1964) with local buffaloe breeds have also recorded higher volumes. The variations in volumes may be due to the breed difference and size of the animal experimented. But in the present study bull and ejaculates have been found to be insignificant in causing any variation in volume which is quite in agreement with the observations of Kuswaha et al.(1955), Prabhu and Bhaya (1950) and Prabhu and Sharma (1962).

The graph (1) shows a negative correlation between volume and semen concentration.

Colour and consistency : The colour and consistency in the present study varied from thick creamy to thin milky and it was in agreement with the description made by Mahmoud (1952)in Egyptian buffaloes, Joshi (1961), Kale (1962) and Kouser (1965) with murrah bulls. But differed with the findings of Viswanath Pillai (1965) in murrah bulls, who could only record milky colour with thick and thin consistency. The percentage of thick creamy was

lesser than that obtained by Joshi (1961), whereas thick milky and milky colours accounted more than his findings. The thickness and creamyness were always associated with more concentration of spermatozoa and followed almost the same description given by Mukherjee and Bhattacharya (1951) with Kumauni hill bulls.

Initial motility : The mean initial motility of all the collections was 3.98 ± 0.116 and is in concurrence with the findings of Kushwaha et al.(1955), Joshi (1961), Kale (1962), Kouser and Viswanath Pillai (1965) who recorded 3.75, 3.84, 4.0 to 4.5 and 4.0 ± 0.5 in murrah buffaloes and Hafez and Darwish (1956), Sayed and Oloufa (1957), Oloufa et al.(1958) with Egyptian buffaloe bulls, Sengar and Sharma (1964) with Utter Pradesh buffaloe bulls where as Prabhu and Bhaya (1950), Prabhu and Bhattacharya (1951), Prabhu and Sharma (1954) and Misra and Sengupta (1964) who recorded 3.68 ± 0.28; 3.07; 2.69 and 1.58 ± 0.09 respectively in other breeds of buffaloes which were definitly lower. The differences may be due to the cold bath given just before collection as stated by Prabhu (1956) or may be due to the effect of season Kuswaha et al.(1955) or it can also be due to the full maturity of the bulls, Raouf (1965). While recording the initial motility it was noted (43.33 percent) in 13 ejaculates out of 30 ejaculates, a very feeble or nil motility immediately after collection,

but the motility increased while examining the same, in bull numbers 137,0/4,4 and 0/8. This might be due to high initial pH as stated by Anderson (1944) and Jean Blain (1953) in bulls. The 'F' test was not statistically significant for the effect of bulls and ejaculate. Similar were the findings of Prabhu and Bhaya (1950) Kushwaha et al.(1955) and Sayed and Oloufa (1957) with different breeds.

The graph (1) indicates that the motility is inversely related to dead percentage of spermatozoa.

Hydrogen ion concentration : (pH). The pH recorded was 7.07 ± 0.03 which was higher and does not tally with the findings of Veeramaniayyar (1944); Kuswaha et al.(1955), Tomar and Desai (1958-59), Kale (1962) Viswanath Pillai (1965) the records of whose were 5.6 to 6.4 ; 6.6 to 6.9; 6.8 to 6.9; 6.4 to 6.7 and 6.43 ± 0.13 in murrah bulls. Only 10 percent of the ejaculates were acidic in nature. This high result may be due to insufficient excercise or might be due to seasonal variation as stated by Prabhu and Guha (1951) Kushwaha et al. (1955) and Oloufa et al.(1958).

This high pH associated with feeble initial motility also leads to suspicion about the fertility rate for the bulls, as stated by Mann (1954) that excessive alkalinity in bulls and rams was often accompanid by low fertility rate.

The graph (1) does not show relationship with other characters studied and no statistical difference between bulls and with in bulls was noted.

Concentration of spermatozoa: The average concentration of spermatozoa per millilitre for bull number 33 was 1070 millions; for bull no.137 was 1079.16 millions; for bull no. 4 was 813.17 millions, for bull no. 0/4 was 1029.17 millions; and for bull no. 0/8 was 1362.17 millions. The overall mean concentration of spermatozoa recorded was 1071.73 ± 70.96 millions per millilitre. This finding was almost in agreement with the findings of Kushwaha et al.(1955), Misra and Sengupta (1964) Viswanath Pillai (1965) who noted 1034.39; 1104 ±352 and 1110.3 ± 154.69 millions per millilitre in murrah bulls, and also with Prabhu and Bhaya (1950), Prabhu and Bhattacharya (1951),in Utter Pradeshi breed and, Madatov (1956) in Russian buffaloes, Sayed and Oloufa (1957), Oloufa et al.(1958) in Egyptian buffaloe bulls and Sengar and Sharma (1964) in local buffaloes of Utter Pradesh. It was higher than the findings of Shukla and Bhattacharya (1949), Sengar and Sharma (1964), in local Utter Pradesh buffaloes; Hafez and Darwish (1956) with Egyptian buffaloes. This may be due to the cold water bath given before collection, as stated by Prabhu (1956). This may also be due to the false mount allowed, to act as a precollection stimulus as reported

by Hale and Almquist (1960) in bulls and lower than the observations made by Roy et al.(1958) and Kouser (1965) in murrah bulls. This may be the effect of summer season as stated by Kushwaha et al, Oloufa et al, Misra and Sengupta (Loc.cit). The results were not having any statistically significant difference between bull and with in bull agreeing with the finding of Prabhu and Bhattacharya (1951).

The graph (1) revealed in inverse relationship to volume, as the concentration increased the volume decreased and vice versa and the reaction time also had the same relationship.

Percentage of dead spermatozoa.

(1) The mean percentage of dead spermatozoa observed during the study was 21.78 ± 1.37 percent. The result agrees with the findings of Joshi (1961), Kale (1962) and Viswanath Pillai (1965) who obtained 21.1; 18 to 25; and 21.07 ± 0.97 percent respectively in murrah bulls Prabhu and Bhaya (1950), Prabhu and Bhattacharya (1951) and Roy (1958) in other breeds; Hafez and Darwish (1956) and Oloufa et al (1958) in Egyptian buffaloe bulls. But the results were higher than the findings of Sayed and Oloufa (1957) in Egyptian buffaloes, Naidu (1960), Sengar and Sharma (1964) in local Utter Pradesh buffaloes, Kouser (1965)

and Saxena (1965) with murrah bulls. The high percentage may be due to high temperatures in summer and excessive load of collection in the season as said by Misra and Sengupta (1964), Sayed Oloufa, Oloufa and Sayed ,Roy (Loc. cit.).

Statistical analysis showed bull and ejaculate are insignificant as far as variation in percentage of dead sperms is concerned as suggested by Prabhu and Bhattacharya (1954).

The graph (1) shows a linear co-relation with volume of semen and concentration of spermatozoa.

