A Study on Keeping Quality of Cow and Buffalo-Bull Semen under Laboratory and Field Conditions.

A Thesis
Submitted to the Magadh University in Partial Fulfilment
of the Requirements for the Degree
of
MASTER OF SCIENCE
(ANIMAL HUSBANDRY)

BY
CHANDRA SHEKHAR PRASAD SINGH
B. V. Sc. & A. H.
Post-Graduate Department of Genetics and Animal Breeding
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Dated the 5th December 1966.

I certify that this study was conducted and thesis prepared under my supervision, by Shri Chandra Shekhar Prasad Singh, a candidate for the degree of Master of Science (A.H.) with Animal Genetics and Breeding as major subject and that this thesis encorporates the results of his independent findings.

J-1. R. Mishra
5/12/66

(H. R. Mishra)

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

The Indian livestock are well-known for their miserably low average production. The sitution may briefly be reviewed as below :

India being an agricultural country, has a large number of livestock. 23% of the total world- bovine population is represented by India alone (175 million cattle and 51.0 million buffaloes-)Chaudhari,1963). But the contribution of this vast population to the national economy is very poor and only 7% of the national income is attributed to it. The average milk-yield of a cow is 369 lbs and that of a buffalo is 1077 lbs per year. This poor standard of production as compared to consumption is definitely a liability on the part of a developing nation like ours. Milk, being generally the main source of protein to children and vegetarian people, has a very vital role to play in the national health. Per head consumption of milk in this country is 4.6 ounces a day in contrast to the minimum nutritional requirement of 10 ounces (Parnerker, 1965). Bihar, on an average, (Cattle and buffaloes taken together) has 43 bovines per 100 human population, but per head milk consumption is only 4 ounces a day.

This challange of under production to the Animal Husbandry industry has to be accepted, otherwise the existance of the industry itself will be at stake in

view of its pace with other industries. The unproductiveness and under-productiveness are due to causes, genetic and environmental both. From the environmental side, with the optimum level of nutrition and management, considerable improvement can be brought about. In order to ensure genetic improvement, the existing potentialities have to be maximised and further efforts made to create greater degree of genetic superiority. Fortunately, Artificial Insemination is known to be one of the best zoo -techniques, that can be made use of to utilise to the maximum, the superior genetic resources for the rapid improvement of livestock within shortest possible time, when a good proven sire can produce hundreds of good calves in a year. In India, too this technique is becoming quite popular. Nishikawa (1964) has reported that about 59 million cows are artificially inseminated every year in the world. In India 376 key village blocks and 64 urban A.I.Centres covering about 2.5 million breedable cows and she-buffaloes have been set up (Gosamwardhan seminar 1963). Bihar state, alone has 107 A.I. Centres, 266 sub-centres and 36 All India Key Village centres (Livestock census of 1961). On an average, Bihar has 316 breeding cows per bull, the range being 60 to 1105 cows per bull. Similarly there are 117 breeding she - buffaloes per buffalo-bull. This ratio varies from 11: 1 to 436 : 1. To make this ratio narrow, and to facilitate breeding work, A.I.centres,

sub-centres and All India Key Village centres and subcentres came into existence in the first five-year plan period and bull distribution programme was started. With increasing necessity of planned breeding and livestock improvement " Semen Banks" were also established and " Crash programme" for cattle development was launched.

All these cattle development and breeding programmes are mostly dependent on the availability of semen from good and superior sires. Diluted semen can be made to travel a long distance from semen banks, A.I.centres or Key Village centres to sub-centres where it is used. The success of programmes depends on the quality and fertilising capacity of the semen at the time of insemination, even though it has been obtained from superior bulls.

Therefore, the quality and keeping quality of semen assume importance since a large number of environmental variables affect this keeping quality, the magnitude of their adverse affects, deserve further attention. From the time semen is collected, till it is used for, other variables, temperature, humidity, modes of transport and shock, seasons of the year (chiefly through temperature, humidity wind velocity and rain fall) too affect its keeping quality either indirectly by their effect on the physiological state of bulls or directly on the metabolic processes of spermatozoa. The age of semen (lapse of time from collection till insemination) is also

an important factor affecting semen quality.

As would appear from the foregoing review of literature, there is not much work done in the tropics to assess the effects of medium of transport on motility and longevity of sperm in the field conditions. In India, diluted semen is carried from the point of collection to the place of insemination through various modes of transport viz. train, jeep, cycle, bus and air. Therefore, these are definitely the major items deciding the success or failure of insemination (assuming other factors to be optimum) and as such informations on these lines are expected to be useful. With this end in view this study has been undertaken. The objective of this work is to:

(1) Study the keeping quality of diluted semen in field conditions i.e. the effect of various modes of transport (viz. Jeep, Train and Cycle) usually employed to transport semen of bulls under field conditions.

