Study On
Comparative Preservability of
Tharparkar And Jersian Bull Semen
IN
Different Seasons.

Thesis

Submitted to the Rajendra Agricultural University Bihar

IN

partial fulfilment of the

requirements for the degree of

MASTER OF SCIENCE (ANIMAL HUSBANDRY)

BY

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1971

Affectionately
Dedicated to my Mother,
who inspired me to go for higher
studies inspite of tremen-
dous difficulties.

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PATNA,

Dated, the 25th February, 1972.

I certify that this Thesis has been prepared under my supervision and guidance by Dr. Janardan Prasad, a candidate for the Degree of Master of Science (A.H.) with Animal Genetics and Breeding as Major subject and that it incorporates the results of his independent findings.

H. R. Mishra
(H.R. MISHRA). 25/2/72

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Abbreviations and Symbols Frequently used.

ml.	=	Millilitre.
N.	=	Number of observations.
N_1	=	Number of observations of 1st treatment.
N_2	=	Number of observations of 2nd treatment.
C.	=	Centigrade.
C.C.	=	Cubic centimetre.
C.V.	=	Coefficient of variation.
Oz.	=	Ounce.
mgm.	=	Milligramme.
i.e.	=	That is.
viz.	=	Namely.
d.f.	=	Degree of freedom.
F.	=	Variance ratio.
t.	=	Test of significance.
pH.	=	Hydrogen ion concentration.
M.S.	=	Mean sum of squares.
N.S.	=	Non-significant.
No.	=	Number.
%	=	Percent.
**	=	Significant at 1% level.
*	=	Significant at 5% level.
S.E.	=	Standard error.
C.D.	=	Critical difference.
Gms.	=	Grams.

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CHAPTER - I

INTRODUCTION

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

In India, milk production of cattle is miserably low. The milk production of cattle being a phenotypic entity, is dependent on genotype and environment both for its expression.

Environment can be improved by the optimal care of feeding and management etc. but unless the genetic potentialities are improved the level of production can not be achieved to a desired level.

The dairy cattle in temperate climate have in general considerably higher yield with other desirable traits. In the tropics, therefore, attempts are being made to cross European breeds (Bos taurus) to the indigenous stocks (Bos indicus) in order to combine the higher yield and other desirable traits of the former with the heat tolerance, resistance to endemic diseases and adaptation capacity and other components of unfavourable environment, of the latter (Mishra, 1965). To maximise the milk production under Indian conditions cross-breeding of exotic cattle to Indian stocks has been recommended and is being followed at present under certain environmental conditions viz. at the Farms, hilly regions with high rainfall, Industrial belts, Intensive Cattle Development Blocks and improved cattle zones with higher consciousness of dairy farming on the part of the breeders.

Consequently in Bihar also cross-breeding with exotic breeds such as Jersey, Ayrshire, Friesian are being tried in different cross-breeding zones of the state but this is limited to the Intensive Cattle Development Blocks, Key Village Organisations,

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Industrial belts, hilly regions and urban areas mainly.

Under the present conditions the only breeding system that could improve milk production in our cattle within the shortest period of time is cross-breeding of our cattle to the exotic stocks following the use of Artificial Insemination with frozen semen or normally diluted semen for the reasons of economy.

The advantages to be derived from Artificial Insemination increase as the keeping qualities of semen is prolonged. The greater the length of time that semen can be stored and still retain its viability and power of fertilisation, the greater would be the value of it for breeding purposes.

Keeping quality of semen is affected by many factors like temperature, humidity, rainfall, seasons of the year etc.

As the semen from both the sources (exotic and Indian bulls) is being used in India and many aspects of its keeping quality are yet to be assessed correctly, it has been considered worthwhile to conduct a study on comparative keeping quality of semen from Indian and exotic bulls kept at Semen Bank, Patna.

The present studies relate to the keeping quality of semen from Tharparkar and Friesian bulls maintained at Semen Bank, Patna, with normal diluent used at the Semen Bank i.e. Egg yolk - citrate diluter, at the refrigerator temperature of 4-5°C with special reference to the following traits :

(1) pH (Hydrogen-ion concentration).

(2) Initial Motility.

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(3) Individual motility.

(4) Estimation of percentage of live spermatozoa.

The subsequent chapters shall provide details of the study under taken.

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CHAPTER - II.

REVIEW OF LITERATURE.

REVIEW OF LITERATURE

Consequent upon the introduction of Artificial Insemination on a large scale as a breeding technique, much work has been done regarding *in vitro* preservation of semen of domestic animals and comparative evaluation of semen quality in respect of different breeds (both between and within) related to many variables. Relevant literature has therefore been briefly reviewed under the following heads that have bearing on the present study viz. -

(a) Preservation of bull semen in general.

(b) Keeping quality of semen as affected by different variables.

(a) Preservation of semen:

The basis of *in vitro* preservation of semen is lowering the metabolic activities of spermatozoa and thus increasing their life-span. Some factors like temperature control, protection from cold shock to the spermatozoa from the lowered temperature and maintainance of pH are of vital interest to the maintainance of normal physiological and biochemical activities of spermatozoa.

Among the early achievements in semen preservation were refrigeration of semen to near zero temperature i.e. slightly above freezing point for suppressing sperm motility and metabolic activities (Wolf, 1921; and Walton, 1930).

Dilution of semen increases the number of females that

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can be bred from a single ejaculate of a sire. Changes associated with the dilution of semen commonly known as "dilution effects" were reviewed by Mann (1954).

Further researches in the field of semen preservation made considerable improvement which include :

(i) Development of substitutes of egg yolk like milk, honey, cream, tomato juice, coconut milk etc. as diluent.

(ii) Inclusion of various supplements like glucose, fructose etc. in the yolk buffer diluents for their increased efficacy.

(iii) Inclusion of various antibiotics such as penecillin and streptomycine-sulfate and sulfanilamides resulting in increased fertility, protective and antibacterial actions in semen diluents.

(iv) Addition of glycerol for its protective action on spermatozoa in the freezing diluents.

Recent investigations include :

(i) Deep-freeze method of semen preservation for longer time at -79°C (Smith and Polge, 1950).

(ii) Illini Variable Temperature diluent for semen preservation at room temperature (Van Demark and Sharma, 1957).

Glucose solution (3 to 5%) was found as the most satisfactory diluent for bull semen (Winters et al, 1938).

Milanov (1938) has investigated the use of gelatinised

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diluents, consisting of gelatin added to glucose phosphate or glucose sulphate diluents. Later tissue extract were tried for preservation of bull semen *in vitro*.

Spermatozoa could be protected from cold-shock by the addition of egg yolk (Lardy and Phillips, 1939), and by gradual cooling (Chang and Walton, 1940).

Rapid cooling on the other hand is well known to be harmful to bull and ram spermatozoa (Willet, Fuller and Salisbury, 1940; Lasley, Mayer and Bogart, 1942).

The use of incorporation of buffer salt in the diluent medium for the maintenance of hydrogen-ion concentration (pH) within limits was indicated by many workers (Phillips and Lardy, 1940).

Egg yolk-phosphate diluents (Phillips and Lardy, 1940) and egg yolk citrate diluent (Salisbury *et al*, 1941) were developed for the preservation of semen.

Lardy and Phillips (1941) reported that motility is sustained chiefly by the glycolytic fructolytic mechanism. The rate of movement varies with the species, the health of the sperm and kind of medium.

The bull spermatozoa preserved at 2°C in embryonic tissue extract prepared from developing chick embryo contained motile spermatozoa for periods upto 46 days (Frank *et al*, 1941).

Swanson and Herman (1941) found the beneficial effects of egg yolk-phosphate on the sperm motility of the bull semen for

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the first 100 hours.

Herman and Swanson (1941) also reported that the greatest loss of motility in stored bull semen occurs during first 24 hours; from 24 to 48 hours the decline was very slight and thereafter it was more or less linear.

Salisbury et al (1941) preserved bull spermatozoa in egg yolk-phosphate and egg yolk-citrate diluent for 2 to 4 days under standard storage conditions, cooling the diluted semen from room temperature to 5°C at the rate of 5°C per 10 minutes. Egg yolk-citrate was found to be superior when stored to 6 days or more but fertility results showed no significant differences between the two diluents with stored semen samples upto 5 days.

Swanson and Herman (1941) showed that referigerator temperature of 40°F was satisfactory for storage of bull semen. They found that semen can be used 1 to 3 days of storage satisfactorily when stored at referigerator temperature.

Although, the fertility results with egg yolk-phosphate and egg yolk-citrate diluents were equally satisfactory (Salisbury et al, 1941; Bratton et al, 1949), the addition of antibiotics resulted in the higher conception rate with egg yolk-citrate than egg yolk-phosphate (Campbell and Edwards, 1955).

Lasley and Bogart (1943) showed that fertility was related to semen volume, concentration of sperms, number of sperms per ejaculate, percentage of live sperms, and percentage of sperms resistant to 10 minutes exposure to 0°C in egg yolk-buffer.

Semen samples having less than 50% live spermatozoa was

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stated to affect fertility. Lasley and Bogart (1943) showed a linear relationship with motility, which was directly related to fertility.

Phillips et al (1943b) observed significant variation in all characters of semen except in volume and motility of spermatozoa.

Lasley (1944) obtained 16.9% dead, 19% non-motile, 12.5% weakly motile and 51.6% progressively motile spermatozoa in a freshly collected semen sample. These figures after 4 days storage were 27.7%, 37.4%, 20.7% and 14.2% respectively. He reported high fertility (69.7%) with semen having higher percentage of motile spermatozoa (61.7%) than (54%) with those having lower percentage of motile spermatozoa.

The pH of freshly ejaculated bull semen depends upon the varying proportions of the several secretions involved. Most normal samples are on acid side of neutrality, varying from about 6.5 to 6.9 with a mean of 6.75 but the pH varies over a wide range from 6.0 or lower to 8.0 or slightly higher. Semen of good quality is usually more acidic (lower pH) than is semen of lower sperm concentration. Since spermatozoa breakdown fructose in the semen to lactic acid, under the anaerobic conditions which usually exist in the narrow collection tubes, the pH of semen is likely to decrease with increased time from collection to measurement. On the other hand bacterially contaminated semen and that containing many dead spermatozoa may evolve ammonia and the pH will then increase (Salisbury and Van Demark, 1961).

Lambert and Mekenzie (1940) reported that the pH of bull

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semen varied from 6.5 to 7.5.

Laing (1945) recorded that initial pH of bull semen above 6.7 indicated low fertility. pH of semen as a matter of fact is not an independent attribute.

The sugar content was not correlated with semen volume, sperm density, initial motility on storage.

The higher the sugar content the lower the pH and better the buffer capacity. The mean reduction in sugar content after 24 hours storage (undiluted) at 10°C was 23.85 ± 1.76%. Higher the initial motility and the sperm density the greater the reduction in the sugar contents. There was no correlation between sugar content and its reduction on storage (Anderson, 1946).

The pH of semen depends upon ionic concentration and buffering capacity of various components present in the seminal plasma. The pH of semen samples from clinically normal bull is strictly acidic. It is only on storage that pH declines towards more acidic side (Tomar, 1970). The pH of semen has been found to increase progressively in successive ejaculates (Prabhu and Sharma, 1953; Sengar and Sharma, 1965; Tomar et al, 1968a). The over all pH is dependent on other semen qualities.

Normal bull semen exhibits a wave like motion. Walton (1952) has described it as follows "The spermatozoa do not move at random in all directions independently of each other but in waves. On examination fairly thick layer of semen under low power of microscope the interplay of waves can be observed; light and dark waves form and disappear and swirl over the field of the focus."

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He believed this phenomenon to be due to the flow orientation of the spermatozoa on ejaculation. As motility is initiated, the spermatozoa tend to move in the direction their heads point, thus sperm oriented in the same direction become seperated in the waves from those that are variously oriented. According to Walton (1952) the phenomenon of sperm rheotaxis may be partially explained on the same basis of flow orientation. Motility has long been considered necessary as a means of spermatozoan transport through the reproductive tract of the female.

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

Rao and Hart (1948) proved motility to be the best criterian for estimating the fertilising capacity of semen.

Lundgren (1948) found a correlation between fertility and motility.

With the clear proof by Van Demark and Hays (1954) that rapid transport of sperm cells from the cervix to the infundibulum (earlier found by Van Demark and Moeller, 1951 to be almost instantaneous even with non-motile spermatozoa) was brought about by oxytocin stimulated contractions of the female reproductive tract.

Motility randomly distributes the sperms cell throughout the area of the female tract in which the ovum is found as a fertilising unit. The evidence is that sperm motility is essential (Asdell and Salisbury, 1941).

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Cooling prolongs the fertilising capacity of spermatozoa. Anderson and Seath (1948) showed that spermatozoal survival was improved by dilution as soon as possible after collection of semen (Maule, 1962).

Semen diluted with salisbury's diluent retained its vitality much longer than undiluted semen (Valerani, 1948).

Schultze et al (1948) obtained on an average of 4.6% reduction in the conception rate per day when semen was stored upto 4 days. Stewart (1950) noted that rate of decline in fertility was associated with the semen quality and fertility level of individual bull.

Carretta (1949) found that fertility was highest following the use of diluted semen stored at 5°C for 4 to 5 hours.

Smith and Polge (1950) recognised that temperature is one of the most important factors in the survival of spermatozoa in vitro and bull semen cooled slowly to about 4°C, can be stored satisfactorily for several days before use. They found that the resistance of spermatozoa to freezing was varying from species to species. The percentage of spermatozoa which servived after storage at a very low temperature was dependent on the rate of cooling and also on the medium in which it was suspended. Glycerol appeared to be outstanding protective agent.

Spermatozoa could survive with restitution of swirling motion, after slow freezing to -79°C in diluents containing mixture of glycerol and 1.25% arabinose, or xylose (Emmens and Blackshaw, 1950).

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Bonadonna (1950) found marked correlation between conception rate and duration of time of storage *in vitro*, upto and even beyond 48 hours.

Rozsa (1950) reported an average of 80,000 bacteria per ml. in the semen sample collected even under strict hygenic measures and that a combination of penicellin and streptomycin could control bacterial growth effectively even when the semen was stored for upto 12 days in yolk-citrate diluent at 4°C (Maule, 1962).