Morphological abnormalities: In the present study the morphological abnormalities were found to be 24.427 percent out of which 13.452 and 10.975 percent were primary and secondary abnormalities out of the six bulls four bulls had above 24 percent and the remaining two bulls had below 20 percent of morphological abnormal spermatozoa. These results were higher than the findings of following workers Prabhu and Bhaya (1950), Prabhu and Bhattacharya (1951,1954), Sayed and Oloufa (1957), Oloufa (1958), Naidu (1960), Sengar and Sharma (1964) in other breeds of buffaloes, Kushwaha et al.(1955) and Viswanath Pillai (1965) who got 7.39 percent and 9.54 percent respectively in their findings in murrah bulls. The abnormalities of head, mid piece, and tail

were noted to be 7.128, 4.192 and 2.132 percent respectively. This high percentage might be due to summer season. It is evident from the findings of Kushwaha et al. Roy (Loc. cit) who observed an increase trend in abnormal percentage of spermatozoa in summer than in spring and autumn. These findings are also a little higher than the observations made in fertile bulls by Lagerlof (1936), Haq (1949) and Rollinson (1951), but lower than those demarked by Blom (1950) and observed by Bishop and Hancock (1955). Hence this much percentage of abnormal spermatozoa may not have any effect on fertility rate, coinciding with no complaints from field stations questioning the fertility of bulls.

The graph (1) shows a linear relationship of abnormal spermatozoa with volume of semen, an increase in volume causing an increase in percentage of abnormal spermatozoa.

Biometrics of spermatozoa: The mean head length, head breadth and tail length including middle piece of unstained spermatozoa were measured to be 6.994 ± 0.015 μ, 4.443 ± 0.019 μ and 54.178 ± 0.102 μ respectively. All the measurements were more than those recorded for epididymal spermatozoa by Mukherjee and Bhattacharya (1949). The head length was lesser than that observed by Mahmoud (1952). Both head length and

head breadth were lesser than the previous records of Guha et al.(1959), Naidu (1960), Venkataswamy and Vedanayagam (1962) and Saxena (1965). The tail length was also less than those recorded by Guha et al. and Venkataswamy and Vedanayagam (Loc.cit.) The differences in head length and head breadth between bulls and with in bulls were not statistically significant. But tail length was significantly different between bulls at one percent level. It seems with the above discussion that measurements of spermatozoa vary from place to place and this may be due to the differences in size and breed of the buffaloe bulls; or age of the bulls as stated by Venkataswamy and Vedanayagam (Loc.cit.) and Mukherjee and Singh (1964). It is opined the role of climate and habit of the bulls may also be responsible for the charges.

Thus this gives a new opening to the further workers to correlate the difference in fertility with biometrics of spermatozoa which may act as a guide for the selection of breeding bulls.

Effect of Preservation on Biometrics of Spermatozoa (With Glucose Sodium Bicarbonate Sulphamezathin Yolk(GBY) and Skim Milk Yolk (SMY) diluters):

(a) At 24 hours : The head length and head breadth of spermatozoa preserved for 24 hours in glucose sodium

bicarbonate yolk diluter and skim milk yolk diluter as per table 7 were lesser than those of fresh semen spermatozoa.

The reduction in head length was 0.159 and 0.177 microns and in head breadth it was 0.358 and 0.269 microns in the two diluters respectively. The analysis of variance (table-8) indicated that both the measurements were significantly different than those of fresh semen spermatozoa, reaching one percent level indicating a highly significant reduction of preserved spermatozoal head dimensions. This table shows no effect of bull and interaction of bull and diluter on the head length of spermatozoa. Unlike on head length, the analysis of variance (table 8) showed a significant effect of bulls on head breadth of spermatozoa preserved for 24 hours, reaching one percent level. This indicated a highly significant effect of bull apart from diluter on the spermatozoa preserved for 24 hours in the diluter. The detailed average head length and head breadth of spermatozoa bull wise was given in table. 9.

The bar diagram made in descending order also confirmed the sigrificant difference of head length and head breadth preserved and fresh spermatozoa further it also indicated that there is no significant difference between the two diluters even though the average head length of the glucose sodium bicarbonate

yolk preserved sperm was nearer to fresh sperm head length than slim milk yolk preserved sperm. This indicated that both the diluters had got the same effect on head length of spermatozoa preserved for 24 hours. The analysis of variance table 8, indicates that there is also a significant difference in head breadth between preserved and fresh semen spermatozoa and the bar diagram indicates a sigrificant difference between the two extenders . As shown in the table 7, reduction in average head breadth of spermatozoa preserved with skim milk yolk diluter was less than that recorded in glucose bicarbonate yolk diluter preserved spermatozoa which was quite opposite to head length that did not show any significant effect. This indicates that skim milk yolk extender is better than glucose bicarbonate sulphamezathin yolk extender in preserving the biometrics of buffaloe bull spermatozoal head at 24 hours preservation.

The preservation of murrah bull semen studied by Joshi (1961) found that out of four extenders he studied, he got best sperm livability results in milk yolk than glucose sodium bicarbonate sulphamezathin, whole milk and reconstituted powdered milk entenders. This supports the present finding in keeping up of the biometrics of the sperm head.

(b) At 48 hours of preservation : The table 7 indicates

that the head length and head breadth of spermatozoa preserved in glucose bicarbonate sulphamezathin yolk and skim milk yolk diluter were lesser than the fresh spermatozoa. The reduction of head length and head breadth of spermatozoa in the former diluter was 0.139 x 0.378 microns and in the later diluter was 0.163 x 0.335 microns. The 'F' test indicated unlike with 24 hours preservation the head length of sperm was significantly affected by bull apart from diluter without any interaction, whereas as per the table 8 head breadth was affected significantly by the bull as well as by the diluter and along with interaction, all these were significantly different from fresh semen spermatozoa reaching one percent level. The bar diagram arranged in descending order also confirmed, the analysis of variance table 8, showing significant difference between fresh semen spermatozoa and preserved spermatozoa measurements. As recorded with 24 hours preservation, no significant difference between the dimensions of glucose sodibicarbonate sulphamezathin yolk preserved spermatozoa head length and skim milk yolk preserved spermatozoa at 48 hours could be seen in table 8, even though the head length of spermatozoa preserved in diluters was significantly less from fresh semen spermatozoa as in table 7. The same table indicated that the average head length in skim milk yolk diluter was nearer to fresh semen spermatozoa. The average head length of spermatozoa

preserved in glucose bicarbonate sulphamezathin yolk did not resemble so much with fresh semen spermatozoa as in the case of skim milk yolk preservation, which was quite opposite with 24 hour of preservation. This indicated that though both diluters differ from fresh spermatozoa, these two diluters acted with same degree of effect at this interval of preservation.

But the reduction in head length of spermatozoa preserved in skim milk yolk diluter after 48 hours was 0.163 microns, which is less than the reduction of head length after 24 hours preservation 0.177 microns i.e. the spermatozoa head length had shown increase with 48 hours preservation over 24 hours preservation, but the average head length of spermatozoa after 48 hours preservation was less than fresh spermatozoa, whereas after 48 hours preservation in glucose sodium bicarbonate diluter the spermatozoal head length has shown a further decrease as compared to 24 hours of preservation. The regaining of head length in skim milk yolk diluter after 48 hours might be due to change in permeability of spermatozoal head length, which may be due to the change in pH of the preservation or it might be due to relaxation of the acrosome which had shrunken more during first 24 hours of preservation.

The table 8 showed a significant difference in the head breadth of preserved spermatozoa and fresh spermatozoa.