(2) Evaluation of semen kept in thermosflask at room temperature for its quality at different intervals, both from field and laboratory conditions.

(3) The effect of seasons on the quality of semen under field and laboratory conditions.

(4) To compare the results obtained under the items from 1 to 3 and draw pertinent conclusions and inferences which may be useful in either planning further work on these lines or organising transportation of semen

under field conditions in the set of environments encountered during the seasons of the year at the places where this study has been conducted. Evaluation of semen and its keeping quality has been planned on the basis of motility and percentage of live spermatozoa.

REVIEW OF LITERATURE

Available relevant literature has been briefly reviewed under the following three heads, that have bearing on the present study viz:

1. Preservation of Semen.
2. Seasonal variation in the quality of semen.

and

3. Transport of semen.

(1) PRESERVATION OF SEMEN

The success of artificial insemination depends on preserving the fertilising capacity of spermatozoa for a longer time *in vitro*. 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 maintenance of pH are of vital interest for the maintenance of normal physiological and biochemical activities of spermatozoa . Works on these lines are briefly reviewed below :

Metabolic activities and motility of spermatozoa were reported to be suppressed when semen was stored at a temperature slightly above the freezing-point(Wolf 1921 and Walton 1930).

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).

Phillips and Lardy (1940) and Salisbury et al (1941) reported that Hydrogen- ion concentration of the diluents can be maintained within limits by incorporating buffer salts in them.

Diluation of semen increases the number of females that can be bred from a single ejaculate of a sire. Changes associated with the dilution of semen commonly refered as " dilution effects" were reviewed by Mann (1954).

Semen preservation made considerable improvement due to further investigations which include :

(a) Minimum number of spermatozoa required for an insemination.

(b) Addition of antibiotics resulting in increased fertility.

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

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

(e) Development of substitutes of egg-yolk , like milk, tomato- juice , coconut-milk and honey etc.

Two recent investigations which are landmarks

in the history of semen preservation include;

1. Deep-freez method of semen preservation for longer time at -79°c (Smith and Polge 1950).

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

3.

BULL SEMEN :

Lasley (1944) obtained 16.9% dead, 19% non-motile-live, 12.5% weakly motile and 51.6% progressively motile spermatozoa in a freshly collected semen sample. These figures after 4 days of 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.

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

Jurgens (1951) reported average duration of life for bull-spermatozoa to be 11.91 days in egg-yolk citrate diluent when stored at +4°c. He recorded on an average of 50 to 60% live spermatozoa up to 7.37 days in yolk- citrate diluent.

Lasley (1951) reported that a stroage period of 4 days in egg-yolk phosphate diluent at 10-12°c the semen sample 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. Using haemocytometer method for determining the percentage of motile and progressively motile spermatozoa and opal blue-eosin staining method for determining the percentage of live spermatozoa, he obtained 4 types of cells in bull semen viz (1) Dead or stained, (2) Live non-motile,(3) Weakly motile and (4) Progressively motile. A highly significant correlation existed between the percentage of live, motile and progressively motile spermatozoa in fresh semen. These characters were correlated with the percentage of live sperms in the semen after storage for 4 days, but no correlation was observed with the percentage of motile spermatozoa with any of these characters after the same period of preservation. On the assumption that cold-shock would kill the weakly motile spermatozoa, (Lasley and Bogart 1943), and cold resistant spermatozoa would be progressively motile, cold-shock was applied in the semen samples and it was found that the average percentage of progressively motile and resistant spermatozoa was almost the same. For every 10% increase in the percentage of progressively motile spermatozoa, corresponding increase of 5.44% in the percentage resistant spermatozoa was noted. The percentage of motile spermatozoa was significantly

correlated with the fertilising capacity (r=0.314). No significant correlation could be found between the progressively motile spermatozoa and fertilising capacity (r =0.167).

Lebreton (1952) obtained 76% conception rate with the semen stored up to 36 hours, where as semen preserved for more than 36 hours resulted in only 66% successful inseminations.

Campbell (1953) observed a decline in the conception rate varying between 3.4 to 4.8% for each day of storage till 4 days. Aamdal (1952) Dzilinski (1958) and Fryer et al.(1958) have reported similar findings.

Schmidt and Kroll (1953) noted an increase in the retention of fertilising capacity of bull semen from 5-6 days to 7-8 days in egg-yolk- citrate diluent when the storage temperature was increased from 4-6°c to 15°c. Rottensten (1953,II) found that 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) studied the fertility of bull semen for 10 days at 4°c. On the results obtained they postulated that 30-40% fertility can be expected from a 10 day old semen sample.