Antibiotics not only controls the bacterial growth but enhances conception rates of some of the bulls of lower fertility. It is best to add the antibiotics to the diluter prior to combining wit with the semen.

The effect of streptomycin has been reported by Biological Society. Streptomycin added to bull semen at the rate of 10 milligram per 10 cubic centimetre semen diluted with egg yolk-citrate and stored for 9 days at 5°C did not influence the percentage of sperm with progressive motion or motility grade when 30 milligram streptomycin was added to 10 cubic centimetre diluted semen the percentage and the motility grade were increased. The addition of streptomycin to diluted semen inhibited the development of *Brucella abortus* and *Bacterium coli*.

Dunn and Bratton (1950) diluted the bull semen in 2.9% citrate sulfanilamide yolk diluent and 2.9% citrate sulphanilamide whole egg diluent and reported 44% and 11% motile spermatozoa after storage of 2 days respectively.

Jurgens (1951) reported average duration of life span

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for bull spermatozoa to be 11.91 days in egg yolk-citrate diluent when stored at + 4°C.

Lasley (1951) found highly significant correlation between the percentage of live motile and progressively motile spermatozoa in fresh semen. He reported that a storage period of 4 days in egg yolk-phosphate diluent at 10 to 12°C semen samples of bull contained 34.9% motile and 14.2% progressively motile spermatozoa in contrast to 64.1% and 51.6% respectively in a fresh semen. The percentage of motile spermatozoa was significantly correlated with the fertilising capacity (r = 0.314).

Fiorentino (1952) found higher survival rate of bull spermatozoa upto 197 hours in semen diluted with skim milk as compared to egg yolk-citrate.

When semen is collected in rapid succession, initial motility declines rapidly after second ejaculation (Prabhu and Sharma, 1953; Hafez and Darwish, 1956; Tomer et al, 1968a).

Campbell and Edward (1954) and Salisbury (1957) have reviewed the work on bull semen diluents used in Artificial Insemination. Subsequent researches have paved the way of preservation of semen at subzero temperature (Polge et al, 1949; Polge and Rowson, 1952), preservation of semen by freeze drying (Bialy and Smith, 1957),(Albright et al, 1958) and preservation of semen at room temperature (Van Demark and Sharma, 1957).

Prabhu et al (1953) estimated that under normal condition, a 35 to 40% conception rate might be expected in India from good quality semen stored upto 10 days, at 4°C.

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Rousell (1954) found a decrease of 5% in conception rate after storage for 24 hours.

Schmidt and Kroll (1953) noted an increase in the retention of fertilising capacity of bull semen from 5 to 6 days to 7 to 8 days in egg yolk-citrate diluent when the storage temperature was increased from 4 to 6°C to 15°C.

Rottensen (1953, II) found that the conception rates averaged 52.4% for the fresh semen and 50.5% for a day old semen.

Bhattacharya and Prabhu (1953) reported no apparent decrease in fertility rate with 76 hours old semen.

Prabhu et al (1953) reported that 30% to 40% fertility can be expected from a 10 days old semen sample stored at 4 to 5°C.

Highest fertility was obtained if the semen was used on the date of collection and there was 3% decline in the fertility within 30 hours of collection (Melrose, 1952b), Willet (1953), Heweston (1955), Rottensen and Anderson (1956) but results obtained by Roussel (1954) revealed a decrease of 5% in fertility for every 24 hours of semen preservation.

The fertilising capacity of semen stored at 5°C averaged 64.4% after 1 to 8 hours, 60.2% after 25 to 32 hours and 51.8% after 49 to 56 hours. The decrease from 1st to 3rd day was highly significant, at the 1% level (Hewetson, 1955).

In addition to the protective action against cold shock, egg yolk contains certain substrates which supports spermatozoal metabolism. Its glucose content can be utilised by spermatozoa

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(Van Tienhoven et al, 1952), and it has been shown by Smith et al (1956) to play a part in the denydrogenase reactions thus maintaining the spermatozoa in a reduced state (Maule, 1962).

The addition of sulfanilamides to diluted semen with egg yolk-citrate increased the percentage of motile spermatozoa but did not influence the survival of spermatozoa in the presence of Bacterium coli and Brucella abortus (Dimitropoulos, 1947).

The level at which the sulfanilamides and antibiotics were added to the diluents was dependent on the diluters which was less in case of boiled milk than other diluent. The motility was slightly reduced when streptomycine was added. Longevity was increased in all treatments (Schmidt, 1954).

Marcus et al (1955) showed that fertility of bull semen declined as the incidence of dead spermatozoa increased and the relationship was highly significant.

Aamdal (1955) found no appreciable differences in pregnancy percentages for 1st, 2nd and total inseminations whether or not semen contained antibiotics.

The conception rate from the semen diluted with egg yolk in combination with antibiotics and sulfanilamide was 54.8% against control 48.5%. When the semen was used at 48 to 60 hours storage it was 2 to 3% higher. The effect was better in the semen of low fertility bull than in that of high fertility bull (Sakala,1957).

✓ Jaiskawsky and Romaniuk (1957) reported that the storage of semen in small test tubes within the thermos flask wrapped in

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damp cotton and surrounded by the ice, gave the best results. The temperature of the semen remained below 5°C for 24 to 120 hours and over 50% motility was maintained for 144 hours.

Degroot and Bekedam (1957) found slightly lower conception rate from older semen than 1st day semen.

Sakala and Turcer (1957) found higher percentage of conception rates from the semen of 2nd day collection than from the 1st day and then it progressively declined.

Schmidt (1956) found that the increase in the protein contents of the ration enhanced the sperm concentrations and percentage survival after 4 days storage at 4°C in refrigerator.

Schmidt et al (1954) found that semen diluted with various extenders and stored at 4°C showed decline trends so far as viable spermatozoa was concerned with increasing dilution rate.

Branton and Prather (1954) reported that liveability of bull spermatozoa was increased by the addition of antibiotics and sulfanilamides. The most destructive antibiotics to sperm was considered to be Terramycin (Grespo Gorcia, 1960).

Though the comparative efficacy of the concentration of live spermatozoa may be predicted with the initial motility of semen (Lasley, 1951; Tomar et al, 1966), the actual evaluation of live spermatozoa percentage is desirable. Semen with more than 30% dead spermatozoa may not be good for preservation. Such semen is liable to reduce fertility rates (Stones et al, 1950; Lasley, 1951; Bonadonna and Kaan, 1955).

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Live spermatozoa declines after second ejaculate (Senger and Sharma, 1965; Tomar et al, 1968a,b). Live percentage does not vary markedly when semen is collected once in a week to every 48 hours (Tomar et al, 1968a,b).

Foote and Bratton (1960) in two experiments reported the presence of 50 and 52% motile spermatozoa in C.U.E. (Cornell University Extender) diluent after a 12 days storage period at 5°C.

Sharma et al (1962) preserved Hariana and Murrah bull semen at 5° to 7°C in referigerater in various diluents. The total time taken for initial motility to come to zero was reckoned as an estimate of full life of spermatozoa. They obtained following results on egg yolk-citrate diluents in different seasons:

Average life of sperms in days:

Season	Hariana bull semen.	Murrah bull semen.
Spring (February to April).	7.6 ± 1.6	4.8 ± 1.1
Summer (May to July).	8.3 ± 1.7	4.9 ± 1.1
Autumn (August to October).	8.1 ± 1.8	6.1 ± 1.4
Winter (November to January).	8.9 ± 2.0	6.3 ± 1.6

Adler et al (1962) found that the semen diluted with homogenised cream and stored at 4°C gave good results. The non-return for 1-5 hours old semen was 70.4%, 69.9% for 6 hours to 12 hours old semen, and 69.2% for 25 to 38 hours old semen. The

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influence of storage time was practically the same for highly fertile and less fertile bulls.

Campbell (1963) showed an average rate of fall in the conception rate for each day of semen age upto 4 days, varying from 3.4 to 8.3%.

Bhattacharya and Prabhu (1953) found that there was no apparent decrease in the fertility with age of semen upto 76 hours.

Saxena (1965) got the following results for motility and percentage of live spermatozoa at different hours of preservation of Hariana bull semen in egg yolk-citrate.

Hours of preservation.	Motility	Percentage of live spermatozoa.
0	87.0 ± 1.1	89.0 ± 1.2
72	76.5 ± 1.3	82.9 ± 1.9
102	67.0 ± 2.0	77.4 ± 2.7

Rao (1965) noted 3.43 to 10.71% of loss in live spermatozoa upto a preservation period of 120 hours. He reported average progressive motility in fresh semen to be 81.66 ± 4.59%. He observed gradual but significant decline in the percentage of motile spermatozoa. He further maintained that 70% motility was maintained upto 48 hours and 49.23% upto 120 hours.

The quality of deep frozen semen depends upon the thawing temperature. Stoye (1966) stated that semen samples thawed at 40°C contained more viable spermatozoa than those thawed at

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lower temperature.

Singh and Sadhu (1966) reported that semen diluted with egg yolk-citrate and stored at 5°C when agitated gave higher percentage of dead spermatozoa than in non-agitated samples. Motility rate of non-agitated sample was higher than agitated one.

Molinari et al (1968) observed in 4034 first inseminations of lowland cows, that pregnancy rate with semen stored at 5°C and frozen semen was 60.43 and 66.92% respectively. Corresponding figures for 2234 inseminations of hill cows were 63.13% and 75.87%. Pregnancy rates did not differ significantly between frozen semen and semen stored in dry ice or in liquid nitrogen.

Giordano (1969) observed highest motility percentage of sperm in semen diluted with salisbury's diluent than other one.

Freezing of semen at -196°C followed by thawing at 45°C yielded reconstituted semen containing 67% live spermatozoa (Balasov, 1969).

Survival of spermatozoa in samples cooled to different temperature and transferred directly to liquid nitrogen varied from 0.3 to 26% (Bugrov et al, 1969).

The average conception rates on 636 cows inseminated with frozen semen on solid CO_2 and 567 cows with frozen semen in liquid nitrogen were 55.5% and 62.7% respectively (Boureanu et al, 1970).

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(b) Keeping quality of semen as affected by different variables:

Semen characteristics vary with species, breed, age, seasons and nutritional standard of the individuals etc. as reviewed here under.

Herman and Swanson (1940) found wide variation in the storage capacity of different ejaculates of the same bulls as well as from different bulls.

Erb et al (1940) found considerable variation in breeding efficiency between sires; 1 to 2½ years age group had the highest efficiency which followed a gradual decline with age. They showed that breeding efficiency may be an inherited character.

Weatherby et al (1940) found slight monthly variation in volume, sperm concentration and longevity of spermatozoa in bull semen.

In U.S.A., Erb et al (1940) reported that the month of May had the highest conception rate (73.4%) in contrast to August (58.2%) having the lowest.

Anderson (1941a) indicated possible breed differences, Dairy bulls giving larger ejaculates than beef bulls. The Friesian gave larger ejaculates than Ayrshires.

Erb et al (1942) recorded significant seasonal differences in semen quality. Semen was found to be superior in spring and inferior in summer. They concluded that this differences were due to the factors characterising season i.e. light, relative

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humidity, general and obscure factors. In their opinion temperature was mostly responsible for the change in the semen quality.

Phillips et al (1943) obtained highest percentage of fertile services in April (59.6%) and lowest in August (40.8%). There were variations between bulls in motility, total concentration and survival on storage.

Seasons appear to have some control on the initial motility (Swanson and Herman, 1944; Mukherjee and Bhattacharya, 1952; Kushwaha et al, 1955; Oloufa et al, 1959; Sen Gupta et al, 1963; Tomar et al, 1966). Humid and hot season was disadvantageous to the production of highly motile sperm. They reported changes in the initial pH in semen of exotic, zebu and buffalo bulls.

Swanson et al (1944) revealed that the motility and viability of bull spermatozoa was lower in winter than in summer and spring due to the effect of season on the physical well being of the bulls.

Anderson (1945) noted considerable variation between bulls in the pH of semen. The semen of fertile bulls had usually a pH of less than 7.0 and that of poor quality bull generally produced semen which was nutral or slightly alkaline.

Mukherjee and Bhattacharya (1947) studied the seasonal effects on seminal characters of Kumani bulls and found the semen was superior in spring than that of summer.

Raps and Cannon (1947) reported significant differences in pH in different seasons.

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Hudopisk (1948) found that the sperm viability was best maintained at 3°C, below this the survival of spermatozoa decreased and after raising the temperature sperm motility slightly increased resulting in increased duration of viability.

Swanson (1951) found no significant difference between months.

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

Mukherjee and Bhattacharya (1952) studied the pH of semen and showed significant variation between years and between months within season.

Johnson and Branton (1953) observed marked seasonal differences in semen quality but no corresponding significance in non-return conception rates could be found.

Casady et al (1953) revealed that high ambient temperature resulted in decreased initial motility, average sperm concentration and total sperm count.

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

Marcus et al (1955) showed that mean incidence of dead spermatozoa differed significantly between bulls. They also showed

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that mean volume of ejaculate and mean concentration of spermatozoa also differed significantly between bulls.

Kushwaha et al (1955) observed the seasonal variation in semen characteristics of six Murrah buffalo bulls but failed to show significant variation between seasons.

Bhattacharya (1957) observed seasonal variation in semen quality.

Hafs et al (1958) noted significant variation among the bulls but did not observe any variation in initial motility of spermatozoa between breeds, Bhatia (1960) noted the average initial motility in Gir and Sahiwal breeds as 4.58 and 3.62 respectively.

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

Patrick et al (1959) subjected bulls to varying conditions of atomospheric temperature and humidity and found no change in semen production and fertility.

Highly significant correlation was observed between initial motility and age of the bull (Lindley et al, 1959).

Ozkoca (1960) found that the best quality of semen samples were obtained from March to May and poorest from July to September.

Maslov (1960) did not find any differences in the

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initial motility of semen collected from bulls of different age groups.

Kodagali (1962) revealed highly significant seasonal variation in seminal characters of Khillar breed of cattle. Semen was best in cold followed by wet and summer.