This difference reached one percent level in 'F' test between bulls and diluters. The bull wise comparative measurements of spermatozoa were as shown in table 9. The bar diagram confirming the differences between fresh and preserved sperm showed a significiant difference in head breadth within the two diluters also here also the average head breadth of spermatozoa preserved in skim milk yolk diluter was nearer to fresh spermatozoa than with the spermatozoa preserved in glucose bicarbonate sulphamezathin yolk diluter i.e. the head breadth of fresh spermatozoa was 4.443 microns as against 4.103 and 4.065 microns in the two diluters respectively. This shows that with regards to head breadth the same trend seen with 24 hours preservation followed here also. This indicates that skim milk yolk diluter is superior to glucose sodium bicarbonate sulphamezathin extender up to 48 hours.

These findings were supported by invitro trials of Joshi (1961), who recorded better survival of spermatozoa in milk yolk than in glucose sodium bicarbonate sulphamezathin yolk extender at 48 hours preservation. But the result disagrees with Kumar (1966) who recorded nosignificant difference in biometrics of sperm head of buffaloe bull semen at 48 hours of preservation with egg yolk citrate, skim milk yolk, tamatojuce and coconut milk diluter keeping egg yolk glucose sodium bicarbonate diluter as a control.

The effects of the two diluters at these intervals was shown in graphs 2 and 3.

(c) At 72 hours of preservation: Also the head length and head breadth preserved in both the diluters decreased shignificantly from that of fresh semen. Table 7 reveals a reduction of 0.195 x 0.422 microns in glucose sodium bicarbonate sulphamezathin yolk diluter and 0.136 x 0.363 microns in skim milk yolk diluter. Table 8 of analysis of variance indicated, as with 48 hours that bull and diluter had shown highly significant effect on head length reaching one percent level without any interaction. The head breadth of sperm was affected by bull and diluter with interaction all reaching to one percent level of significance and differed significantly from fresh semen. The bar diagram also confirmed the analysis of varience findings, but here, unlike with 24 and 48 hours preservation, the bar diagram has shown that both the diluters differed significantly in preserving he ad length ; spermatozoa preserved in skim milk yolk diluter had a head length of 6.858 microns as compared with the head length of spermatozoa preserved in glucose sodium bicarbonate sulphamezathin diluter which was 6.779 microns, the trend was as with 48 hours preservation, only indicating that the former diluter is better than the later diluter to preserve head length nearer to fresh semen spermatozoa.

But the reduction in head length after 24 hours, 48 hours preserved in skim milk yolk diluter was more than that of spermatozoa preserved upto 72 hours i.e. the head length was reduced by 0.177; 0.163 and 0.136 microns respectively indifferent periods of preservation from that of fresh semen spermatozoa. This shows that the head length of the spermatozoa preserved in skim milk yolk diluter has more shrinkage after 24 hours preservation, which was regained at 48 and 72 hours preservation to certain extent, but differed significantly even after 72 hours with fresh spermatozoal head length, where as in glucose bicarbonate sulphamezathine diluter the reduction in head length was gradual from 24,48 to 72 hours preservation i.e. 0.358; 0.378 and 0.422 microns respectively with regards to head breadth also the same trend as recorded with 48 hours preservation was noted. The skim milk yolk diluter preserved the head breadth nearer to fresh spermatozoan's head breadth than glucose sodium bicarbonate sulphamezathin yolk diluter i.e. 0.363 and 0.422 microns respectively in the two diluter.

This obviously indicated that skim milk yolk diluter is better than glucose sodium bicarbonate diluter to preserve spermatozoal head shape nearer to that of fresh semen spermatozoa, this was supported by the invitro preservation of Joshi (1961) who recorded better liablity of buffaloe bull spermatozoa in milk yolk than egg yolk glucose sodium bicarbonate sulphamezathin diluter.

PART - VII.

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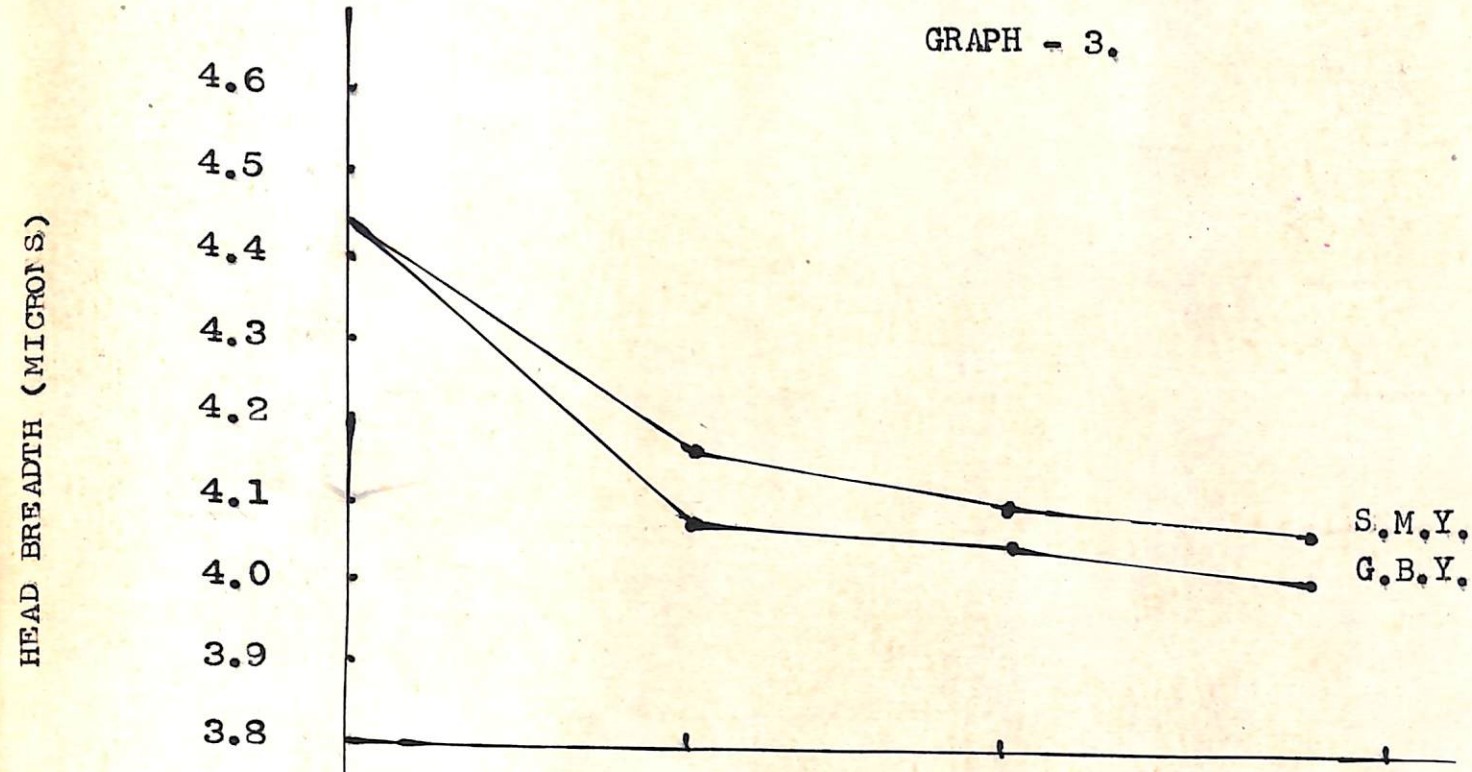
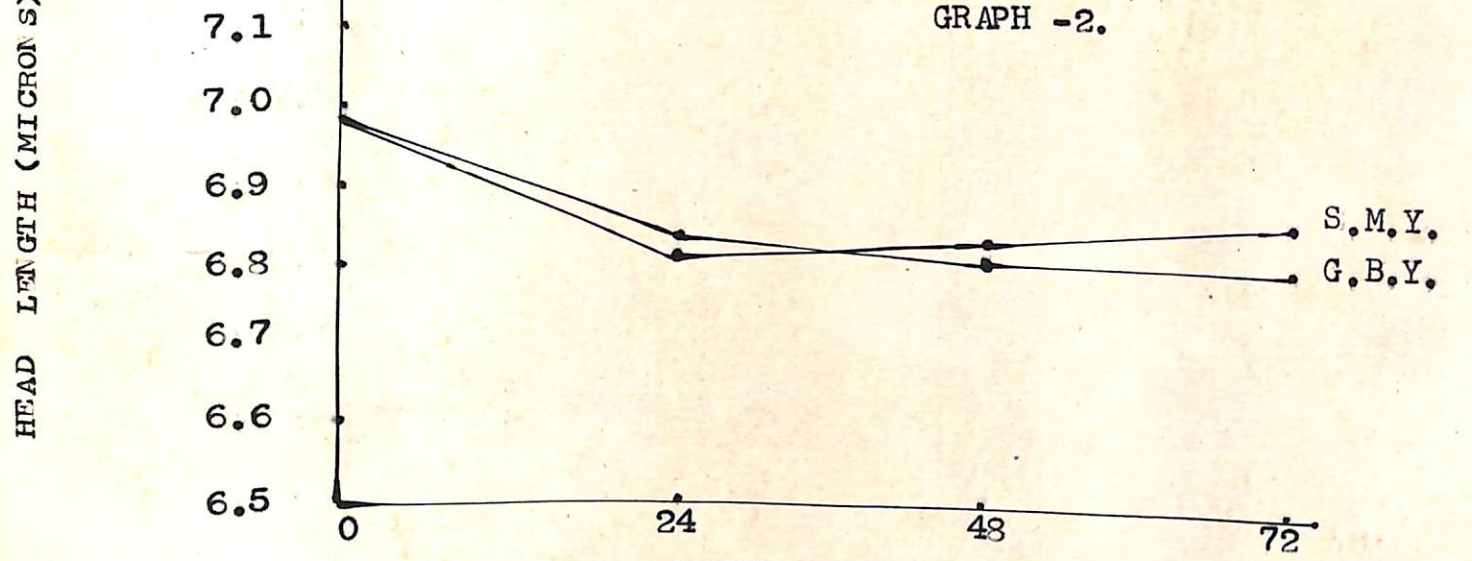
GRAPH - 3.