It is known that highest fertility results are

obtained if the semen is used on the date of collection and there is a little decline(3%) in the fertility within 30 hours of collection (Melrose 1952 b), Willett (1953), Heweston (1955), Rettenstem and Andersen (1956), but results obtained by Roussel (1954) reveal a decrease of 5% in fertility for every 24 hours of semen preservation.

Jaiskowsky and Romaniuk (1957) studied 3 methods of semen preservation in thermosflask at room temperature (20-30°c). They reported that the storage of semen in small test-tubes within the thermosflask, wrapped in damp cotton and surrounded by ice gave the best results. The temperature of semen remained below 5°c for 24-120 hrs and over 50% motility was maintained for 144 hours.

De-Groot and Bekedam (1957) found slightly lower conception rate from older semen than 1st day semen. Storage time had no significant effect on fertility of the diluted semen preserved for 4 days.

Sakala and Turcer (1957) found that with bull semen diluted in egg-yolk citrate when used for insemination for 4 successive days after collection, the conception rate was found to be higher with the inseminations made on second day than on the first day and then it progressively declined.

Morozov et al. (1961) studied the quality of semen samples kept at 0°c. They found that the

temperature of the diluted semen in a container kept directly in the ice reached 0°c within 25 minutes whereas that of the sample wrapped in cotton-wool was at 1.2°c in 2 hours. The conception rate from the first sample after 3 days of storage was 71.5% and from the wrapped one stored for 3,4 or 5 days conception rate was 87.9, 87.5 and 50.0% respectively.

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

Hour of preservation	Motility	Percentage 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

Sharma et al. (1962) preserved Hariana and Murrah semen at 5-7°c in referigerator in various diluents. The total time taken for intial motility to come to zero was reckoned as estimated of full life of spermatozoa. They observed following results on egg-yolk citrate diluent in different seasons.

Average life of sperm in days

Season	Hariana bull semen	Murrah bull semen
Spring (Feb - April)	7.6 ± 1.6	4.8 ± 1.1
Summer (May- July)	8.3 ± 1.7	4.9 ± 1.1

Season	Hariana bull semen	Murrah bull semen
Autumn (Aug-Sept)	8.1 ± 1.8	6.1 ± 1.4
Winter (Nov-Jan)	8.9 ± 2.0	6.3 ± 1.6

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

BUFFALO BULL :

Shrivastava and Prabhu (1956) compared the keeping quality of buffalo semen in 6 diluents and noted that kampschmit's glucose- sodium- bicarbonate-egg yolk diluent (1951, 1953 b) with sulphanilamide gave the best results for a period of one week. They reported 32.6% live spermatozoa at the end of preservation for one week.

Gokhale (1958) observed that the average survival time for buffalo spermatozoa in egg-yolk sodium citrate glucose diluent was 180.04 hours.

Mahajan (1960) noted a 15% decline in the motility after 72 hours of preservation.

Mahajan and Sharma (1960) recommended 20%

egg yolk to be used in sodium bicarbonate-glucose diluent as they did not find any special advantage on preservation quality by adding higher levels of egg-yolk. They found sodium bicarbonate glucose and egg-yolk dilutor to be superior to commonly used semen diluents ie egg-yolk phosphate, yolk citrate, yolk-boiled milk and yolk-glycine dilutors. They observed that in 1:10 dilution, the spermatozoa survived for 16 days and after 10 days of storage time 22% of the spermatozoa were found to be alive. They concluded that buffalo semen could be preserved for 3-4 days in in Egg-yolk bicarbonate diluent with desired motility rating.

Tomar and Desai (1961) found that the diluent containing whole egg-glucose fructose maintained 60% motility of buffalo-semen for 4.9 ± 0.215 days and whole life for 27.95 ± 0.809 days. These workers in another study in the same year observed that buffalo-semen kept in referigerator maintained +++ motility for 6.15 days in egg yolk glucose fructose- sodium bicarbonate diluent and the average sperm life was 23.2 days. The egg yolk-glucose- fructose- sodium citrate and Potassium- carbonate diluent maintained +++ motility for 7 days, the average sperm life was recorded to be 23.85 days in this diluent.

Saxena (1965) obtained following results for percentage of motility and live spermatozoa of Murrah semen at various hours of preservation.

Hours of preservation	Average motile spermatozoa %	Average % of live spermatozoa
0 hr.	86.0 ± 1.0	88.3 ± 1.2
72 hr.	79.2 ± 1.9	80.9 ± 2.2
120 hour	68.8 ± 1.7	76.4 ± 2.3

Between bull differences were significant at 1% and 5% levels of probability.