Sharma and Saha (1962) found no seasonal effect on semen quality of Hariana bulls.

Kelly and Hurst (1963) found highest conception rates in April and October and lowest from June to September.

Horic and Ishikura (1964) showed that bull semen was of best quality in spring and poorest in late summer.

Dimitriev (1964) and Malberg (1965) were of the opinion that initial motility increased as the bulls grew in age.

Milicevic (1965) observed that high environmental temperature was associated with reduced ejaculate, where as low temperature adversely affected motility, pH, viability and sperm concentration. Highest fertility was recorded in autumn and spring, (temperature range from 12.2 to 34°C).

Nikulenko (1965) studied the different attributes of semen in Black pied, Jersey and Red Danish bulls and found that semen of Red Danish and Jersey bulls had higher sperm concentration, better survivability of spermatozoa and lower percentage of dead sperms than that of Black pied.

Mukherjee and Singh (1966) found that the percentage of

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dead spermatozoa in the samples did not vary significantly between bulls.

Singh (1966) observed considerable variation among bulls.

Singh (1966) observed that bull semen was better preserved in rainy season that summer and significant seasonal variation were recorded in all characters.

Sinha and Prasad (1966) studied seasonal, individual and year variation in the semen characteristics and reaction time of 30 Tharparkar bulls, 8 Hariana and 1 Taylor bulls. Highly significant variation in average initial motility was noted between seasons, bulls and years for Tharparkar bulls. Highly significant variation in average reaction time was seen for Hariana and Tharparkar bulls but not for Taylor bulls.

pH of semen was higher during hot humid season when sperm motility and density were comparatively inferior (Tomar et al, 1966). Seasonal variation in live percentages were reported lower during hot humid season in Hariana bull.

Age group does not affect the production of semen but bulls of over 3 years of age may produce higher percentage of live spermatozoa (Singh et al, 1967).

Rosenhalm and Mackk (1967) found no effect of season on semen quality.

Nadarajh (1968) studied the semen characteristics of Jersey, Ayshire and Friesian bulls. Motility was 4.1 ± 0.4, 3.2 ± 0.48 and 3.8 ± 0.54 and sperm concentration ($x10^6$) was 1346 ±

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458, 872.9 ± 254, and 995 ± 65.4 per ml. In Jersey and Ayrshire, the two characters did not differ significantly. Age of the bull did not affect semen characters significantly but motility was highly significantly correlated (0.60) with 60 to 90 days non-return rate to 1st insemination.

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

Dessouky and Juma (1968) studied the different attributes of semen and seasonal variation in the Holstein Friesian bulls. The study was made on volume, density, pH, and motility. None of these characters was significantly affected by age of bull or by season. All the characters were closely correlated with one another.

Mishra et al (1969) studied the seasonal variation in spermatozoan characteristics of Hariana and Tharparkar bulls.

The study was conducted during three seasons viz. rainy, winter and summer for measurements of head length, middle piece and tail in each seasons. There were significant seasonal differences in all the measurements. Sizes were smaller in rainy season than those of winter and summer.

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Resistance of spermatozoa to freezing to -196°C was lowest in May (29.54%), and highest in August (69.56%) and September (67.74%); (ILJINSKAJA, 1970).

Tomar and Kanajia (1970) studied the seasonal variation in reaction time and semen characteristics of Hariana bulls. Initial motility, sperm density, percentage of abnormal spermatozoa and methyline blue reduction time varied significantly between seasons. Semen quality was best in winter followed by spring, autumn and summer.

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C H A P T E R - III.

MATERIALS AND METHODS

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MATERIALS AND METHODS

In the present study five Tharparkar and four Friesian bulls, aged about 7 years and 1½ years respectively, maintained at Semen Bank, Patna, were used. Bulls were selected randomly.

Bulls of Tharparkar herd were obtained from Government Cattle Farm, Patna, and the Friesians from the "Holstein Friesian Association of America", Brattlebord, Vermont. Friesian bulls were brought to Patna on 22.12.1970 from Aarey Milk Colony, Bombay, as these bulls were earlier kept at Bombay. Informations regarding their age could be gathered from the register of Government Cattle Farm, Patna in case of Tharparkar bulls and in case of Freisian from the Registration card of each bull supplied by the Holstein Friesian Association of America to the Government of Bihar through the Ministry of Food and Agriculture, Government of India.

Bulls of both breeds were under identical environments so far as housing, management, feeding etc. were concerned.

The house was pucca built with concrete floor and asbestes roof with bilateral byre having sufficient ventilation for air and light. Each animal was placed in separate stall in the byre, the stall being seperated by the pucca wall.

Bulls were given daily exercise and water bathing in the morning hours.

Meteorological datas :

The temperature, humidity and rainfall were as follows

during the period of study i.e. from July'71 to December'71.

TABLE NO. 3.1

Showing temperature, rainfall and humidity.

Months	Temperature		Rainfall total in mm.	Humidity in percent
	Maximum Av.	Minimum Av.		
July'71.	31.9°C	24.6°C	385.9	79
August'71.	30.2°C	24.5°C	503.7	78
September'71.	31.9°C	24.4°C	108.0	77
October'71.	29.3°C	21.1°C	224.7	73
November'71.	27.8°C	14.4°C	-	71
December'71.	24.4°C	9.8°C	-	77

These datas were obtained from the Agriculture Research Institute, Patna and Central Potato Research Station, Patna.

Health status:

Bulls were vaccinated against Hoemorrhagic septicaemea, Anthrax, Black quarter and Foot and Mouth disease but the Friesian bulls got the sudden attack of Foot and Mouth disease even after vaccination. Daily temperature records were being maintained for each bull and heifer of the Semen Bank, Patna.

Feeding schedule:

The feeding schedule of the bulls, was also kept the same. Three kilograms of concentrate mixture was given to each bull, in addition to Bhusa, green fodder, mineral mixture and

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salts etc. The composition of ration was as follows for each bull with minor adjustments according to the availability of feeds and fodders in the Government Cattle Farm, Patna, but the nutritional standards were not changed.

Composition of ration:

Wheat Bran -	1 Kilogram.
Groundnut cake -	1 "
Maize -	1 "
Bhusa -	5 "
Green fodder -	5 "
Mineral Mixture -	1% of the total ration.
Common salt -	2 oz. per animal.per day.
Vitablend AD_3 (Glaxo)-	1 gram per animal per day.

Green fodders such as Para, Napier and Jwar were given as per availability in the Farm.

Procedure of experiment:

The experiment was conducted in two seasons viz. Rainy and Winter. The rainy season was considered to last for 3 months from July to September, while the winter season ranged from October to December.

A similar duration for season was maintained by Tomar et al (1966).

The plan of work originally designed was as follows:

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TABLE NO. 3.2

Showing the number of observations to be studied within each breed per season.

Breed	No. of bulls	No. of collections per bull per month for 3 months.	No. of samples per collection.	No. of traits to be studied.	Total samples for each trait	Grand total for all the traits per season.
Tharparkar.	6	4x3 = 12	6	4	6x4x3x6 =432	432x4=1728
Friesian.	4	4x3 = 12	6	4	4x4x3x6 =288	288x4=1152

TABLE NO. 3.3

Showing the number of observations to be studied for each bull in a season.

No. of collection per bull per month for 3 months.	No. of samples per collection.	No. of observation under each trait per bull.
4x3 = 12	6	12x6 = 72

The six samples were assigned to six intervals viz., 0 hour, 24 hours, 48 hours, 72 hours, 96 hours and 120 hours randomly.

The number of collections actually obtained in each season was as follows:

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TABLE NO. 3.4

Showing the number of collections actually obtained in each season.

Breed	Season		Total
	Rainy	Winter	
Tharparkar.	53	58	111
Friesian.	38	37	75

Due to illness and other adverse circumstances lesser number of collections was obtained from Friesian bulls as compared to the Tharparkar group. The lesser number of observations obtained from both the herds as compared to the original plan was due to non-mounting of certain bulls on the scheduled dates of collection.

Before proceeding for recording experimental data, the techniques to be followed for evaluation of semen quality were standardised. An anaestrus cow and a buffalo bull acted as dummies for collection of semen from bulls of Tharparkar breed whereas anaestrus heifers were used for collection of semen from the bulls of Friesian herd. Only one attendent took the collection throughout the period of study. For each bull seperate Artificial Vagina was used. The temperature of Artificial Vagina for bulls was maintained between 45°C-55°C. After collection the neat semen was immediately examined for motility and then diluted.

Egg yolk-citrate (Salisbury et al, 1941) was used for diluting the bull semen at Semen Bank, Patna.

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The citrate buffer for bull semen consisted of the following: -

Crystalline sodium citrate -	2.94 gram
Glass distilled water -	100 c.c.

2 parts of this buffer was mixed with one part of egg yolk to constitute the diluent. To each 100 c.c. of the diluter, thus prepared, (a) 1 lakh unit of penicillin-G-sodium and (b) 100 mgm. of streptomycin sulphate were added. Semen was diluted at the rate of 1 in 20.

After dilution, 6 smaller test tubes measuring 4"x½" were taken. Each was wrapped in cotton with a label bearing bull number, date of collection and dilution rate.

Eachof the 6 test tubes was inserted in an outer tube measuring 4"x¾". The inner tubes were corked finally and kept in thermosflask, 3/4 filled with ice. All the conditions were maintained uniform for each sample of semen.

The thermosflask was brought from the Semen Bank to the College Laboratory for further examination.

The 6 samples from each collection were assigned to six intervals (viz. 0 hour, 24 hours, 48 hours, 72 hours, 96 hours and 120 hours) randomly.

Out of the six samples thus assigned, five were kept in the referigerator for preservation at 4°C in a beaker with a outer label on the beaker written with a grease pencil.

The semen in one test tube which was assigned for 0 hour

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was examined immediately after the arrival in the laboratory for the pH (hydrogen-ion concentration), motility, both initial i.e. mass, individual motility and percentage of live spermatozoa.

The first examination was treated as the evaluation of semen at 0 hour, after collection. Further five samples from each collection of each bull were examined at intervals of 24 hours, 48 hours, 72 hours, 96 hours and 120 hours respectively. Any sample which gave less than 40% live spermatozoa count was discarded. Abnormal samples were not encountered during the experimental period.

Each sample was put to the following four tests for the purpose of semen evaluation at each interval.

1. pH (hydrogen-ion concentration).
2. Initial Motility.
3. Individual Motility.
4. Estimation of live spermatozoa (in percentage).

1. DETERMINATION OF pH :

A sample of 0.1 c.c. of thoroughly mixed semen was taken in two test tubes, 14.9 c.c. of glass distilled water was added to each of them by means of a pipette. In one of the test tubes 0.5 c.c. of brom thymol blue indicater was added. After thoroughly mixing the fluid in these test tubes, it was transferred to the smaller test tubes seperately and placed in the holes of Lovibond disc comparater. The one containing the indicater was placed in the right hand side hole of the comparater and the other in the left hand side hole. The colour of the test tube was then

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matched with the standard coloured glass filted in the disc of the comparater by rotating the disc of the comparater. In this way pH was determined. The middle point of the range was noted for the sample in which the figure was in between the two numbers of the disc comparater.

2. INITIAL MOTILITY OF SPERMATOZOA:

The live spermatozoa are motile but all the immotile sperms are not dead. Many non-motile sperms samples gain motility when treated suitably with diluents (Joshi et al, 1967) Tomar et al, 1968a, b). Spermatozoan motility is judged as gross or mass motility and as individual motility. The first way of evaluation is rapid. It is commonly used for scoring the initial motility of semen samples immediately after collection. In the present study a drop of semen was spread uniformly over a clean, dry and grease free glass slide maintained at 37°C. Motility was graded under low power microscope, after seeing the mass movement of spermatozoa. On the basis of activity, semen was graded grossly under five categories (Blom, 1946) as follows :-

(i) + 5 Motility - Very quick wave motions in the field, were noted, there were waves after waves. Swirles were noted with mass movement of spermatozoa.

(ii) + 4 Motility - Swirles were comparatively not so rapid as in + 5 grade. They were observed to more towards the extremities.

(iii) + 3 Motility - Swirles were scattered in the field.

(iv) + 2 Motility - Swirles were absent, individual movements

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of sperms were more evident in the field.

(v) + 1 Motility - No wave motions were observed in the field.

Zero motility was given to the sample in which sperms were completely non-motile.

The initial motility was further sub-divided into +5, +4.5, +4, +3.5, +3, +2.5, +2, +1.5 and +1 grades for more precise recording. For the sake of statistical analysis these grades were recorded as follows :-

+ 5	=	100%
+ 4.5	=	90%
+ 4	=	80%
+ 3.5	=	70%
+ 3	=	60%
+ 2.5	=	50%
+ 2	=	40%
+ 1.5	=	30%
+ 1	=	20%

3. <u>INDIVIDUAL MOTILITY</u> :

The percentage of spermatozoan motility was determined as follows by hoemocytometer method (Lasley, 1951).

<u>Preparation of buffer solution</u> :

To maintain the motility, the same buffer solution was prepared that was used in the diluent. The composition was :

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Crystalline sodium citrate dihydrate - 2.94 grams.

Glass distilled water - 100 c.c.

Procedure :

The tube containing the sample was taken out from the refrigerator, and was kept in a beaker containing water at room temperature for half an hour. This was done to bring the semen to room temperature. Similarly, the buffer solution was also brought to the room temperature by similar treatment. A few drops of semen was taken in a clean, watch glass. From this, it was sucked upto 0.5 mark of the R.B.C. diluting pipette. It was diluted to 200 times by sucking the buffer solution upto 101 mark in the R.B.C. pipette. The centre of the pipette was rotated for thorough mixing. A few drops were discarded and then a drop of diluted semen was placed on the previously focussed counting chamber of hoemocytometer under high power (40x) objective. The number of non-motile and weakly motile spermatozoa in all the 25 big squares were counted and recorded. All the spermatozoa that showed movement but not in straight line (a characteristic for progressively motile spermatozoa) were recorded as weakly motile. Those having slow movements in smaller circles were recorded as weakly motile but as they were in the process of dying these were recorded as non-motile. Weakly motile and non-motile were grouped in the category of non-motile because only two categories were recorded either motile or non-motile. Reverse movement of spermatozoa i.e. tail ahead and head behind was also recorded. Apart from these, following categories of non-motile spermatozoa were seen which include (i) head static and oscillating tail. (ii) Tail

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static and oscillating head. (iii) Head constantly moving in circle with tail in the centre. (iv) Tail moving in the circle and head in the centre.