GRAPH SHOWING THE EFFECT OF DILUTERS ON HEAD BREADTH.

GRAPH -2.



- HOURS OF PRESERVATION -

GRAPH SHOWING THE EFFECT OF DILUTERS ON HEAD LENGTH.

The analysis of variance run after 72 hours preservation between bulls and diluters between bull and interval, and between interval and diluter supported the findings and the statistical analysis carried out at different intervals, as shown in table 8,13,14 and 15. The graphs no.2 and 3 also explaines the same.

The present work agrees to some extent with the statistical findings of Mukherjee and Dott (1960) who recorded significant decrease of head length and head breadth of bull sperm in one of the two extender used and with the findings of Saxena (1965) who also recorded a significant decrease of head length of Hariana bull spermatozoa after 72 hours preservation; Rathore and Mukherji (1961) and Rathore (1965) who recorded a significant decrease in head length and breadth after 72 hours of preservation in both the diluters they used. But the present result did not tally with the statistical findings of Naidu (1960), Saxena (1965) and Kumar (1966) who found no significant difference in preserved and unpreserved spermatozoa in Utter Pradesh local and murrah buffaloe bulls and Saxena (Loc.cit) in ram spermatozoa also who got non significant results.

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CHAPTER - VI.

S U M M A R Y.

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5. Hydrogen ion concentratio
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7) Percentage of dead sperm

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S U M-M A R Y .

The reaction time in murrah buffaloes bulls and certain characteristics of their semen were studied. The influence of glucose sodium bicarbonate sulphamezathin yolk and skim milk yolk diluters on the morphology of spermatozoa was recorded. Six first ejaculates were collected at 4 days intervals from each of the five murrah bulls taken for this experiment.

1) Reaction time (a) The mean reaction time recorded was 152.6 ± 27.17 seconds.

b) An inverse relationship between reaction time and concentration of spermatozoa and a linear relationship for reaction time and percentage of abnormal spermatozoa was noted.

2) Volume of the ejaculate : The mean volume was noted to be 3.27 ± 0.38 milliliter per ejaculate.

3) Colour and consistency. Noted as

a) Thick creamy, Thick milky, milky and thin milky.

c) Thick consistency was observed in 56.66 percent and thin consistency in 43.44 percent of ejaculates.

4. Initial motility: (a) The mean initial motility recorded was 3.98 ± 0.116.

(b) 43.33 percent of ejaculates were having feeble or no motility immediately after collection of semen but improved in motility while examining under microscope.

c) The motility was noted to be inversely related to dead percentage of spermatozoa.

5. Hydrogen ion concentration (pH): The average pH noted was 7.07 ± 0.03.

6. Sperm concentration: (a) The average sperm concentration studied was 1071.73 ± 70.96 millions per millilitre.

b) Concentration varied with colour and consistency of the ejaculates.

c) Concentration was found to be inversely related to the volume of ejaculate and reaction time.

7) Percentage of dead spermatozoa.

a) The percentage of dead spermatozoa was 21.78 ± 1.87 percent.

b) A linear carrelation between percentage of dead spermatozoa and volume of ejaculate was noted.

8. Morphological abnormalities:

a) A total of 24.427 percent of abnormal spermatozoa could be recorded, on an average with 13.452 and 10.975 percent of primary and secondary abnormalities

respectively.

No significant effect of bull and ejaculate were noticed on the characters noted against numbers 1,2 and 4 to 8.

8) Biometrics of spermatozoa.

a) The average head length, head breadth and tail length of spermatozoa measured were 6.994 ± 0.015; 4.443 ± 0.019 and 54.178 ± 0.102 microns respectively with a spermatozoal length of 61.17 ± 0.207 microns on an average.

b) Neither bull nor the ejaculate influenced the head length and head breadth significantly, but a highly significant difference was found in tail length between bulls.