Kumar (1965) compared the efficacy of 5 groups of diluents on their capacity of preserving semen 144 hours at 3 ± 1°c. These include, Egg-yolk glucose sodibicarb, Egg-yolk-citrate, Skim-milk series, tomato juice series and coconut-milk series. He found that the values of motility and percentage of live spermatozoa were higher in egg-yolk glucose sodibicarb diluent.

Kouser (1965) reported the following results on preserving semen at different hours in egg-yolk- sodium biocarbonate diluent in referigerator at 3±1°c.

Hour of preservation	Motility %	Percentage of dead spermatozoa.
0	+++.81 ± 0.01	18±0.1
24	+++.62 ± 0.08	27 ± 0.1
48	+++.34 ± 0.06	35 ± 0.2
72	++.85 ± 0.16	42 ± 0.2
96	++.23 ± .20	56 ± 0.2

(II) SEASONAL VARIATION IN QUALITY OF SEMEN

COW -BULL

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.

Erb et al. (1942) further recorded significant seasonal differences in the semen quality; semen was found to be superior in quality in spring and inferior in summer. They concluded that this difference in quality was due to the factors characterising seasons ie. light, relative humidity and general obscure factors. In their opinion, atmospheric temperature was mostly responsible for the change in semen quality.

Phillips et al.(1943) obtained highest percentage of fertile services in the April (59.6%) and lowest in August (40.8%).

Swanson et al.(1944) revealed that the motility and useful viability of bull spermatozoa was lower in winter than in summer and spring. They concluded that the seasonal effect was mainly due to the effect of seasonal weather on the physical well being of the bull.

Anderson (1945) and Mercier (1946) claimed that temperature variation had less effect on spermatogenesis. In their view spermatogeneses was largely affected by the hours of day light to which the animal was exposed.

Mukherji and Bhattacharya (1947) studied the seasonal effects on seminal characters of kumauni bulls and observed that the semen produced in the spring was superior to that of summer.

Hudopisk (1948) found that the sperm vaibility was best maintained at 3°c, below this, the survival of supermatozoa decreased and after raising the temperature, sperm motility increased resulting in decreased duration of viability. Johnston and Branton (1953) observed marked seasonal differences in semen quality, but no corresponding significance in non return conception rates could be found.

Results obtained by the work of Casady et al. (1953), revealed that high ambient temperature resulted in decreased initial motility, average sperm concentration and total sperm count. A gradual recovery was noted when the temperature was lowered. High temperature resulted in increased blood temperature, a factor known to cause reduction in semen quality.

Burgess (1953) got no monthly or seasonal variation in the conception rates.

Prabhu and Bhattacharya (1953) studied 9734 insiminations between 1946 to 1948 from 20 bulls, at Calcutta, Patna and Bangalore artificial insemination centres. They observed that a significant difference in conception rate existed between one artificial insemination

centre to another. No significant difference between months was found.

Schindler (1954) recorded lowest conception rate in the month of September accompanied by the lowest spermatozoal concentration and survival rates.

Singh et al (1958) observed that from March to July a maximum number (68.5%) of Hariana bulls are saxually active. This number goes below (52.5%) in the month of August with the increase in humidity and flies. September to January is the worst period, so far as the ability of donating semen is concerned. When only 52.6% of the bulls are sexually active.

Results obtained by Brown (1959) reveal the adverse effect of temperature on semen quality. Significant portion of variation was contributed by bulls, months and year on semen quality. Semen quality was better from December to April and poor in July- October.

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

Basirov (1960) noted inhibition of libido and ejaculation in buffalo- bull and a great reduction in these two attributes in cow- bulls, when exposed to a temperature of 45°- 50°c. Submormal temperature was found to have no effect on semen characters.

Kodagali (1962) recorded highly significant seasonal variation in seminal characters of khillar breed of cattle. Semen was the best in cold, followed by wet and summer seasons.

Sharma and Saha (1962) could not find any seasonal effect on the semen quality of Hariana bulls.

Kelly and Hurst (1963) using deep frozen semen, recorded higher number of successful inseminations (47.1%) in cooler conditions than those having high environmental temperature (30.1%). They obtained highest conception rates in April and October and lowest from June to September.

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

Milićvic(1965) observed that high environmental temperature was associated with reduced ejaculate, whereas low temperature adversely affected motility, pH, viability and sperm concentration. Highest fertility was recorded in autumn and spring (temperature range 12.2-34°c).

Tripathi (1965) found no seasonal variation in initial motility of Hariana and Murrah-bull's semen.

BUFFALO- BULL SEMEN:

Kaleff (1942) reported seasonal variation in

semen quality. He explained that seasonal variation in buffalo-semen is due to the fact that Buffalo is sensitive to extremes of cold and heat.