The counting chamber was then placed in the freezing chamber of the refrigerator. After the diluted suspension of spermatozoa under coverslip had frozen, the counting chamber was taken out of the refrigerator and kept at room temperature, or thawed directly in the sunlight. One to one and half hours time was allowed to keep the counting chamber in the refrigerator since it was found to be convenient. After the frozen suspension had melted, the total number of spermatozoa in the same 25 big squares were counted and recorded. This included both the number of motile and non-motile spermatozoa. The sample assigned for 0 hour was also treated in the same way except that it was not taken out from the refrigerator as it was immediately examined after bringing in the laboratory from the Semen Bank.

The percentage of motile spermatozoa was calculated as follows :-

(a) $\frac{\text{No. of non-motile spermatozoa before freezing}}{\text{Total no. of spermatozoa after freezing}} \times 100$

= percentage of non-motile spermatozoa.

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

4. <u>ESTIMATION OF LIVE SPERMATOZOA (IN PERCENTAGE)</u> :

<u>Preparation of stain</u>:

Eosin-Nigrosin stain as advocated by Swanson <u>et al</u> (1951)

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was used. It was prepared as follows :-

Eosin (water soluble) -	1 gram
Nigrosin (B.D.H.) -	5 grams
Sodium citrate dihydrate-	3 grams
Glass distilled water -	100 c.c.

This solution was kept on water bath for half an hour and filtered after cooling.

Preparation of slides:

On a grease free, clean dry slide a small drop of diluted semen and a large drop of stain was put leaving one inch space from one end so that the ratio between the semen and the stain was 1:2 (Swanson et al, 1951). The semen and the stain droplets were well mixed by gently blowing air through a pipette. A second clean slide was immediately put on this leaving the space uncovered by the stain. A soft cloth was applied at the edges of slides by applying a gentle pressure on the slide so as to remove excess of stain. The two slides were seperated quickly by using sliding action. Exposure of semen to stain was made for 1-2 minutes. The smears were air-dried and immediately examined under oil-immersion lens (100x). Live spermatozoa did not take any stain and were colourless. The back ground was violet with dead spermatozoa taking red stain of eosin. Those taking partial stain anteriorily or posteriorily were taken as dead, as majority of the workers are of the view that partially stained spermatozoa are on the way to death(Perry, 1960; Maule, 1962). Percentage of live spermatozoa was determined by counting 200 spermatozoa in the field taking

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diagnally across the slide (Asdell, 1955). The formula for calculation was as follows :-

$$\frac{\text{Total number of live spermatozoa}}{\text{Total number of live + dead spermatozoa}} \times 100$$

= percentage of live spermatozoa.

At each intervals as assigned earlier all the four tests were done on the samples and then it was discarded.

Procedure of analysis :

The mean values of pH (Hydrogin-ion concentration) together with S. E. and C. V. % were calculated intervalwise in each breed and season respectively.

All the observations obtained for live spermatozoa percentage and motility were transformed first by Sin $^{-}$P transformation prior to statistical analysis of the data (Snedecor, 1967). The transformed values were subjected to analysis of variance (Snedecor, 1967) for knowing the effects of breeds, seasons and intervals (Hours of preservation). The transformed datas were re-transformed into percentages for presentation of Means, S.E's and C.V. values. In case, where mean differences were found significant by analysis of variance, they were further tested by calculating the critical differences, according to the formula given below for knowing the roles of specific variables.

Critical differences = S.E. of differences between two treatments mean X t value at 5% or 1% level of significant for error degrees of freedom.

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S.E. of differences between two treatment means =

$$\sqrt{\text{Mean sum of squares for error degree of freedom} \times \left(\frac{1}{N_1} + \frac{1}{N_2}\right)}$$

Where N_1 and N_2 are the number of observations in each treatment.

Mean, S.E., C.V.% were calculated and analysis of variances run as per Snedecor (1967).

Details about analysis of variance:-

Analysis of variance was run to see the effects of seasons in each breed seperately, and also on the basis of pooled data. To see the effects of breeds pooled data was used, but in case of initial motility, individual motility and percentage of live spermatozoa seperate analysis was also done breedwise in each season. Analysis of variance to see the effect of intervals (Hours of preservation) was done on pooled data in each season seperately.

Tables, graphs, and Micro-photgraphs have been used for the interpretations, comparisons and drawing inferences.

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C H A P T E R - IV.

RESULTS AND DISCUSSION.

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RESULTS AND DISCUSSION

Altogether 53 collections from 5 Tharparkar bulls and 38 collections from 4 Friesian bulls were obtained during rainy season. In winter season, the number of collections from Tharparkar and Friesian bulls were 58 and 37 respective (Table 3.4).

The six samples from each collection were assigned to six different intervals (hours of preservation), i.e. 0 hour, 24 hours, 48 hours, 72 hours, 96 hours and 120 hours respectively as planned earlier.

Four seminal characters which have direct bearing on the quality of semen were studied. They are pH (hydrogen-ion concentration), initial motility, individual motility, and estimation of percentage of live spermatozoa. All the four traits were studied at each interval as assigned earlier.

The number of observations in Rainy and winter seasons from both the breeds, i.e. Tharparkar and Friesian were 296, 333 and 184, 184 respectively. The total number of observations was 997.

The study was conducted in two seasons, viz. rainy season and winter season and the results obtained have been placed below traitwise:-

pH (Hydrogen-ion concentration) :

The average pH of Tharparkar and Friesian bull semen at 0 hour in rainy season was 6.8 ± 0.036 and 6.8 ± 0.045 respec-

-tively (Table 4.1). There was decline in pH during hours of preservation. At 120 hours of preservation, the average pH for both the breeds was 6.4 ± 0.04 and 6.36 ± 0.06 respectively.

In winter season the average pH of bull semen for both the breeds at 0 hour was 6.84 ± 0.01 and 6.67 ± 0.02 respectively. There was decline in pH during hours of preservation. At 120 hours of preservation the averages were 6.24 ± 0.012 and 6.3 ± 0.03 for Tharparkar and Friesian bulls. The average decline in pH from 0 hour to 120 hours of preservation during rainy season was 0.4 for Tharparkar bull semen and that for Friesian bull semen being 0.44. In winter season, the decline was 0.60 and 0.37 for Tharparkar and Friesian respectively (Table 4.1 and 4.2).

Breeds did not seem to influence the pH of semen significantly (Table 4.3) but seasons affected pH highly significantly in both the breeds when studies were made on each breed seperately as well as when the same was done on pooled data for both the breeds(Table 4.4, 4.5 and 4.6). Different intervals (hours of preservation) also seemed to affect the pH of semen significantly in both the seasons (Table 4.7 and 4.8). The differences between hours of preservation revealed non-significant differences between 72 - 96 hours, 72 - 120 hours and 96 - 120 hours in the rainy season only, but all other differences between the hours of preservation turned out to be statistically significant (Table 4.9 and 4.10).

On storage of semen, pH declines towards more acidic side (Tomar, 1970).

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Erb et al (1942) recorded significant seasonal differences in semen quality. Swanson and Herman (1944), Mukherjee and Bhattacharya (1952), Kushwaha et al (1955), Oloufa et al (1959), Sen Gupta et al (1963), Tomar et al (1966) have also reported significant changes in the initial pH in semen of exotic, zebu and buffalo bulls.

Significant seasonal effects on pH of semen were observed by Raps and Cannon (1947). Mukherjee and Bhattacharya (1952) found the effects of years as well as months on the pH of semen to be significant. Bhattacharya (1957) also corroborated the significant seasonal differences in the pH of semen. Significant seasonal variation on all the semen characters was recorded by Singh (1966). He also observed better preservability of bull semen in the rainy season over that in the summer season.

The results of present study as regards the significant seasonal effects are in agreement with the findings of Raps and Cannon (1947), Mukherjee and Bhattacharya (1952), Erb et al (1942), Tomar et al (1966), Kushwaha et al (1955), Sen Gupta et al (1963) and Singh (1966), but it differs with the findings of Dessouky and Juma (1968), Milicevic, Varadin and Borjanovic (1968).

The seasonal differences in the pH might be due to the change in the availability of feeds and fodders as well as variability of climatic factors, as the pH is easily influenced by the factors like temperature, humidity and physical well being of the individual. Besides, a look at the raw data on pH studies indicates greater variability in respect of Friesian bull semen as compared to that of Tharparkar breed.

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TABLE NO. 4.1

Showing the means with their S.E's and C.V.% of pH (hydrogen-ion concentration) at different hours of preservation of bull semen during Rainy season.

Hours of preser-vation.	Breeds					
	THARPARKAR			FRIESIAN		
	N	Mean ± S.E.	C.V.%	N	Mean ± S.E.	C.V.%
0 hour	53	6.8± 0.036	3.8	38	6.8 ± 0.045	4.1
24 hours	53	6.7 ± 0.033	3.57	36	6.6 ± 0.039	3.46
48 hours	52	6.56± 0.093	3.2	36	6.53± 0.034	3.06
72 hours	52	6.48± 0.09	3.08	33	6.49± 0.037	3.38
96 hours	51	6.44± 0.032	3.57	29	6.47± 0.04	3.4
120 hours	35	6.4 ± 0.04	3.59	12	6.36± 0.06	3.3

TABLE NO. 4.2

Showing the means with their S.E's and C.V.% of pH (hydrogen-ion concentration) at different hours of preservation of bull semen during Winter season.

Hours of preser-vation	Breeds					
	THARPARKAR			FRIESIAN		
	N	Mean ± S.E.	C.V.%	N	Mean ± S.E.	C.V. %
0 hour	58	6.84±0.01	1.3	37	6.67±0.02	1.9
24 hours	58	6.7 ±0.01	1.4	37	6.6 ±0.02	1.8
48 hours	58	6.56±0.013	1.5	37	6.5 ±0.01	1.69
72 hours	58	6.4 ±0.012	1.5	35	6.36±0.01	1.5
96 hours	57	6.32±0.013	1.55	28	6.35±0.015	1.3
120 hours	44	6.24±0.012	1.29	10	6.3 ±0.03	1.58

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TABLE NO. 4.3

Analysis of variance showing the effects of breeds in respect of pH (on pooled data).

Sources of variation	d. f.	M.S.	F.
Between breeds	1	0.06	1 N.S.
Within breeds	995	0.06	
Total	996		

N.S. denotes non-significant.

TABLE NO. 4.4

Analysis of variance to the see the effects of seasons on pH in Tharparkar herd.

Source of variation	d.f.	M.S.	F.
Between seasons	1	0.53	8.4**
Within seasons	627	0.063	
Total	628		

** denotes significant at 1% level.

TABLE NO. 4.5

Analysis of variance to see the effects of season on pH in Friesian herd.

Source of variation	d.f.	M.S.	F.
Between season	1	0.83	16.9**
Within season	366	0.049	
Total	367		

** denotes significant at 1% level.

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TABLE NO. 4.6

Analysis of variance to see the effects of seasons on pH (pooled data).

Source of variation	d.f.	M.S.	F.
Between season	1	1.26	21.3**
Within season	995	0.059	
Total	996		

** denotes significant at 1% level.

TABLE NO. 4.7

Analysis of variance to see the effects of intervals (hours of preservation) in respect of pH during Rainy season.

Source of variation	d.f.	M.S.	F.
Between interval (hours of preservation).	5	2.12	39.25**
Within interval (hours of preservation).	474	0.054	
Total.	479		

** denotes significant at 1% level.

TABLE NO. 4.8

Analysis of variance to see the effects of intervals (hours of preservation) in respect of pH during Winter season.

Source of variation	d.f.	M.S.	F.
Between interval (hours of preservation).	5	3.08	250.4**
Within interval (hours of preservation)	511	0.0123	
Total.	516		

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TABLE NO. 4.9

Showing critical differences between different intervals (hours of preservation) in respect of pH in Rainy season.

Comparison between hours of preservation.	(N_1-N_2)	Mean difference	Calculated C.D. at 5%	Calculated C.D. at 1%
0-24	91-89	0.15**	0.066	0.087
0-48	91-88	0.28**	0.066	0.087
0-72	91-85	0.34**	0.066	0.087
0-96	91-80	0.37**	0.068	0.09
0-120	91-47	0.44**	0.078	0.10
24-48	89-88	0.13**	0.066	0.087
24-72	89-85	0.19**	0.066	0.087
24-96	89-80	0.22**	0.068	0.09
24-120	89-47	0.29**	0.078	0.10
48-72	88-85	0.06**	0.066	0.087
48-96	88-80	0.09*	0.068	0.09
48-120	88-47	0.16**	0.078	0.10
72-96	85-80	0.03 N.S.	0.07	0.09
72-120	85-47	0.01 N.S.	0.082	0.10
96-120	80-47	0.07 N.S.	0.082	0.10

N_1-N_2 denote number of observations at different hours of preservation.

N.S. denotes non-significant.

* denotes significant at 5% level.

** denotes significant at 1% level.

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TABLE NO. 4.10

Showing critical differences between different intervals (hours of preservations) in respect of pH in Winter season.

Comparison between hours of preservation.	(N_1-N_2)	Mean difference	Calculated C.D.at 5%	Calculated C.D.at 1%
0-24	95-95	0.14**	0.039	0.0516
0-48	95-95	0.25**	0.039	0.0516
0-72	95-93	0.36**	0.039	0.0516
0-96	95-85	0.44**	0.041	0.054
0-120	95-54	0.53**	0.047	0.061
24-48	95-95	0.11**	0.039	0.0516
24-72	95-93	0.22**	0.039	0.0516
24-96	95-85	0.30**	0.041	0.054
24-120	95-54	0.39**	0.047	0.0619
48-72	95-93	0.11**	0.039	0.0516
48-96	95-85	0.19**	0.041	0.054
48-120	95-54	0.28**	0.047	0.0619
72-96	93-85	0.08**	0.041	0.054
72-120	93-54	0.17**	0.047	0.0619
96-120	85-54	0.09**	0.047	0.0619

N_1-N_2 denotes number of observations in different hours of preservation.