10). The effect of glucose sodium bicarbonate sulphamezathin yolk and skim milk yolk was studied on the biometrics of spermatozoa at intervals of 24,48 and 72 hours of preservation : The influence of preservation with the diluters for 24 and 48 hours has been studied in this experiment. This does not appear to have been noted by any previous worker. A total of 5400 spermatozoa were measured in studying the effect of diluters.

i) At 24 hours of preservation: the head length and head breadth were 6.834 ± 0.009 and 4.085 ± 0.013 microns in glucose sodium bicarbonate sulphamezathin

yolk diluter and 6.817 ± 0.01 and 4.174 ± 0.14 microns in skim milk yolk diluter. A highly significant drop in head length and head breadth was noticed between preserved and fresh semen spermatozoa. No effect of bull was noted on the head length but both the dilutershad highly significant effect on head length without any significant difference between them.

A highly significant effect of bull and diluter was noted in the drop of head breadth with a significant intraction.

Skim milk yolk diluter was found to be better than glucose sodium bicarbonate yolk diluter.

ii) At 48 hours preservation: The head length and head breadth of glucose sodium bicarbonate sulphamezathin yolk diluter and skim milk yolk diluter preserved spermatozoa were 6.805 ± 0.010; 4.065 ± 0.010 and 6.831 ± 0.010 and 4.108 microns respectively.

iii) The preserved spermatozoa had shown a highly significant decrease in measurements than fresh semen spermatozoa. A significant effect of bulls was noted in decrease of head length and head breadth spermatozoa preserved in diluters, but diluter did not play any role. In head breadth it was quite opposite,

showing highly significant difference with interaction; where the bull as well as the diluter played their role. Here also the skim milk yolk diluter proved to be better than glucose sodium bicarbonate sulphamezathin yolk diluter.

iii) At 72 hours preservation : The mean head length and head breadth in glucose sodium bicarbonate sulphamezathin and skim milk diluter were 6.799 ± 0.011, 4.021 ± 0.014 and 6.858 ± 0.077, 4.030 ± 0.144 microns respectively.

A highly significant difference was found in head length and head breadth between preserved and fresh semen spermatozoa. Effect of bull and diluters was found to be highly significant with significiant interaction only in case of head breadth.

The head length and head breadth of spermatozoa had shown a decrease in glucose sodium bicarbonate sulphamezathin yolk than skim milk yolk diluter proving the later diluter as a better one than the former diluter for preservation of spermatozoal head shape up to 72 hours.

The skim milk yolk diluter has shown a marked decrease in head length of spermatozoa at 24 hours preservation which was to some extent, the lost decrease was regained subsequently at 48 and 72 hours of

preservation, with glucose sodium bicarbonate sulphamezathin diluter, the decrease in head length of preserved spermatozoa had increased as the interval increase from 24 to 48 to 72 hours.

In case of head breadth, the trend of decrease in breadth was observed to be increased as the preservation interval increase from 24,48 to 72 hours. Except in 24 hours preservation in skim milk yolk diluter the head length of spermatozoa was nearer to fresh semen spermatozoa than with that of glucose sodium bicarbonate sulphamezathin yolk diluter. The head breadth of spermatozoa preserved in skim milk yolk diluter was noted to be nearer to fresh spermatozoa than the other diluter. So the skim milk yolk diluter is considered to be a better extender than glucose sodium bicarbonate sulphamezathin yolk diluter for preserving buffaloe bull semen up to 72 hours.

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CHAPTER -VII.

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CHAPTER -VIII.
APPENDIX.

APPENDIX

TABLE -1.

CHARACTERS OF BUFFALOE BULL SEMEN

(Bull wise)

Bull No.	CHARACTER								
	Reaction time in (Seconds)	Volume of Ejacu mi. ml.	pH	Initial motility	Concen-tration in(milli-ons/ml.	% dead sperm	PERCENTAGE OF ABNORMAL SPERMATOZOA Primary	Secondary	Total
33	169.00	2.933	7.07	3.75	1070.00	27.38	16.573	13.276	29.849
137	185.00	2.366	7.03	4.00	1079.16	19.33	12.160	6.053	18.213
4	212.17	5.066	7.17	4.17	818.17	16.99	14.996	14.776	29.772
0/4	129.17	3.000	7.07	3.83	1029.17	24.11	13.938	10.498	24.436
0/8	67.67	3.000	7.00	4.25	1362.17	20.55	9.606	10.277	19.883
AVERAGE	152.60	3.27	7.07	3.98	1071.73	21.78	13.452	10.975	24.427

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TABLE -2

RESULTS OF STATISTICAL ANALYSIS OF DIFFERENT CHARACTERISTICS OF BUFFALOE BULL SEMEN.

Sl. No.	Character	Mean	Range		S.D.	S.E.	C.V.
			Minimum	Maximum			
1.	React.time	152.60	15	685	148.603	27.170	97.40
2.	Vol.of ejac.	3.27	0.7	9.5	2.0795	0.380	63.50
3.	Int.motil.	3.98	2.5	4.5	0.6360	0.116	15.98
4.	pH.	7.07	6.8	7.4	0.1610	0.030	2.27
5.	Sperm con-centration Million/ml.	1071.73	409.0	1980.0	388.734	70.960	36.36
6.	% of dead sperm.	21.78	8.00	54.0	9.260	1.870	42.44
7.	% Abnormal sperm .	24.43	10.00	47.333	-	-	-
(a)	Primary Abnormal.	13.45	6.00	35.333	-	-	-
(b)	Secondary Abnormal	10.98	1.667	22.667	-	-	-
8.	Biometrics of sperm						
	(a) Head length (Microns)	6.994	5.500	9.625	0.45	0.015	0.214
	(b) Head breadth (Microns)	4.443	2.750	5.500	0.58	0.019	0.432
	(c) Tail length (Microns)	54.178	41.250	63.250	3.07	0.102	0.190
	(d) Total sperm length. (Microns).	61.173	48.125	71.500	6.22	0.207	0.340

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T A B L E 3.

TABLE SHOWING DIFFERENT TYPES OF ABNORMAL SPERMATOZOA IN BUFFALOE BULL SEMEN.

(Percentages)

Bull No.	PRIMARY SPERM ABNORMALITIES									SECONDARY SPERM ABNORMALITIES						
	Pyriform head	Tapering head.	Narrow head.	Dwarf head.	Giant head.	Short thick head.	Other abnormal heads.	Abnormally stained head.	Large abnormal heads.	Loose normal heads.	Sperm with proximal droplet	Sperm with distal droplet	Bent tail	Detached Galea capiti	Total	Grand Total.
38	2.055	0.277	0.666	2.611	0.333	-	-	1.388	0.166	0.111	2.222	-	6.244	0.500	16.573	1.111	1.666	0.555	9.333	0.611	13.276	29.849
13	2.222	0.555	0.222	1.500	2.444	0.055	0.055	0.388	0.166	-	0.888	-	3.388	0.277	12.160	0.500	0.388	0.166	4.222	0.777	6.053	18.213
4	1.333	0.777	0.111	1.055	4.611	0.222	-	0.055	0.333	-	4.222	-	0.833	1.444	14.996	0.500	0.166	0.888	12.722	0.500	14.776	29.772
0/4	2.333	1.555	0.166	1.611	0.666	0.277	0.166	0.277	0.222	0.055	1.111	0.111	4.888	0.500	13.938	1.994	0.333	3.166	4.944	0.111	10.498	24.436
0/8	1.666	0.388	0.111	1.333	0.722	0.111	-	0.222	0.055	-	2.055	0.055	2.222	0.666	9.606	2.611	0.222	0.611	6.833	-	10.277	19.883
AVERAGE.	1.922	0.710	0.255	1.622	1.755	0.133	0.044	0.466	0.188	0.033	2.099	0.033	3.515	0.677	13.452	1.333	0.555	1.077	7.611	0.399	10.975	24.427

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TABLE 3.