Results obtained by Malkani (1954) and Kushwaha et.al.(1955) suggest that the semen quality of Murrah and Surati buffaloes in India is optimal in spring. Kushwaha et al.(1955) recorded significant seasonal variation in the initial motility of buffalo semen. They also observed that Murrah buffalo failed to retain the quality of semen at a high level during winter though Malkani (1954) did not find very marked degradation of semen quality of winter.

Singh et al.(1958) pointed out that 89.3% of the buffalo- bulls were very active from August to February so far as the capacity of donating semen is concerned. During March the peak starts declining as 71.4% of the bulls function properly. The period of April to June was the worst as only 47.3% of the bulls work properly. After the onset of rains in July bulls start getting better and 61.6% of bulls were vigorous semen-donators.

Dharampal (1961) reported mean survival of usable semen to be 1 day from November to February against 2.35 days for the rest of the period. He noted that maximum temperature, relative humidity and rainfall had no appreciable effect on sperm survival. A rise in minimum temperature from 11.2°c. to 13.8°c. increased

the storage period of usable semen having more than 50% motile spermatozoa from 1 day to 3.29 days.

Sharma et al.(1962) found significant seasonal difference in the average full life of spermatozoa in the semen samples collected from Murrah buffalo bulls.

Sen Gupta et al. (1963) reported that the semen of buffalo was best during spring and worst during summer. It improved considerably with the onset of rainy season.

Mishra et al.(1965) observed that heat-stress resulted in the loss of libido and production of very poor quality of semen in summer. This deleterious effect was of temporary nature and both the attributes improved in autumn and spring.

(III) TRANSPORT OF SEMEN

Milovanov (1932) reported that semen should not travel a distance which takes more than two hours journey and not more than 6 hours should lapse between collection and insemination.

In England, Cows have been successfully impregnated with the semen sent from Denmark by air (Edwards, Walton and Siebenga, 1938).

Lambert and Mckenzie (1940) reported successful inseminations with semen lifted from Beltsville, Maryland to Piran in the province of Buenos Aires with a total time loss of seven days between collection and insemination.

Smirnov-ugerjumov (1940) recorded a reduction in the spermatozoal activity when undiluted semen samples travelled for a distance up to 0.6 to 5.9 miles in thermosflask at 15 to 20°c.

Hronopulo (1940) noted that transport distance of 10 km. and 11 to 34 km. had no marked effect for the semen stored for 4 hours on its fertility rate(48 and 46 % respectively). But a distance over 35 km.reduced the fertilising capacity to 29.5%.

Ayyar (1944) reported that hand-shaking of a semen sample during the transport killed the spermatozoa.

Herman et al.(1944) found that the capacity of the semen to withstand storage was one of the limiting factors for the success of its long distance shipping, and semen from 40% of the sires studied at Missauri station, was found unfit for transport, though otherwise found suitable if utilised within 6-20 hours. Semen having less than 20-45% progresively motile spermatozoa resulted in only 43% conception after transhipment.

Prince and Almquist (1948) studied the effect of mechanical agitation on the livability of spermatozoa. They found that this effect was maximum on the semen sample kept in quarter filled test-tubes and decreased as the amount of semen increased in the test tubes i.e. half filled, three quarter filled and completely full test tubes. Livability was found to decrease with the increase in agitation length.

TABLE No. 2:1

Showing the effect of mechanical agitation on the livability of bull spermatozoa (Prince et al.1948)

Fullness of tubes	Length of Agitation	Percentage of motile spermatozoal 15 ejaculates after storage at 5°c for				
	Hours	Before storage	4days	8days	12days	16days	20days
Filled	0	63	49	43	32	17	5
	6	63	52	42	31	16	7
	12	63	50	41	29	16	7
	24	63	51	42	30	15	5

Contd..

Fullness of tubes	Length of Agitation	Percentage of motile spermatozoa (15 ejaqualate, after storage at 5°c for				
	Hours	Before storage	4days	8days	12days	16days	20days
3/4 filled	0	63	51	39	28	17	7
	6	63	47	32	23	9	3
	12	63	43	31	16	6	3
	24	63	41	27	13	6	1
½ filled	0	63	51	42	26	13	7
	6	63	41	29	19	6	1
	12	63	38	27	13	4	0
	24	63	36	19	11	4	1
¼ filled	0	63	49	26	13	5	3
	6	63	39	21	9	3	0
	12	63	31	21	11	3	1
	24	63	27	10	5	2	1

Letard et al.(1949) reported that when diluted semen in incompletely filled tubes was put to artificial agitation for 4-5 hours the spermatozoal motility was increased, and the motility was not appreciably affected when the tubes were completely filled with diluted semen and hermatically sealed even after 5 hours agitation. But when the undiluted semen was put to 300-400 agitations per minute in ½ or ¼ filled tubes a

reduction of 10% in the motility was recorded in ½ hour and complete ceasation after 1 hour.