** denotes significant at 1% level.

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Initial Motility:-

At 0 hour of collection the average initial motility of semen was 85.31 ± 0.015, 85.87 ± 0.009 and 84.54 ± 0.042, 79.30 ± 0.049 in Tharparkar and Friesian during rainy and winter season respectively (Table 4.11 and 4.12).(Fig. No. 1 and 2).

After 120 hours of preservation the averages were 32.13 ± 0.012, 35.60 ± 0.008, and 28.95 ± 0.044, 29.81 ± 0.027 in Tharparkar and Friesian respectively. Sharp decline in the initial motility during different hours of preservation was noticed. From 0 hour to 120 hours there were decline of 53.18, and 50.27 in Tharparkar herd and 55.59 and 49.49 in Friesian herd during rainy and winter seasons respectively (Table 4.47).

From these figures it appeared that in winter, the keeping quality seemed to be better than that in the rainy season (Table 4.11 and 4.12).

Though on pooled data breeds were found to have no significant effects on this trait, the data in winter season revealed significant breed differences on the trait. The Tharparkar bull semen seemed to have a little superiority over that of the Friesian during winter. The probable cause of it might be due to the environmental effects on these bulls of exotic origin (Table 4.13, 4.14 and 4.15).

In both Holstein-Friesian and Tharparkar herds, seasons were found to have non-significant effects on this trait. Even on pooled data non-significant seasonal effect was recorded (Table 4.16, 4.17 and 4.18).

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Hours of preservation turned out to be highly significant in both the seasons. Critical difference test revealed that all the hours of preservation varied significantly among themselves (Table 4.19, 4.20, 4.21 and 4.22).

Decline in motility in stored bull semen was also reported by Swansen and Herman (1941). Gradual but significant decline in the percentage of motile spermatozoa was observed by Rao (1965) with bull semen.

Months did not have any significant effects on motility of spermatozoa according to Swanson (1951). Kushwaha et al (1955) observed no significant seasonal variation in this trait. With Hariana bulls Sharma and Saha (1962) corroborated the findings of Kushwaha et al (1955).

The present findings in this trait are in agreement with the findings of Swanson (1951), Kushwaha et al (1955) and Dessouky and Juma (1968) so far as seasonal variation is concerned but it differs from ~~the~~ those of Anderson (1945), Kodagali (1963), Sinha and Prasad (1966), and Singh (1966).

The significant differences between different hours of preservation conform to the findings of Swanson and Herman (1941), Rao (1965) and Singh (1966).

The cause of this decline in the motility might be due to the duration of time during preservation in which the spermatozoa utilised the restored energy.

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TABLE NO. 4.11

Showing the means with their S.E's and C.V. of initial motility at different hours of preservation of bull semen during Rainy season.

Hours of preservation.	Breeds					
	THARPARKAR			FRIESIAN		
	N	Mean ± S.E.	C.V.%	N	Mean ± S.E.	C.V.%
0 hour	53	85.31±0.015	1.7	38	84.54±0.042	3.57
24 hours	53	70.67±0.007	0.79	36	68.34±0.007	3.01
48 hours	52	62.17±0.006	0.67	36	59.52±0.025	3.44
72 hours	52	53.52±0.009	1.98	33	50.54±0.02	3.17
96 hours	51	43.06±0.009	2.78	29	41.97±0.019	3.32
120 hours	35	32.13±0.012	3.47	12	28.95±0.044	5.12

TABLE NO. 4.12

Showing the means together with their S.E's and C.V. of initial motility at different hours of preservation of bull semen during winter season.

Hours of preservation.	Breeds					
	THARPARKAR			FRIESIAN		
	N	Mean ± S. E.	C.V.%	N	Mean ± S.E.	C.V.%
0 hour	58	85.87±0.009	1.06	37	79.30±0.049	4.68
24 hours	58	72.9 ±0.001	0.89	37	65.61±0.022	2.78
48 hours	58	63.87±0.006	0.92	37	56.51±0.019	2.89
72 hours	58	53.98±0.007	1.25	35	48.23±0.017	2.93
96 hours	57	45.22±0.006	1.4	28	40.30±0.009	1.73
120 hours	44	35.60±0.008	2.03	10	29.81±0.027	2.39

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TABLE NO. 4.13

Analysis of variance to see the effects of breeds in Rainy season in respect of initial motility.

Source of variation	d.f.	M. S.	F.
Between breed.	1	12.4164	<1
Within breed.	478	126.1772	
Total.	479		

TABLE NO. 4.14

Analysis of variance to see the effects of breeds on initial motility in Winter season.

Source of variation	d.f.	M.S.	F.
Between breed.	1	516.5767	4.7*
Within breed.	515	109.6507	
Total.	516		

* denotes significant at 5% level.

TABLE NO. 4.15

Analysis of variance to see the effects of breeds on initial motility on pooled data.

Source of variation	d.f.	M. S.	F.
Between breed.	1	187.8869	1.5 N.S.
Within breed.	995	117.938	
Total.	996		

N.S. denotes non-significant.

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TABLE NO. 4.16

Analysis of variance to see the effects of seasons on initial motility in Tharparkar herd.

Source of variation	d.f.	M.S.	F.
Between season.	1	80.7289	<1
Within season.	627	117.6698	
Total.	628		

TABLE NO. 4.17

Analysis of variance to see the effects of season on initial motility in Friesian herd.

Source of variation	d.f.	M.S.	F.
Between season.	1	266.1350	2.25 N.S.
Within season.	366	118.0960	
Total.	367		

N.S. denotes non-significant.

TABLE NO. 4.18

Analysis of variance to see the effects of seasons on initial motility on the basis of pooled data.

Source of variation	d.f.	M. S.	F.
Between season.	1	5.7577	<1
Within season.	995	119.1267	
Total.	996		

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TABLE NO. 4.19

Analysis of variance to see the effects of intervals (hours of preservation) on initial motility in Rainy season.

Source of variation	d.f.	M.S.	F.
Between interval.	5	10090.8142	484.5**
Within interval.	474	20.8252	
Total.	479		

** denotes significant at 1% level.

TABLE NO. 4.20

Analysis of variance to see the effects of intervals (hours of preservation) on initial motility in Winter season.

Source of variation	d.f.	M.S.	F.
Between interval.	5	9401.9449	471.1**
Within interval.	511	19.9535	
Total.	516		

** denotes significant at 1% level.

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TABLE NO. 4.21

Showing critical differences between different intervals (hours of preservation) in respect of initial motility in Rainy season.

Comparison between intervals.	(N_1-N_2)	Mean differences	Calculated C.D. at 5%	Calculated C.D. at 1%
0-24	91-89	10.57**	1.33	1.75
0-48	91-88	15.79**	1.33	1.75
0-72	91-85	20.84**	1.33	1.75
0-96	91-80	26.42**	1.35	1.78
0-120	91-47	33.17**	1.58	2.08
24-48	89-88	5.22**	1.33	1.75
24-72	89-85	10.27**	1.35	1.78
24-96	89-80	15.85**	1.37	1.80
24-120	89-47	22.60**	1.60	2.11
48-72	88-85	5.05**	1.35	1.78
48-96	88-80	10.63**	1.37	1.80
48-120	88-47	17.38**	1.60	2.11
72-96	85-80	5.58**	1.39	1.83
72-120	85-47	12.33**	1.60	2.11
96-120	80-47	6.75**	1.62	2.14

** denotes significant at 1% level.

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TABLE NO. 4.22

Showing critical differences in different intervals (hours of preservation) in respect of initial motility in Winter season.

Comparison between intervals.	(N_1-N_2)	Mean differences	Calculated C.D. at 5%	Calculated C.D. at 1%
0.24	95-95	9.11**	1.25	1.65
0-48	95-95	14.60**	1.25	1.65
0-72	95-93	19.93**	1.27	1.67
0-96	95-85	24.65**	1.29	1.70
0-120	95-54	29.99**	1.48	1.96
24-48	95-95	5.49**	1.25	1.65
24-72	95-93	10.82**	1.27	1.67
24-96	95-85	15.54**	1.29	1.70
24-120	95-54	20.88**	1.48	1.96
48-72	95-93	5.33**	1.27	1.67
48-96	95-85	10.05**	1.29	1.70
48-120	95-54	15.39**	1.48	1.96
72-96	93-85	4.72**	1.31	1.72
72-120	93-54	10.06**	1.48	1.96
96-120	85-54	5.34**	1.50	1.98

** denotes significant at 1% level.

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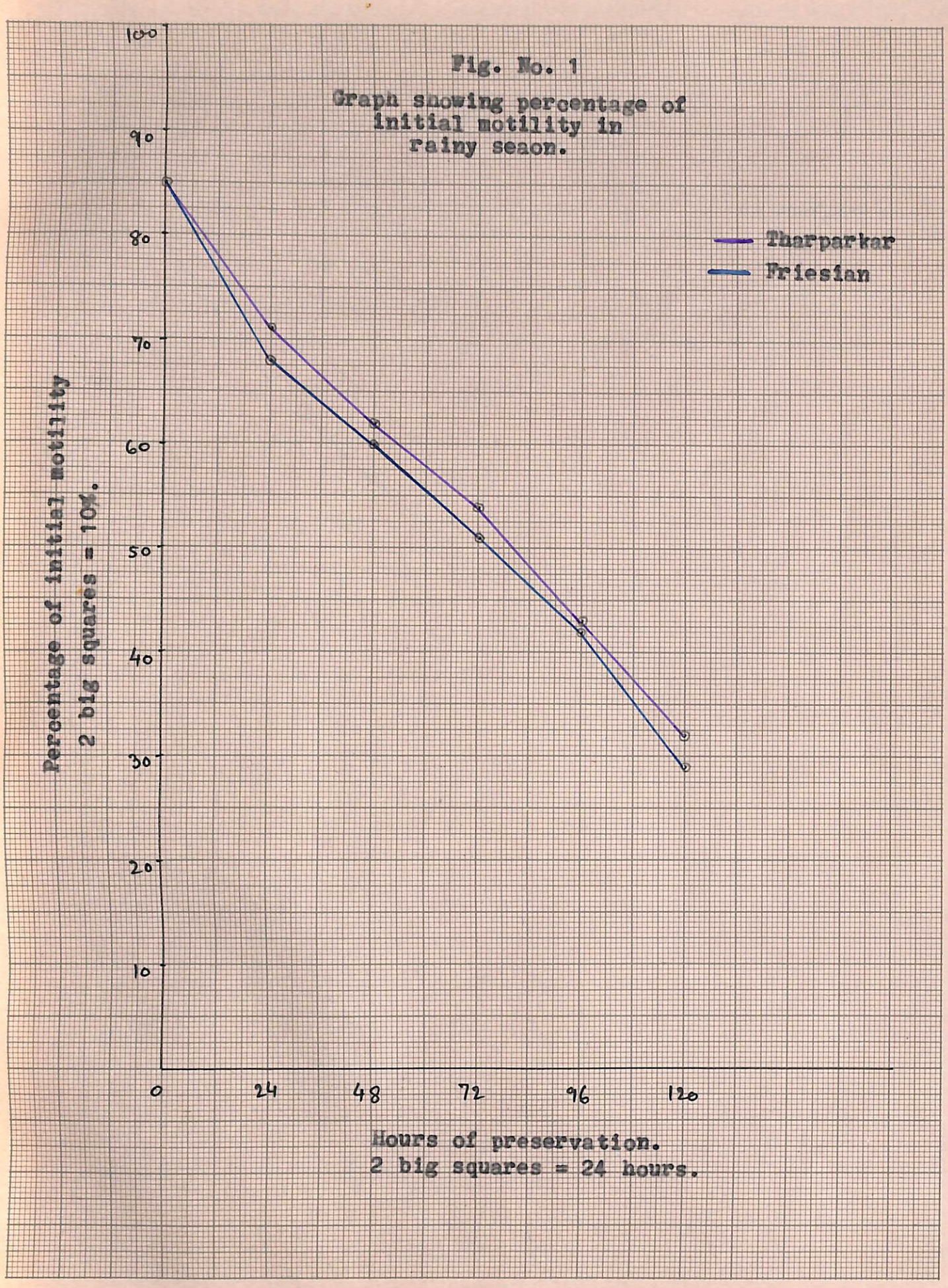
Fig. No. 1
Graph showing percentage of initial motility in rainy seaon.
Tharparkar
Friesian
Percentage of initial motility
2 big squares = 10%.
100
90
80
70
60
50
40
30
20
10
0
24
48
72
96
120
Hours of preservation.
2 big squares = 24 hours.