TABLE SHOWING DIFFERENT TYPES OF ABNORMAL SPERMATOZOA IN BUFFALOE BULL SEMEN.

(Percentages)

Bull No.	PRIMARY SPERM ABNORMALITIES								SECONDARY SPERM ABNORMALITIES									
	Pyriform head	Tapering head.	Narrow head.	Dwarf head.	Giant head.	Short thick head.	Other abnormal heads.	Abnormally stained head.	Loose abnormal heads.	Sundry aborti forms	Normal heads with highly coild tail	Double forms	Abaxially attached middle piece	Middle piece defects	Total	Loose normal heads.	Sperm with proximal droplet	Sperm with distal droplet	Bent tail	Detached Galea capiti	Total	Grand Total.
38	2.055	0.277	0.666	2.611	0.333	-	-	1.388	0.166	0.111	2.222	-	6.244	0.500	16.573	1.111	1.666	0.555	9.333	0.611	13.276	29.8
13	2.222	0.555	0.222	1.500	2.444	0.055	0.055	0.388	0.166	-	0.888	-	3.388	0.277	12.160	0.500	0.388	0.166	4.222	0.777	6.053	18.2
4	1.333	0.777	0.111	1.055	4.611	0.222	-	0.055	0.333	-	4.222	-	0.833	1.444	14.996	0.500	0.166	0.888	12.722	0.500	14.776	29.7
0/4	2.333	1.555	0.166	1.611	0.666	0.277	0.166	0.277	0.222	0.055	1.111	0.111	4.888	0.500	13.938	1.994	0.333	3.166	4.944	0.111	10.498	24.4
0/8	1.666	0.388	0.111	1.333	0.722	0.111	-	0.222	0.055	-	2.055	0.055	2.222	0.666	9.606	2.611	0.222	0.611	6.833	-	10.277	19.8
AVERAGE.	1.922	0.710	0.255	1.622	1.755	0.133	0.044	0.466	0.188	0.033	2.099	0.033	3.515	0.677	13.452	1.333	0.555	1.077	7.611	0.399	10.975	24.4

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0.555	0.222	1.500	2.444	0.055	0.055	0.388
0.777	0.111	1.055	4.611	0.222	-	0.055
1.555	0.166	1.611	0.666	0.277	0.166	0.277
0.388	0.111	1.333	0.722	0.111	-	0.222
0.710	0.255	1.622	1.755	0.133	0.044	0.466

0.333	-										
0.222	0.055	1.111	0.111	4.888	0.500	13.938	1.994	0.333	3.166	4.944	0.111
0.055	-	2.055	0.055	2.222	0.666	9.606	2.611	0.222	0.611	6.833	-
0.166	0.033	2.099	0.033	3.515	0.677	13.452	1.333	0.555	1.077	7.611	0.399

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TABLE - 4

TABLE SHOWING ANALYSIS OF VARIENCE
OF
BUFFALOE BULL SEMEN

(Different Characters)

Source of varience.	Degree of freedom	CHARACTERS											
		REACTION TIME		VOLUME OF EJACULATE		INITIAL MOTILITY		pH		SPERM CONCENTRATION		PERCENTAGE OF DEAD SPERM .(+)	
		Mean squs..	F. Ratio	Mean squs..	F. Ratio.	Mean squs..	F. Ratio.	Mean squs..	F. Ratio.	Mean squs..	F. Ratio.	Mean squs..	F. Ratio.
Between Bulls.	4	13944	0.93	6.4	2.1	0.21	0.48	0.02	0.01	779	0.004	50.40	0.501
Between Ejaculates.	5	45510	2.36	6.6	2.2	0.47	1.07	0.06	0.02	81199	0.410	61.90	0.626
Error.	20	19242.7	-	3.0	-	0.43	-	0.20	-	199666	-	98.78	-

(+) Figures transferred into angles.

TABLE-5

TABLE SHOWING THE BIOMETRICAL ANALYSIS OF BUFFALOS BULL SPERMATOZOA

(Neat semen Bull wise)

Bull No.	Av. head length.	S.D.	S.E.	Head length-Range Minimum (Microns)	Maximum (Microns)	C.V.	Av. head breadth (Microns)	S.D.	S.E.	Headbreadth-Range Minimum (Microns)	Maximum (Microns)	C.V.
38	6.982	0.480	0.036	5.500	9.625	0.68	4.438	0.578	0.434	4.125	5.500	0.97
137	7.089	0.406	0.303	5.500	9.625	0.42	4.721	0.699	0.052	2.750	5.500	1.10
4	7.028	0.561	0.042	6.875	9.625	0.59	4.293	0.451	0.336	4.125	5.500	0.76
0/4	6.936	0.380	0.027	5.500	8.250	0.38	4.377	0.524	0.039	4.125	5.500	0.89
0/8	6.936	0.341	0.025	5.500	9.625	0.36	4.388	0.540	0.040	4.125	5.500	0.91
AVERAGE	6.994	0.450	0.015	5.775	9.350	0.214	4.443	0.580	0.019	3.850	5.500	0.432

TABLE -5 (CONTD.)

Bull No.	Tail length (Microns)	S.D.	S.E.	Tail length-Range Minimum (Microns)	Maximum (Macrons)	C.V.	Sperm length (Microns)	S.D.	S.E.	Sperm length-Range Minimum (Microns)	Maximum (Microns)	C.V.
38	54.114	6.618	0.489	41.250	63.250	0.90	61.906	4.752	0.354	48.125	70.125	0.57
137	54.656	2.733	0.204	48.125	60.500	0.31	61.745	2.174	0.165	55.000	70.125	0.26
4	54.908	2.098	0.156	48.125	60.500	0.28	61.936	2.130	0.159	55.000	70.125	0.25
0/4	54.175	2.106	0.158	48.125	61.875	0.29	61.111	3.151	0.235	55.000	70.125	0.38
0/8	53.037	3.593	0.268	48.125	61.875	0.50	59.973	1.754	0.131	55.000	71.500	0.22
AVERAGE.	54.178	3.070	0.102	46.750	61.600	0.90	61.173	6.220	0.207	53.625	71.200	0.340

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TABLE -6

TABLE SHOWING ANALYSIS OF VARIENCE
OF
HEAD LENGTH , HEAD BREADTH AND TAIL LENGTH OF SPERMATOZOA
(Neat Semen).

Source of varience.	Degree of Freedom	Head length.		Head breadth		Tail length	
		Mean Squares	F.Ratio	Mean Squares	F.Ratio	Mean Squares	F.Ratio
Between Bulls.	4	0.764	0.09	4.235	0.37	91.99	6.00**
Between Ejaculates.	5	0.160	0.002	2.676	0.21	20.04	1.24
Error.	20	8.286	-	12.729	-	15.327	-

** Significant at one percent level.

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T A B L E -7

TABLE SHOWING STATISTICAL ANALYSIS OF HEAD LENGTH AND HEAD BREADTH of BUFFALOE BULL SPERMATOZOA - NEAT SEMEN AND PRESERVED SEMEN.