In Italy, successful inseminations have been made from semen lifted from North and South America (Bonadonna 1949). The same author, (1951) observed no effect of transport and storage time up to 48 hours, on the fertility of semen. He found no relation between fertility, period of insemination and time of insemination.

Aslanjan (1952) found no change in ram semen after being transported by aeroplane, motor car or cycle, and submitted to various degrees of vibration and various altitudes for 5 hrs - 5 days.

In India, successful inseminations have been made with Hariana semen sent either way between Izatnagar and Calcutta. In addition, over 250 inseminations have been performed at Cochin, Ernakulam and Colombo from semen transporated by air from Sindhi bulls kept at Indian Veterinary Research Institute, Izatnagar(Prabhu et al.1953).

Adler (1960) reported higher conception rate with the semen transported in large glass vials in comparison to that of small ones.

Lysak (1965), studied the effect of jerking and shaking on the semen quality in relation to road surface and type of transport and reported that the semen samples being transported or subjected to conditions simulating

those during transport, sperm motility was greater after transport over 223 km. by automobile than by motor cycle. In each case motility was greater in ½ filled than in ¼ filled flasks. Sperm survival was greater after shaking for 6-12 hrs than after subjection to vibration for 6-12 hours and therefore greater after transport over a smooth road in a vehicle fitted with shock absorbers than over a rough road over a motar cycle.

MATERIALS AND METHODS

In the present study, six Murrah and six Tharparkar bulls, maintained at semen bank, Patna were used. The exact age of the bulls could not be known. Bulls of both species were approximately of the same age group. 15 lbs of hay was provided per animal per day. The animals were fed 6 lbs of concentrate -mixture daily. The composition of the concentrate mixture was as follows :

Groundnut cake-	-	2 lbs
Wheat bran	-	2 lbs
Crushed maize	-	2 lbs
Common salt	-	1 oz
Mineral mixture	-	1 oz

The experiment was conducted in two seasons - viz summer and rainy. The summer trial lasted from May to June and rainy from July to August. Two collections were taken from a bull in a week. Two collections from each bull were obtained in each season thus, altogether 24 collections from cow bulls were studied. 9 collection in each season from 6 Murrah buffalo-bulls were obtained, thus a total of 18 collection from buffalo- bulls were taken. Due to adverse weather lesser number of collection could be available from buffalo-bulls.

Before proceeding for experimental observations,

the techniques to be followed for evaluation of semen quality were standardised. An anaestrus cow and a buffalo acted as dummies for collection of semen from bulls and buffalo- bulls respectively. For each bull separate A.V. was used. The temperature of A.V. for bulls was maintained between 50-55°c and between 40-45°c for buffalo bulls. After collection the neat semen was immediately examined for motility and then diluted.

Egg yolk- citrate (Salisbury et al.1941) and Egg yolk - Sodium bicarbonate- glucose (Kampschmidt et al. 1951 and 1953) are the dilutors used for diluting the bull and buffalo-semen respectively for artificial insemination in Bihar. These two diluents were used in the present study for diluting bull and buffalo-semen respectively. The citrate buffer for bull semen consisted of

Crystalline Sodium citrate	- 2.94 gms
Distilled water	- 100 cc

Two parts of this buffer was well mixed with one part of egg-yolk to constitute the diluent. To each 100 cc of the dilutor, thus prepared, (a) 1 lakh units of Penicillin G Sodium and (b) 100 mg of Streptomycin-sulphate were added. Semen was diluted at the rate of 1 : 25.

Buffalo semen dilutor was prepared as follows:

(a)	Sodium bicarbonate	-	1.3 gms
	Glass distilled water	-	100 cc.
(b)	Glucose anhydrous	-	5 gms
	Sulphanilamide powder	-	0.3 gms
	Glass distilled water	-	100 cc

One part of (a) was mixed with 4 parts of (b) to constitute the buffer. One part of this buffer was mixed with 2 parts of egg yolk to make the dilutor. To each 100 cc of this dilutor (i) Pencillin G Sodium 1 lakh units and (ii) Streptomycin sulphate 100 mgms were added. Dilution of semen was at the rate of 1:15.