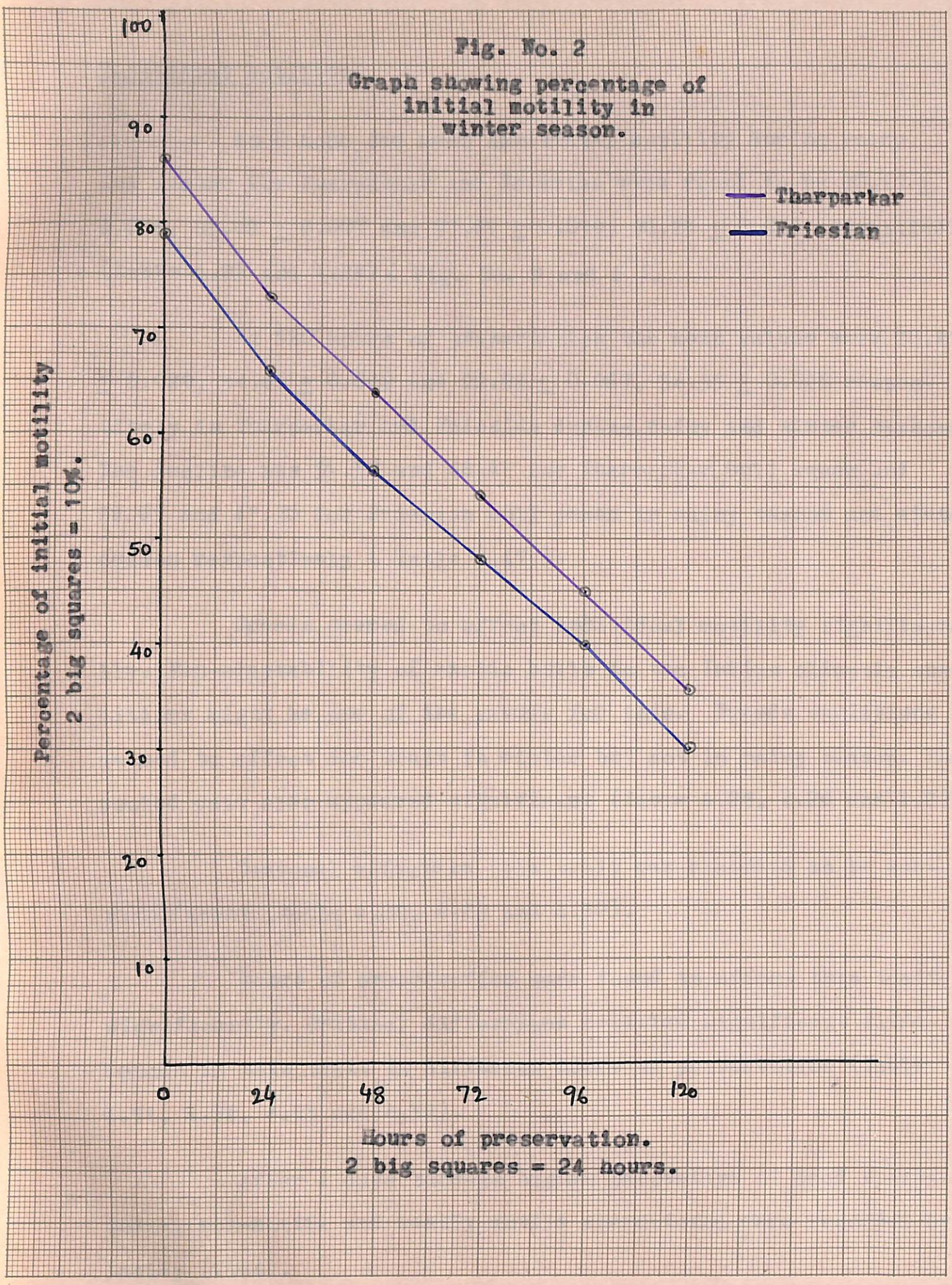
Fig. No. 2
Graph showing percentage of initial motility in winter season.
Tharparkar
Friesian
Percentage of initial motility
2 big squares = 10%.
100
90
80
70
60
50
40
30
20
10
0
24
48
72
96
120
Hours of preservation.
2 big squares = 24 hours.

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Individual motility:

At 0 hour the mean individual motility during rainy and winter for Tharparkar and Friesian were found to be 80.17 ± 0.008, 83.02 ± 0.007 and 77.61 ± 0.039, 76.23 ± 0.029 respectively (Table 4.23 and 4.24).(Fig. No. 3 and 4).

At 120 hours of preservation the mean values were 28.87 ± 0.029, 34.61 ± 0.008 and 22.94 ± 0.067, 34.53 ± 0.026 respectively in Tharparkar and Friesian during rainy and winter seasons. The decline was 51.30 and 48.41 for Tharparkar bull semen and 54.67 and 41.70 for Friesian bull semen in rainy season and winter season respectively (Table 4.47).

Breeds seemed to affect individual motility significantly. Non-adaptability of exotic bulls to the Indian climatic conditions might be one of the causes for this. Breeds variations seemed to affect the trait in winter but not in rainy season. The pooled data also appeared significant (Table 4.25, 4.26 and 4.27).

Seasonal effects on this trait was recorded to be non-significant (Table 4.28, 4.29 and 4.30).

Hours of preservation were found to affect this trait significantly. Critical differences revealed significant differences in this trait in different hours of preservation (Table 4.31, 4.32, 4.33 and 4.34).

After 4 days storage higher percentage of dead spermatozoa, non-motile and weakly motile spermatozoa were recorded by Lasley (1944).

In Hariana bull semen Saxena (1965) obtained 87.0 ± 1.1% motility. Non-significant effect of months on this trait was recorded by Swanson (1951).

Patrick et al (1959) found no significant effect of temperature and humidity in semen production and fertility.

Rossner (1955) found seasonal variation in all characters except concentration and forward movement of spermatozoa in which no seasonal changes were observed.

Kushwaha et al (1955) observed seasonal variation in semen characteristics of six Murrah buffalo bulls but failed to show significant variation between seasons.

Nikulenko (1965) studied different attributes of semen in Jersey, Red Danish and Black pied bulls and found significant differences between breeds in this trait.

Sharma and Saha (1962) found no seasonal effect on semen quality of Hariana bulls.

Rosenhelm and Mackk (1967) found no effect of season on semen quality.

Dessouky and Juma (1968) studied the different attributes of semen and seasonal variation in Holstein-Friesian bulls. No seasonal effect was observed.

The present results are in agreement with the findings of Lasley (1944), Saxena (1965), Swanson (1951), Patrick et al (1959), Rossner (1955), Kushwaha et al (1955), Nikulenko (1965),

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Sharma and Saha (1962), Rosenhelm and Mackk (1967) and Dessouky and Juma (1968) but differs from the findings of Weatherby et al (1940), Erb et al (1942), Phillips et al (1943), Swanson et al (1944), Mukherjee and Bhattacharya (1947) and Singh (1966).

TABLE NO. 4.23

Showing the means with their S.E's and C.V.% of individual motility at different hours of preservation of bull semen during Rainy season.

Hours of preservation.	Breeds					
	THARPARKAR			FRIESIAN		
	N	Mean ± S.E.	C.V.%	N	Mean ± S.E.	C.V.%
0 hour	53	80.17±0.008	0.92	38	77.61±0.039	3.84
24 hours	53	69.36±0.008	1.18	36	65.88±0.03	3.57
48 hours	52	64.00±0.006	0.896	36	55.78±0.048	7.34
72 hours	52	52.20±0.012	2.78	33	46.73±0.036	6.27
96 hours	51	39.87±0.014	4.51	29	38.60±0.029	5.54
120 hours	35	28.87±0.029	9.45	12	22.94±0.067	9.5

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TABLE NO. 4.24

Showing the means with their S.E's and C.V.% of individual motility at different hours of preservation of bull semen during Winter season.

Hours of preservation.	Breeds					
	THARPARKAR			FRIESIAN		
	N	Mean ± S.E.	C.V.%	N	Mean ± S.E.	C.V.%
0 hour	58	83.02±0.007	0.64	37	76.23±0.029	2.9
24 hours	58	71.83±0.007	0.92	37	64.75±0.017	2.03
48 hours	58	62.58±0.007	0.99	37	54.91±0.017	2.61
72 hours	58	53.47±0.006	1.15	35	47.78±0.018	3.19
96 hours	57	44.47±0.007	1.58	28	39.41±0.012	2.11
120 hours	44	34.61±0.008	2.01	10	34.53±0.026	2.015

TABLE NO. 4.25

Analysis of variance to see the effects of breeds on individual motility in Rainy season.

Source of variation	d.f.	M.S.	F.
Between breed.	1	53.8011	<1
Within breed.	478	121.6406	
Total.	479		

TABLE NO. 4.26

Analysis of variance to see the effects of breeds on individual motility in Winter season.

Source of variation	d.f.	M.S.	F.
Between breed.	1	441.6453	4.6*
Within breed.	515	95.5948	
Total.	516		

* denotes significant at 5% level.

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TABLE NO. 4.27

Analysis of variance to see the effects of breeds on individual motility on pooled data.

Source of variation	d.f.	M. S.	F.
Between breed.	1	426.9194	3.94*
Within breed.	995	108.3147	
Total.	996		

* denotes significant at 5% level.

TABLE NO. 4.28

Analysis of variance to see the effects of seasons on individual motility in Tharparkar herd.

Source of variation	d.f.	M. S.	F.
Between season.	1	387.5010	3.58 N.S.
Within season.	627	108.7539	
Total.	628		

N.S. denotes non-significant.

TABLE NO. 4.29

Analysis of variance to see the effects of seasons on individual motility in Friesian herd.

Source of variation	d.f.	M. S.	F.
Between season.	1	10.0949	<1
Within season.	366	107.0679	
Total.	367		

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TABLE NO. 4.30

Analysis of variance to see the effects of seasons on individual motility on pooled data.

Source of variation	d.f.	M. S.	F.
Between season.	1	329.0689	3.03 N.S.
Within season.	995	108.4131	
Total.	996		

N.S. denotes non-significant.

TABLE No. 4.31

Analysis of variance to see the effects of intervals (hours of preservation) on individual motility in Rainy season (pooled data).

Source of variation	d.f.	M.S.	F.
Between interval	5	9023.3550	326.9**
Within interval.	474	27.5976	
Total.	479		

** denotes significant at 1% level.

TABLE NO. 4.32

Analysis of variance to see the effects of intervals (hours of preservation) on individual motility in Winter season (pooled data).

Source of variation	d.f.	M. S.	F.
Between interval.	5	8216.8584	260.4**
Within interval.	511	31.5494	
Total.	516		

** denotes significant at 1% level.

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TABLE NO. 4.33

Showing critical difference between different intervals (hours of preservation) in respect of individual motility in Rainy season.

Comparison between hours of preservation	(N_1-N_2)	Mean differences	Calculated C.D.at 5%	Calculated C.D.at 1%
0-24	91-89	7.28**	1.52	2.01
0-48	91-88	12.83**	1.52	2.01
0-72	91-85	17.77**	1.54	2.03
0-96	91-80	23.93**	1.56	2.06
0-120	91-47	31.30**	1.84	2.42
24-48	89-88	5.55**	1.54	2.03
24-72	89-85	10.49**	1.54	2.03
24-96	89-80	16.65**	1.56	2.06
24-120	89-47	24.02**	1.84	2.42
48-72	88-85	4.94**	1.54	2.03
48-96	88-80	11.10**	1.56	2.06
48-120	88-47	18.47**	1.84	2.42
72-96	85-80	6.16**	1.58	2.08
72-120	85-47	13.53**	1.86	2.45
96-120	80-47	7.37**	1.88	2.47

** denotes significant at 1% level.

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TABLE NO. 4.34

Showing critical difference between different intervals (hours of preservation) in respect of individual motility in Winter season.

Comparison between intervals.	(N_1-N_2)	Mean differences	Calculated C.D.at 5%	Calculated C.D.at 1%
0-24	95-95	7.55**	1.58	2.08
0-48	95-95	13.23**	1.58	2.08
0-72	95-93	18.02**	1.60	2.11
0-96	95-85	22.93**	1.64	2.16
0-120	95-54	27.76**	1.86	2.45
24-48	95-95	5.68**	1.58	2.08
24-72	95-93	10.47**	1.60	2.11
24-96	95-85	15.38**	1.64	2.16
24-120	95-54	20.21**	1.86	2.45
48-72	95-93	4.79**	1.60	2.11
48-96	95-85	9.70**	1.64	2.16
48-120	95-54	14.53**	1.86	2.45
72-96	93-85	4.91**	1.64	2.16
72-120	93-54	9.74**	1.88	2.47
96-120	85-54	4.83**	1.90	2.50

** denotes significant at 1% level.

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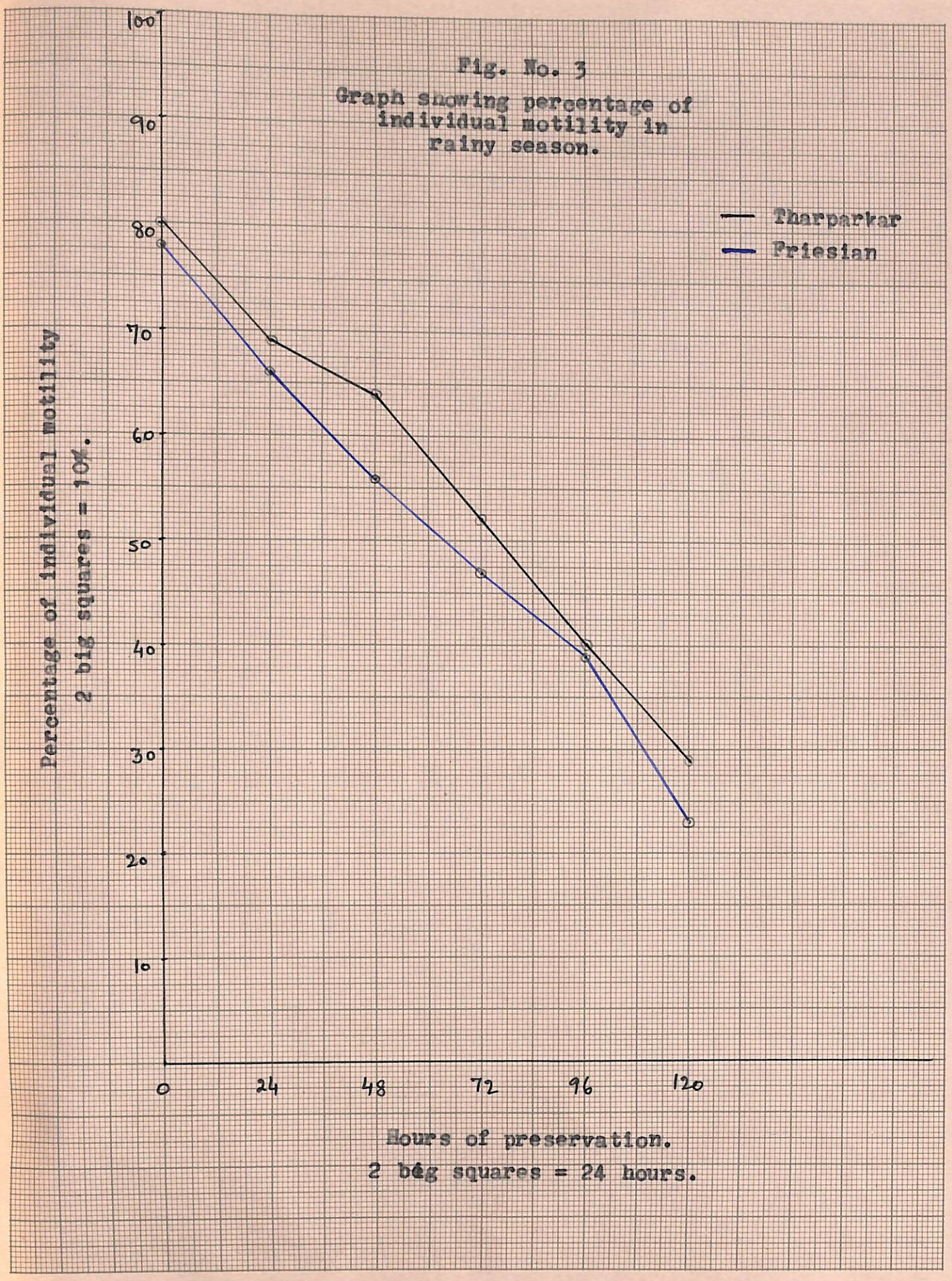
Fig. No. 3
Graph showing percentage of individual motility in rainy season.
Tharparkar
Friesian
Percentage of individual motility
2 big squares = 10%.
100
90
80
70
60
50
40
30
20
10
0
24
48
72
96
120
Hours of preservation.
2 big squares = 24 hours.