	HEAD LENGTH OF SPERMATOZOA (Microns).							HEAD BREADTH OF SPERMATOZOA (Microns)						
	Neat semen	24 Hours preserved		48 Hours preserved		72 Hours preserved		Neat semen	24 Hours preserved		48 Hours preserved		72 Hours preserved.	
		GBY Diluter	SMY Diluter.	GBY. Diluter	SMY Diluter.	GBY Diluter.	SMY Diluter.		GBY. Diluter.	SMY. Diluter.	GBY. Diluter.	SMY. Diluter.	GBY. Diluter.	SMY. Diluter.
Mean	6.994	6.835	6.817	6.805	6.831	6.799	6.858	4.443	4.085	4.174	4.065	4.108	4.021	4.080
S.D.	0.450	0.274	0.297	0.303	0.268	0.340	0.222	0.580	0.400	0.411	0.320	0.438	0.430	0.442
S.E.	0.015	0.009	0.100	0.010	0.100	0.011	0.077	0.019	0.013	0.140	0.010	0.140	0.014	0.144
C.V.	0.214	0.140	1.500	0.150	1.500	0.016	1.120	0.432	0.320	3.330	0.240	3.400	0.350	3.530

B A R D I A G R A M.

HOURS OF PRESERVATION.	DIFFERENCE OF SPERM HEAD LENGTH (Microns)			C.D.	DIFFERENCE OF SPERM HEAD BREADTH (Microns)			C.D.
	Neat semen.	Preserved Semen Diluter.			Neat semen.	Preserved Semen Diluter.		
		GBY	SMY			SMY	GBY	
24	6.994	6.835	6.817	0.108	4.443	4.174	4.085	0.043
		SMY	GBY					
48	6.994	6.831	6.805	0.032	4.443	4.108	4.065	0.042
72	6.994	6.858	6.799	0.023	4.443	4.080	4.021	0.048

T A B L E .8

TABLE SHOWING THE ANLYSIS OF VARIENCE
of
HEAD LENGTH AND HEAD BREADTH OF BUFFALOE BULL SPERMATOZOA
BETWEEN NEAT SEMEN AND PRESERVED SEMEN
(At different intervals)

Source of varience.	Degree of freedom	HEAD LENGTH OF SPERMATOZOA.						HEAD BREADTH OF SPERMATOZOA.					
		24 Hours preserved		48 Hours preserved		72 Hours preserved		24 Hours preserved		48 Hours preserved		72 Hours preserved	
		Mean squares	F. Ratio.	Mean squares	F. Ratio.	Mean squares	F. Ratio.	Mean squares	F. Ratio.	Mean squares	F. Ratio.	Mean squares	F. Ratio.
BETWEEN BULLS.	4	0.880	0.64	1.052	8.766 **	0.887	7.31 **	4.713	21.92 **	5.202	25.62 **	4.790	17.48 **
BETWEEN DILUTER.	2	8.550	6.218 **	9.49	79.08 **	10.075	82.334 **	31.190	147.07 **	38.490	189.61 **	47.005	171.17 **
INTERACTION.	8	0.233	0.169	0.108	0.900	0.1706	1.4064	0.834	3.88 **	0.777	3.822 **	0.9265	3.34 **
ERROR .	2685	1.375	-	0.120	-	0.1213	-	0.215	-	0.203	-	0.274	-

** Significant at one percent level.

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T A B L E -9

TABLE COMPARING HEAD LENGTH AND HEAD BREADTH
of
BUFFALOE BULL SPERMATOZOA IN FRESH AND PRESERVED SEMEN
(At different intervals-Bull wise)

Bull No.	HEAD LENGTH OF SPERMATOZOA (Microns)							HEAD BREADTH OF SPERMATOZOA (Microns)							
	Neat semen	24 Hours preserved		48 Hours preserved		72 Hours preserved		Neat semen	24 Hours preserved		48 Hours preserved		72 Hours preserved		
		GBY. Dilutor.	SMY Dilutor.	GBY. Dilutor.	SMY Dilutor.	GBY. Dilutor.	SMY Dilutor.		GBY. Dilutor.	SMY. Dilutor.	BY. Dilutor.	SMY. Dilutor.	GBY. Dilutor.	SMY. Dilutor.	
38	6.982 ± 0.036	6.791 ± 0.269	6.844 ± 0.032	6.766 ± 0.027	6.820 ± 0.018	6.768 ± 0.028	6.860 ± 0.027	4.438 ± 0.434	4.506 ± 0.0269	4.125 ± 0.022	4.026 ± 0.029	4.033 ± 0.027	3.942 ± 0.035	4.003 ± 0.028	
137	7.089 ± 0.303	6.867 ± 0.018	6.867 ± 0.013	6.844 ± 0.015	6.833 ± 0.013	6.829 ± 0.015	6.844 ± 0.019	4.721 ± 0.052	4.163 ± 0.320	4.308 ± 0.034	4.156 ± 0.011	4.260 ± 0.039	4.148 ± 0.038	4.171 ± 0.356	
4	7.028 ± 0.042	6.875 ± 0.034	6.867 ± 0.025	6.844 ± 0.015	6.837 ± 0.020	6.852 ± 0.017	6.860 ± 0.009	4.293 ± 0.336	4.079 ± 0.030	4.133 ± 0.025	4.064 ± 0.025	4.056 ± 0.055	3.965 ± 0.038	4.094 ± 0.022	
0/4	6.936 ± 0.027	6.791 ± 0.017	6.776 ± 0.027	6.776 ± 0.027	6.791 ± 0.017	6.745 ± 0.030	6.806 ± 0.022	4.377 ± 0.039	4.094 ± 0.024	4.178 ± 0.036	4.049 ± 0.026	4.094 ± 0.019	4.018 ± 0.028	4.072 ± 0.036	
0/8	6.936 ± 0.025	6.852 ± 0.017	6.814 ± 0.021	6.876 ± 0.025	6.814 ± 0.021	6.760 ± 0.028	6.837 ± 0.017	4.388 ± 0.040	4.033 ± 0.031	4.125 ± 0.032	4.033 ± 0.008	4.094 ± 0.036	4.033 ± 0.014	4.056 ± 0.037	
AVERAGE.	6.994 ± 0.015	6.835 ± 0.009	6.817 ± 0.100	6.805 ± 0.010	6.831 ± 0.100	6.799 ± 0.011	6.858 ± 0.077	4.443 ± 0.019	4.085 ± 0.013	4.174 ± 0.140	4.065 ± 0.010	4.108 ± 0.140	4.021 ± 0.014	4.080 ± 0.144	

T A B L E - 10.