4 smaller tubes measuring 4"x½" were taken. Each was wrapped in cotton and with a label bearing bull no, collection time, dilution rate and mode of transport ie. jeep, train cycle or control. Each of these 4 tubes was inserted in an outer tube measuring 4" x 3/4". Now 2 cc. of diluted bull or buffalo-semen from a collection was taken in each of the inner tubes. The inner tubes were finally corked. Each tube, less than ¼ filled with diluted semen was kept in thermosflask. Thermosflasks were of 1350 cc. capacity and approximately filled with ice and marked for the mode of transport. Out of these 4 flasks.

One was brought in the laboratory and immediately examined for motility and percentage of live spermatozoa. This constituted the control.

One thermosflask travelled a distance of 82 miles by jeep (treatment no : 1). The road was pitched but in poor condition and full of jerking. The jeep was of willy make and in old condition it had already covered a distance of 6,00,000 kilometers. The average speed of the jeep was 40 km per hour.

Another thermosflask containing the semen travelled a distance of 38 miles by a passenger train (treatment no.2). The speed of the train is around 35 miles per hour.

The fourth thermosflask travelled a distance of 18 miles by an old cycle. The road though, pucca was full of ditches and bumps resulting in considerable jerking.

Three samples that travelled by jeep, train and cycle were brought back in the laboratory after 12 hours of dispatch. The temperature of semen in thermosflask was $3 \pm 1^{\circ}$c. The evaluation of these samples was made along with the control for the quality of semen. Subsequent evalutions were made after every 12th hour till 96th hour (ie. 12th, 24th, 36th, 48th, 60th, 72 nd, 84th and 96th hour).

So, from each sub-sample (travelled by a particular mode) from a collection 9 observations were taken. Each sample was put to the following two tests for purpose of semen evaluation :

(1) Estimation of live spermatozoa (in percentage)
(2) Motility of the spermatozoa.

Estimation live spermatozoa

Preparation of stain :

Eosin-Nigrosin stain as advocated by Swanson et al.(1951) was used. It was prepared as follows :

Eosin (water soluble) - 1 gm
Nigrosin (BDH.) - 5 gms

Sodium citrate di-hydrate 3 gms; volume was made 100 cc by adding distilled water. This solution was kept on water bath for 30 minutes and filtered after cooling.

Preparation of slides :

On a grease free 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 either 1:1 or 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 the slides by applying a gentle pressure on the slide so as to remove excess of stain. The two slides were separated quickly by using sliding action. Exposure of semen to stain on an average was 1 to 2 minutes. Slides were marked with grease pencil for bull number, mode of transport (control, jeep, train, cycle) and hour of examination. The smears were air-dried and examined under oil immersion. Live spermatozoa did not take stain and were colourless. The back ground was violet with dead spermatozoa taking red stain(Micro photograph no. 1). Those taking partial stain anteriorly or posteriorly were taken as dead, as majority of the workers were of the view that partially stained spermatozoa were on the way to death. Percentage of live spermatozoa were determined by counting 300 spermatozoa per slide on random basis.

Motility of spermatozoa

The percentage of spermatozoan motility either progressive or weak was determined by haemocytometer method (John F. Lasley 1951) which is as follows:

Preparation of buffer solutions :

To maintain the motility of the spermatozoa, during motility estimation same buffer solution was

prepared that was used in diluent :

Bull semen :

Crystalline sodium citrate di-hydrate 2.94 gms.
Glass distilled water - 100 cc

Buffalo- semen :

(a) Sodium bicarbonate - 0.26 gms
Glass distilled water - 20 cc
(b) Glucose anhydrous - 4 gms
Sulphanilamide powder - 0.24 gms
Glass distilled water - 80 cc

(a) was mixed with (b) to constitute the buffer. Buffer solutions were freshly prepared and used. Old solutions gave fluctuating results.

Procedure :

The tubes containing semen samples were taken out of thermosflask and kept in a 500 cc. beaker containing water at room temperature for 30 minutes. This was done to bring the the semen at room temperature. Similarly, buffer solutions were also brought at room temperature before use. A few drops of semen were taken in a clean watch glass. From this, it was sucked up to 0.5 mark of RBC. diluting pipette. This semen was diluted 200 times by sucking the respective buffer solution

up to 101 mark. The contents of the pipette were thoroughly mixed by rotation. A few drops were discarded and then a drop was placed on the previously focussed counting chamber of haemocytometer in high power (40 x objective). The number of non-motile and weakly motile spermatozoa in all the 25 large squares was 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. In buffalo semen specially, a number of spermatozoa were found moving in circles. Those having slow movement in smaller circle were recorded as weak motile. Reverse movement of spermatozoa ie. 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 static and oscillating head (iii) head constantly moving in circle with tail in the centre (iv) tail moving in a circle and head in the centre.