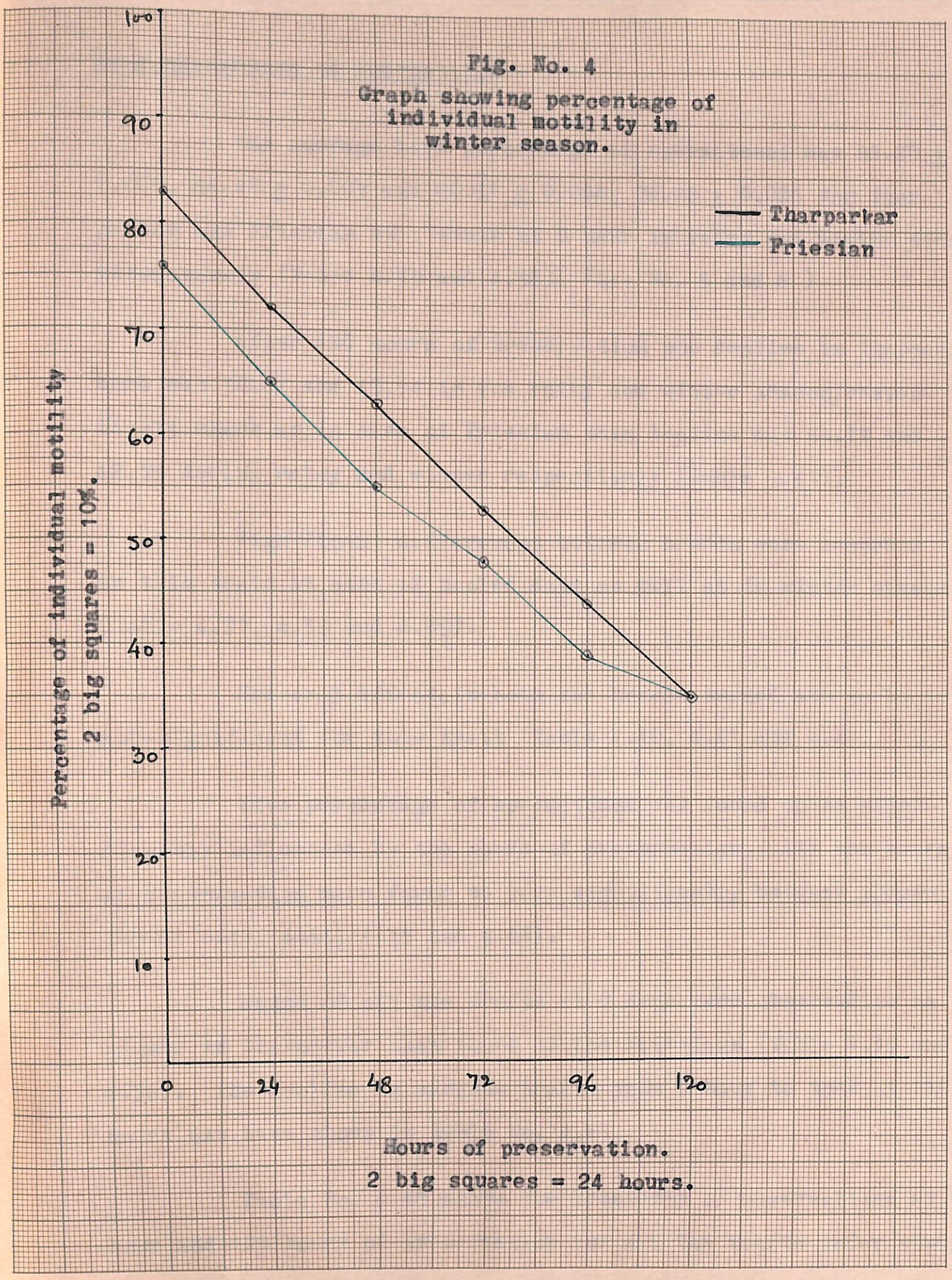
Fig. No. 4
Graph showing percentage of individual motility in winter season.
Tharparkar
Friesian
Percentage of individual motility
2 big squares = 10%.
100
90
80
70
60
50
40
30
20
10
0
24
48
72
96
120
Hours of preservation.
2 big squares = 24 hours.

Live percentage of spermatozoa:-

The average live percentage of spermatozoa was 82.20 ± 0.009, 84.47 ± 0.007 and 80.31 ± 0.044, 76.88 ± 0.026 for Tharparkar and Friesian bull semen at 0 hour in rainy and winter seasons respectively (Table 4.35 and 4.36)(Fig. No. 5 and 6).

After 120 hours of preservation the decline in Tharparkar herd was 50.62 and 49.34 in the rainy and winter seasons respectively where as in case of Friesian this decline was 55.18 and 42.78 during rainy and winter respectively (Table 4.47).

Significant differences between breeds were recorded in this trait in case of pooled data as well as in winter season (Table 4.37, 4.38 and 4.39).

Seasonal effects in both the breeds turned out to be non-significant for this trait (Table 4.40, 4.41 and 4.42).

Live percentage of spermatozoa was found to be significantly influenced by the hours of preservation. All the hours of preservation were found to vary significantly between themselves (Table 4.43, 4.44, 4.45 and 4.46).

Means of all the traits at 0 hour were observed to be higher in Tharparkar than those of the Friesians (Table 4.35 and 4.36). The decline in various traits from 0 hour to that of 120 hours has been shown in Table No. 4.47.

Table No. 4.48 shows the frequency of observations at 0 hour and 120 hours of preservation for all the traits under study.

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Samples with less than 40% live sperms were discarded. It was thus evident that the keeping quality of Tharparkar bull semen appeared a bit superior to that of the Friesian.

Iasley (1944) obtained more percentage of dead spermatozoa after 4 days storage of semen. Saxena (1965) got 89.0 ± 1.2% of live spermatozoa at 0 hour of preservation.

Swanson (1951) recorded non-significant effect of months on this trait.

The result of the present study conform with the findings of other workers.

Patrick et al (1959) also recorded non-significant effect of seasons. Sharma and Saha (1962) also recorded similar results.

Breed variations have been observed by Nikulenko (1965) on this trait. The results of present study tally with the findings of the above author.

Non-significant seasonal effects on semen quality under the present study was found to be in consonance with the results of Rosenhelm and Mackk (1967) and Dessouky and Juma (1968) in Holstein-Friesian bulls. They recorded non-significant effects of age of bulls and season on this trait.

Non-significant seasonal variation in this trait is in agreement with the findings of Sharma and Saha (1962) and Patrick et al (1959) and above authors but it differs from the findings of Mukherjee and Bhattacharya (1952) and Anderson (1945).

The non-significant seasonal effects might be due to less

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marked variation in the climatic factors in both winter and rainy seasons.

TABLE NO. 4.35

Showing means with their S.E's and C.V.% of live percentage of spermatozoa at different hours of preservation of bull semen during Rainy season.

Hours of preservation.	Breeds					
	THARPARKAR			FRIESIAN		
	N	Mean ± S.E.	C.V.%	N	Mean ± S.E.	C.V.%
0 hour	53	82.20±0.009	0.92	38	80.31±0.044	3.97
24 hours	53	70.31±0.009	1.4	36	68.10±0.032	3.65
48 hours	52	62.45±0.007	1.06	36	58.46±0.034	4.83
72 hours	52	53.58±0.009	1.75	33	49.25±0.029	4.66
96 hours	51	42.40±0.013	3.67	29	38.97±0.032	6.22
120 hours	35	31.58±0.019	5.69	12	25.13±0.078	9.98

TABLE NO. 4.36

Showing means with their S.E's and C.V.% of live percentage of spermatozoa at different hours of preservation of bull semen during Winter season.

Hours of preservation.	Breeds					
	THARPARKAR			FRIESIAN		
	N	Mean ± S.E.	C.V.%	N	Mean ± S.E.	C.V.%
0 hour	58	84.47±0.007	0.67	37	76.88±0.026	2.52
24 hours	58	72.87±0.008	0.98	37	65.20±0.017	2.03
48 hours	58	63.80±0.007	0.95	37	55.28±0.017	2.78
72 hours	58	54.83±0.007	1.24	35	48.43±0.015	2.65
96 hours	57	45.33±0.006	1.44	28	39.28±0.01	1.93
120 hours	44	35.13±0.007	1.73	10	34.10±0.021	1.69

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TABLE NO. 4.37

Analysis of variance showing the effect of breeds on live percentage of spermatozoa in Rainy season.

Source of variation	d.f.	M.S.	F.
Between breed.	1	28.4470	
Within breed.	478	120.2329	<1
Total.	479		

TABLE NO. 4.38

Analysis of variance showing the effects of breeds on live percentage of spermatozoa in Winter season.

Source of variation	d.f.	M. S.	F.
Between breed.	1	682.3330	6.89**
Within breed.	515	98.8921	
Total.	516		

** denotes significant at 1% level.

TABLE NO. 4.39

Analysis of variance showing the effect of breeds on live percentage of spermatozoa (pooled data).

Source of variation	d.f.	M.S.	F.
Between breed.	1	510.4667	4.6*
Within breed.	995	109.1976	
Total.	996		

* denotes significant at 5% level.

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TABLE NO. 4.40

Analysis of variance showing the effects of seasons on live percentage of spermatozoa in Tharparkar herd.

Source of variation	d.f.	M. S.	F.
Between season.	1	193.9087	1.77 N.S.
Within season.	627	109.0016	
Total.	628		

N.S. denotes non-significant.

TABLE NO. 4.41

Analysis of variance to see the effects of seasons on live percentage of spermatozoa in Friesian herd.

Source of variation	d.f.	M. S.	F.
Between season.	1	56.8891	
Within season.	366	109.4447	<1
Total.	367		

TABLE NO. 4.42

Analysis of variance to see the seasonal effects on live percentage of spermatozoa (pooled data).

Source of variation	d.f.	M.S.	F.
Between season.	1	50.4845	
Within season.	995	109.6599	<1
Total.	996		

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TABLE NO. 4.43

Analysis of variance to see the effects of intervals (hours of preservation) in Rainy season (pooled data).

Source of variation	d.f.	M. S.	F.
Between interval.	5	9134.4556	366.07**
Within interval.	474	24.9526	
Total.	479		

** denotes significant at 1% level.

TABLE NO. 4.44

Analysis of variance to see the effects of intervals (hours of preservation) in Winter season (pooled data).

Source of variation	d.f.	M.S.	F.
Between interval.	5	8536.9952	488.6**
Within interval.	511	17.4693	
Total.	516		

** denotes significant at 1% level.

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TABLE NO. 4.45

Showing critical differences between different intervals (hours of preservation) in respect of live percentage of spermatozoa in Rainy season.

Comparison between intervals	(N_1-N_2)	Mean differences	Calculated C.D.at 5%	Calculated C.D.at 1%
0-24	91-89	8.03**	1.45	1.90
0-48	91-88	12.21**	1.45	1.90
0-72	91-85	18.39**	1.47	1.93
0-96	91-80	24.56**	1.48	1.96
0-120	91-47	31.32**	1.74	2.29
24-48	89-88	5.18**	1.47	1.93
24-72	89-85	10.36**	1.47	1.93
24-96	89-80	16.53**	1.50	1.98
24-120	89-47	23.29**	1.76	2.32
48-72	88-85	5.18**	1.48	1.96
48-96	88-80	11.35**	1.50	1.98
48-120	88-47	18.11**	1.76	2.32
72-96	85-80	6.17**	1.50	1.98
72-120	85-47	12.93**	1.76	2.32
96-120	80-47	6.76**	1.78	2.34

** denotes significant at 1% level.

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TABLE NO. 4.46

Showing critical differences between different intervals (hours of preservation) in respect of live spermatozoa percentage in Winter season.