TABLE COMPARING HEAD LENGTH AND HEAD BREADTH
of
BUFFALOE BULL SPERMATOZOA IN PRESERVED SEMEN
(At 24 hours of preservation-Bull wise)

Bull No.	GLUCOSE YOLK DILUTER.								SKIM MILK YOLK DILUTER.							
	Head length of spermatozoa (Microns).				Head breadth of spermatozoa (Microns).				Head length of spermatozoa (Microns).				Head breadth of spermatozoa (Microns).			
	Mean	S.D.	S.E.	C.V.	Mean	S.D.	S.E.	C.V.	Mean	S.D.	S.E.	C.V.	Mean	S.D.	S.E.	C.V.
38	6.791	0.361	0.269	0.40	4.056	0.382	0.029	0.70	6.844	0.426	0.032	0.40	4.125	0.291	0.022	0.52
137	6.867	0.230	0.018	0.26	4.163	0.438	0.327	0.78	6.876	0.176	0.013	0.19	4.308	0.469	0.034	0.79
4	6.875	0.460	0.034	0.49	4.079	0.408	0.030	0.71	6.867	0.334	0.025	0.36	4.133	0.331	0.025	0.59
0/4	6.791	0.231	0.017	0.25	4.094	0.319	0.024	0.58	6.766	0.357	0.027	0.39	4.178	0.486	0.036	0.86
0/8	6.852	0.229	0.017	0.24	4.033	0.426	0.031	0.77	6.814	0.284	0.021	0.31	4.125	0.430	0.032	0.77
AVERAGE.	6.835	0.274	0.009	0.14	4.085	0.400	0.013	0.32	6.817	0.297	0.100	01.50	4.174	0.411	0.140	3.33

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T A B L E - 11.

TABLE COMPARING HEAD LENGTH AND HEAD BREADTH
of
BUFFALOE BULL SPERMATOZOA IN PRESERVED SEMEN
(At 48 hours of preservation-Bull wise)

Bull No.	GLUCOSE YOLK DILUTER								SKIM MILK YOLK DILUTER.							
	Head length of spermatozoa (Microns.)				Head breadth of spermatozoa (Microns.)				Head length of spermatozoa (Microns).				Head breadth of spermatozoa (Microns).			
	Mean	S.D.	S.E.	C.V.	Mean	S.D.	S.E.	C.V.	Mean	S.D.	S.E.	C.V.	Mean	S.D.	S.E.	C.V.
38	6.776	0.357	0.027	0.40	4.026	0.385	0.029	0.70	6.829	0.247	0.018	0.30	4.033	0.363	0.027	0.69
137	6.844	0.202	0.015	0.22	4.156	0.142	0.011	0.26	6.833	0.178	0.013	0.19	4.260	0.523	0.039	0.89
4	6.844	0.203	0.015	0.22	4.064	0.342	0.025	0.61	6.837	0.269	0.020	0.49	4.056	0.466	0.035	0.78
0/1	6.776	0.357	0.027	0.39	4.049	0.403	0.030	0.44	6.791	0.231	0.017	0.25	4.094	0.250	0.019	0.45
0/8	6.876	0.334	0.025	0.36	4.033	0.113	0.008	0.26	6.814	0.284	0.021	0.31	4.094	0.487	0.036	0.88
AVE-RAGE.	6.805	0.303	0.010	0.15	4.065	0.320	0.010	0.24	6.831	0.268	0.100	1.50	4.108	0.438	0.140	4.40

TABLE - 12.

TABLE COMPARING HEAD LENGTH AND HEAD BREADTH
of
BUFFALOE BULL SPERMATOZOA IN PRESERVED SEMEN
(At 72 hours of preservation-Bull wise)

Bull No.	GLUCOSE YOLK DILUTER								SKIM MILK YOLK DILUTER.							
	Head length of spermatozoa (Microns).				Head breadth of spermatozoa (Microns).				Head length of spermatozoa (Microns).				Head breadth of spermatozoa (Microns).			
	Mean	S.D.	S.E.	C.V.	Mean	S.D.	S.E.	C.V.	Mean	S.D.	S.E.	C.V.	Mean	S.D.	S.E.	C.V.
38	6.768	0.369	0.028	0.40	3.942	0.468	0.035	0.88	6.860	0.357	0.027	0.40	4.003	0.382	0.029	0.70
137	6.829	0.194	0.015	0.20	4.148	0.503	0.038	0.86	6.844	0.250	0.019	0.27	4.171	0.480	0.036	0.81
4	6.852	0.229	0.017	0.24	3.965	0.508	0.038	0.88	6.860	0.145	0.009	0.01	4.094	0.289	0.022	0.51
0/4	6.745	0.403	0.030	0.44	4.018	0.370	0.028	0.68	6.806	0.300	0.022	0.32	4.072	0.490	0.036	0.87
0/8	6.760	0.381	0.028	0.42	4.033	0.184	0.014	0.33	6.837	0.222	0.017	0.24	4.056	0.509	0.037	0.91
AVE-RAGE.	6.799	0.340	0.011	0.02	4.021	0.43	0.014	0.35	6.858	0.222	0.077	1.12	4.080	0.442	0.144	3.53

T A B L E - 13.

TABLE SHOWING ANALYSIS OF VARIENCE
of
BUFFALOE BULL SPERMATOZOAL HEAD MEASUREMENTS

(Between Bull and diluter after preserving 72 Hours)

SOURCE OF VARIENCE.	Degree of freedom.	Sperm head length (Microns)		Sperm head breadth (Microns)	
		Mean squares	F. Ratio.	Mean squares	F. Ratio.
BETWEEN BULLS.	4	1.343	19.296**	7.583	40.55**
BETWEEN DILUTERS.	2	11.716	168.33**	51.568	275.76**
INTERACTION.	8	23.515	337.86**	0.858	4.267**
ERRORX	2685	0.0696	-	0.187	-

** Significant at one percent level.

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T A B L E - 14.

TABLE SHOWING ANALYSIS OF VARIENCE
of
BUFFALOE BULL SPERMATOZOAL HEAD MEASUREMENTS

(Between Bull and interval after preserving 72 Hours)

SOURCE OF VARIENCE.	Degree of freedom.	Sperm head length (Microns)		Sperm head breadth (Microns)	
		Mean squares	F. Ratio.	Mean squares	F. Ratio.
BETWEEN BULLS.	4	1.340	13.467**	7.555	34.763**
BETWEEN INTERVALS.	3	7.670	76.833**	34.116	158.357**
INTERACTION.	12	0.1584	1.592	0.451	2.081*
ERROR.	6280	0.0995	-	0.2167	-

** Significant at 1% level.
* Significant at 5% level.

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T A B L E - 15.

TABLE SHOWING ANALYSIS OF VARIENCE
of
BUFFALOE BULL SPERMATOZOAL HEAD MEASUREMENTS

(Between diluter and interval after preserving 72 Hours)

SOURCE OF VARIENCE	Degree of freedom.	Sperm head length (Microns)		Sperm head breadth (Microns).	
		Mean squares	F. Ratio.	Mean squares	F. Ratio.
BETWEEN DILUTERS.	1	0.90	10.84**	5.36	32.09**
BETWEEN INTERVALS.	2	0.245	2.95	2.849	17.06**
INTERACTION.	2	0.355	4.18*	0.245	1.46
ERROR.	5394	0.083	-	0.167	-

** Significant at 1% level.
* Significant at 5% level.

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