Comparison between intervals.	(N_1-N_2)	Mean differences	Calculated C.D.at 5%	Calculated C.D.at 1%
0-24	95-95	7.68**	1.17	1.54
0-48	95-95	13.36**	1.17	1.54
0-72	95-93	18.05**	1.17	1.54
0-96	95-85	23.27**	1.21	1.59
0-120	95-54	28.20**	1.39	1.83
24-48	95-95	5.68**	1.17	1.54
24-72	95-93	10.37**	1.17	1.54
24-96	95-85	15.59**	1.21	1.59
24-120	95-54	20.52**	1.39	1.83
48-72	95-93	4.69**	1.17	1.54
48-96	95-85	9.91**	1.21	1.59
48-120	95-54	14.84**	1.39	1.83
72-96	93-85	5.22**	1.21	1.59
72-120	93-54	10.15**	1.39	1.83
96-120	85-54	4.93**	1.41	1.85

** denotes significant at 1% level.

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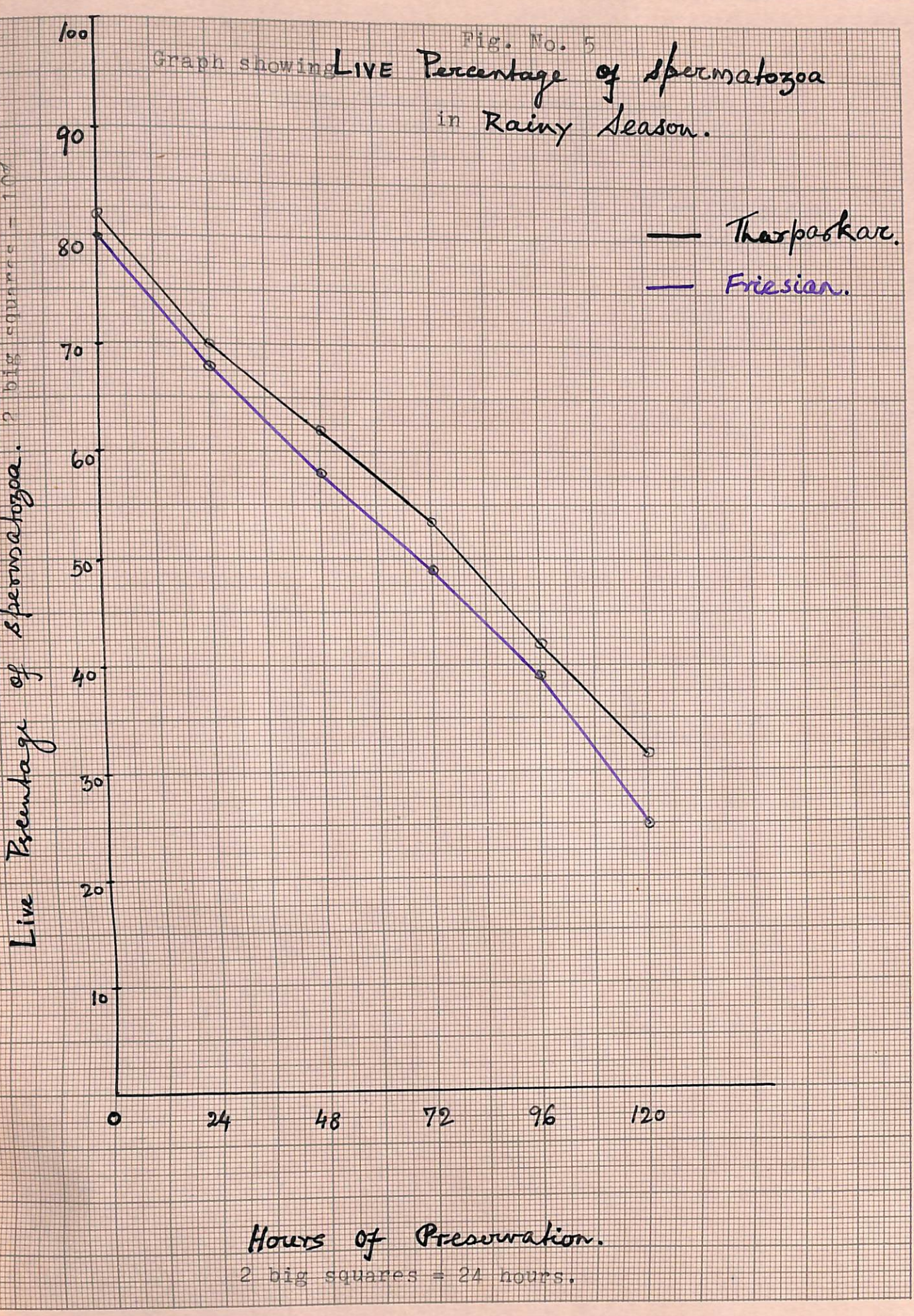
Fig. No. 5

Graph showing LIVE Percentage of spermatozoa in Rainy Season.



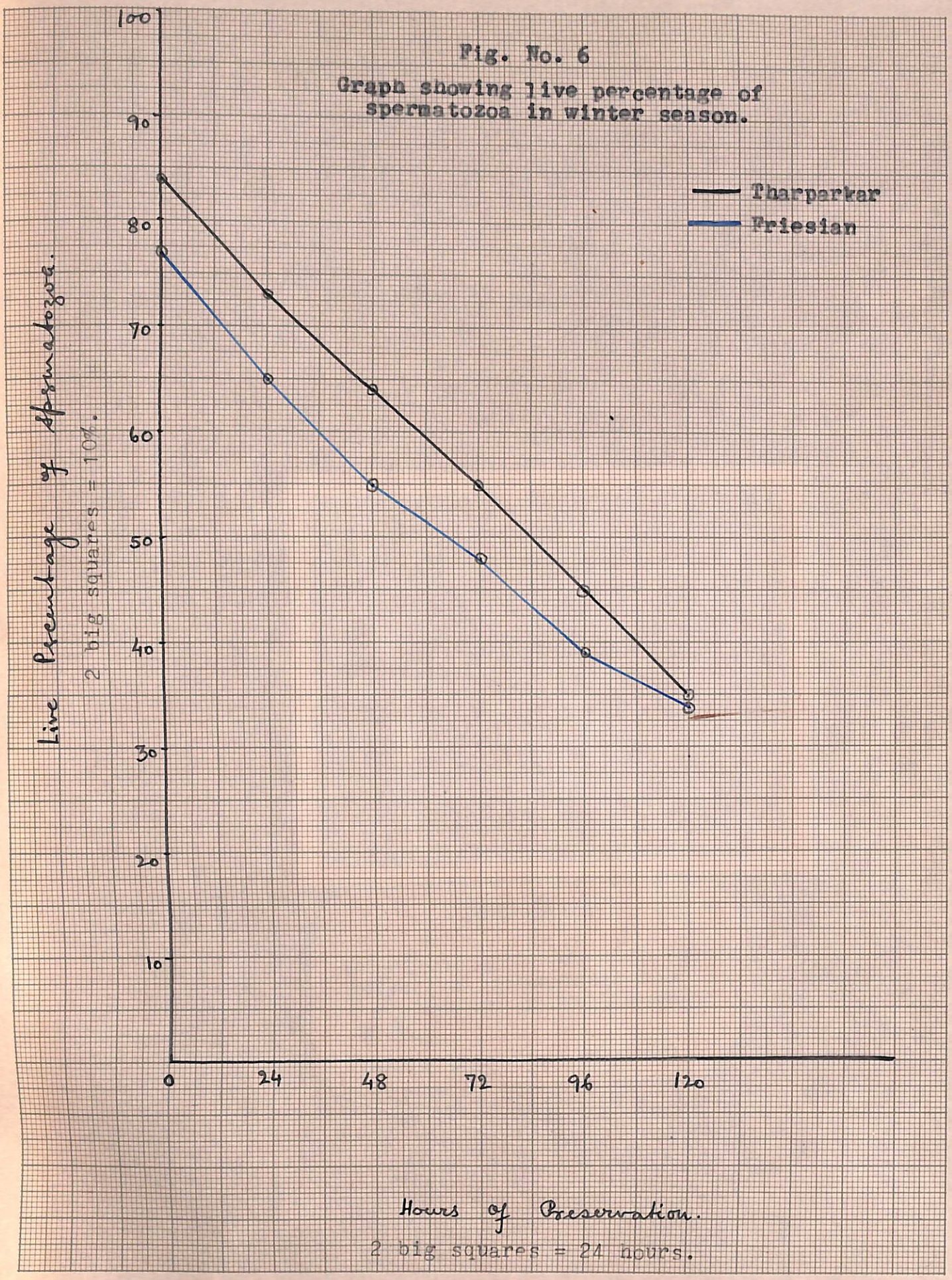


Fig. No. 6

Graph showing live percentage of spermatozoa in winter season.

Plate No. 1

Microphotograph showing larger number of livespermatozoa (colourless) of bull semen (x320).

(Eosin nigrosin staining).

TABLE NO. 4.47

Showing the decline in various characters such as pH, initial motility, individual motility and live percentage of spermatozoa after 120 hours of preservation in the referigerater at 4-5°C for bull semen in Rany and Winter seasons.

Breed / Characters	Seasons					
	RAINY			WINTER		
	Value at 0 hour	Value at 120 hours	Difference	Value at 0 hour	Value at 120 hours	Difference
THARPARKAR						
pH.	6.8	6.4	0.4	6.84	6.24	0.60
Initial motility.	85.31	32.13	53.18	85.87	35.60	50.27
Individual motility.	80.17	28.87	51.30	83.02	34.61	48.41
Live % of spermatozoa.	82.20	31.58	50.62	84.47	35.13	49.34
FRIESIAN						
pH.	6.8	6.36	0.44	6.67	6.3	0.37
Initial motility.	84.54	28.95	55.59	79.30	29.81	49.49
Individual motility.	77.61	22.94	54.67	76.23	34.53	41.70
Live % of spermatozoa.	80.31	25.13	55.18	76.88	34.10	42.78

TABLE NO. 4.48

Showing the frequency of observations at 0 hour and 120 hours for all the traits under study in Rainy and Winter seasons in both the breeds.

Breed	Seasons			
	RAINY		WINTER	
	N_1 (0 hour)	N_2 (120 hours)	N_1 (0 hour)	N_2 (120 hours)
Tharparkar.	53	35	58	44
Friesian.	38	12	37	10

N_1 & N_2 denotes number of observations.

TABLE NO. 4.47

Showing the decline in various characters such as pH, initial motility, individual motility and live percentage of spermatozoa after 120 hours of preservation in the referigerater at 4-5°C for bull semen in Rany and Winter seasons.

Breed / Characters	Seasons					
	RAINY			WINTER		
	Value at 0 hour	Value at 120 hours	Difference	Value at 0 hour	Value at 120 hours	Difference
THARPARKAR						
pH.	6.8	6.4	0.4	6.84	6.24	0.60
Initial motility.	85.31	32.13	53.18	85.87	35.60	50.27
Individual motility.	80.17	28.87	51.30	83.02	34.61	48.41
Live % of spermatozoa.	82.20	31.58	50.62	84.47	35.13	49.34
FRIESIAN						
pH.	6.8	6.36	0.44	6.67	6.3	0.37
Initial motility.	84.54	28.95	55.59	79.30	29.81	49.49
Individual motility.	77.61	22.94	54.67	76.23	34.53	41.70
Live % of spermatozoa.	80.31	25.13	55.18	76.88	34.10	42.78

TABLE NO. 4.48

Showing the frequency of observations at 0 hour and 120 hours for all the traits under study in Rainy and Winter seasons in both the breeds.

Breed	Seasons			
	RAINY		WINTER	
	N_1 (0 hour)	N_2 (120 hours)	N_1 (0 hour)	N_2 (120 hours)
Tharparkar.	53	35	58	44
Friesian.	38	12	37	10

N_1 & N_2 denotes number of observations.

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CHAPTER - V.

SUMMARY

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SUMMARY

This study was undertaken to assess the comparative keeping quality of semen of Friesian and Tharparkar bulls stationed at Semen Bank, Patna.

Preservability of bull semen was studied at the refrigerator temperature of 4-5°C with Egg Yolk Citrate diluent from 0 hour to 120 hours of preservation in the rainy and winter seasons extending over a total period of 6 months.

The evaluation was made on the basis of examination of pH, initial motility, individual motility and live percentage of spermatozoa.

pH :-

The mean pH at 0 hour for Tharparkar and Friesian during rainy and winter season was 6.8 ± 0.036, 6.8 ± 0.045, 6.84 ± 0.01 and 6.67 ± 0.02 respectively. The figures for the same trait at 120 hours were 6.4 ± 0.04, 6.36 ± 0.06, 6.24 ± 0.012 and 6.3 ± 0.03 respectively.

Significant seasonal variation in semen pH was recorded. Hours of preservation was also found to have significant effects on this character whereas breed effects turned out to be non-significant. The differences between hours of preservation revealed non-significant effects between 72-96 hours, 72-120 hours, and 96-120 hours in rainy season while at other intervals the

effects turned out to be statistically significant.

Initial motility :-

The mean percentage of initial motility at 0 hour in Tharparkar and Friesian during rainy and winter season was 85.31 ± 0.015, 84.54 ± 0.042, 85.87 ± 0.009 and 79.30 ± 0.049 respectively. The same at 120 hours was 32.13 ± 0.012, 28.95 ± 0.044, 35.60 ± 0.008 and 29.81 ± 0.027 respectively.

The initial motility was not affected by the breeds in rainy season but significant breed effects were recorded in winter season. Non-significant seasonal effects were recorded. Hours of preservation affected this trait significantly and critical differences showed highly significant variations between hours of preservation.

Individual motility :-

The averages at 0 hour for Tharparkar and Friesian in rainy and winter seasons were 80.17 ± 0.008, 77.61 ± 0.039, 83.02 ± 0.007 and 76.23 ± 0.029 respectively. The same at 120 hours were 28.87 ± 0.029, 22.94 ± 0.067, 34.61 ± 0.008 and 34.53 ± 0.026 respectively.

Significant breed effects were recorded for winter season as well as for the data for the whole period under the study but seasonal effects appeared to be non-significant. Hours of preservation affected this trait significantly and highly significant variations were recorded between intervals.

Live percentage of spermatozoa :-

The mean percentage of spermatozoa at 0 hour for Tharparkar and Friesian during rainy and winter season was 82.20 ± 0.009, 80.31 ± 0.044, 84.47 ± 0.007 and 76.88 ± 0.026 respectively. The same at 120 hours was 31.58 ± 0.019, 25.13 ± 0.078, 35.13 ± 0.007 and 34.10 ± 0.021 respectively.

Breeds significantly affected this character during winter season as well as during the entire period but seasonal effects appeared to be non-significant. Hours of preservation affected this trait significantly and critical differences showed highly significant variations between intervals.

The present study revealed that Tharparkar bulls had a little edge over those of the Friesian in respect of preservation of semen under different hours of preservation under the conditions, the study was made.

As the extent of utilisation of exotic bulls for cross breeding in different parts of our country is progressively increasing, the present study is expected to be of importance in the breeding programmes where diluted semen needs preservation.

However, further works on these lines covering various ramifications with larger number of observations, are needed to make the study more reliable.

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