The counting chamber was then placed in the freezing chamber of a referigerator. After the diluted suspension of the spermatozoa under coverslip had frozen, the counting chamber was taken out of the referigerator and kept at room temperature . One to one and half hours time was allowed to keep the counting chamber in the referigerator since it was

found to be convenient. Increased time resulted in the accumulation of undue amount of water over and under the coverslip which made the results to fluctuate considerably. After the frozen suspension had melted, the total number of spermatozoa in the same 25 big squares was counted and recorded. This number included the numbers of weak and progressively motile spermatozoa.

The percentages of weakly motile, progressively motile and total motile spermatozoa were calculated as follows :

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

= Percentage of non-motile spermatoza.

(B) $\frac{\text{No. of weakly motile spermatozoa before freezing}}{\text{Total number of spermatozoa after freezing}} \times 100$

= Percent weakly motile spermatozoa.

The percentage of progressively motile spermatozoa was calculated by subtracting the percentages of non-motile and weakly motile spermatozoa (A + B) from 100. Similarly, the percentage of total motile spermatozoa (weakly + progressively motile) was determined by subtracting the percentage of non-motile spermatozoa from 100.

During the experimental period, a record of maximum and minimum temperature of the room in which the samples were kept was maintained. A record of ambient temperature, humidity and total rain fall was obtained from the central Potato Research Institute, Phulwarisarif, situated in the same locality.

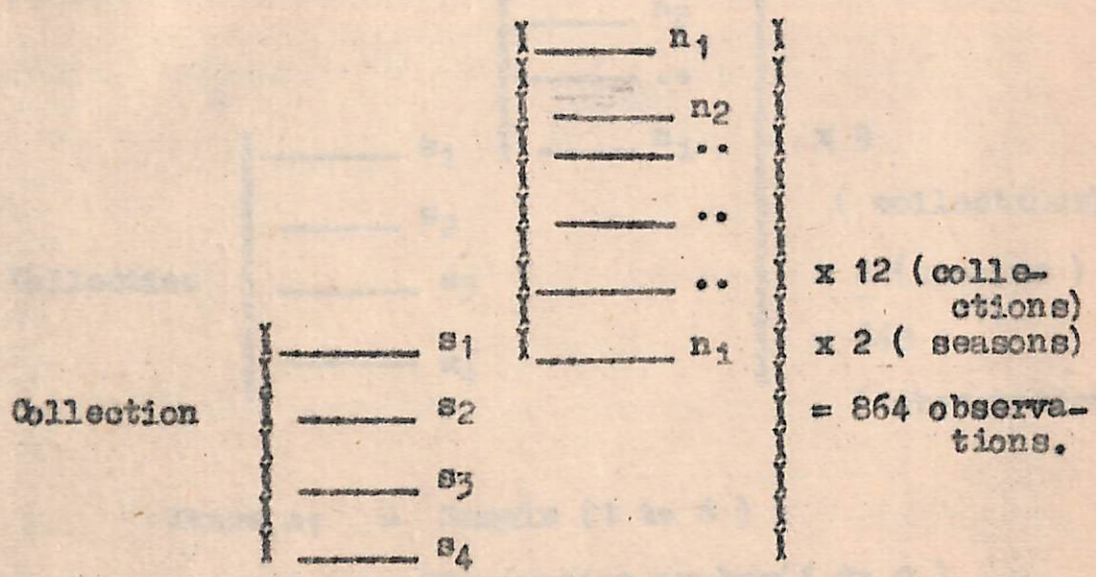
In both the seasons altogether 24 collections were taken for bulls and 864 observations were made for each of the three characters ie. total motility, progressive motility and percentage of live spermatozoa. Similarly out of 18 collections from buffalo- bulls 648 observations were made for each of the characters including percentage of live spermatozoa, percentage of total motile and percentage of active motile spermatozoa.

These figures of observations (864 and 468) can be explained as follows :

Semen from one collection (ejaculate) was divided into 4 equal samples (control, jeep, train and cycle). The sample in the thermos kept at room temperature in the laboratory was used as control which also served as one of the modes. Each sample was studied at 0, 12, 24, 36, 48, 60, 72, 84 and 96 hours of preservation, thereby giving 9 observation for each character in each mode. Since there were two collections

from each bull in each seasons and there were 6 bulls used in this study, the total number of collections came to 12 in one season. This, gave 12 x 9 = 108 observations in one season for one mode. Since there were 24 collections in all in both the seasons, (12 collections in each season)the total number of observations in both the seasons became 216, this was the number of observations for one mode. Since there were 4 modes, the total number of observations turned out to be 216 x 4 = 864. Therefore,for each character in both the seasons, for all the six bulls, (two collections from each bull in each season) gave 864 observations for bull semen.

The design of the study may be illustrated as shown below :

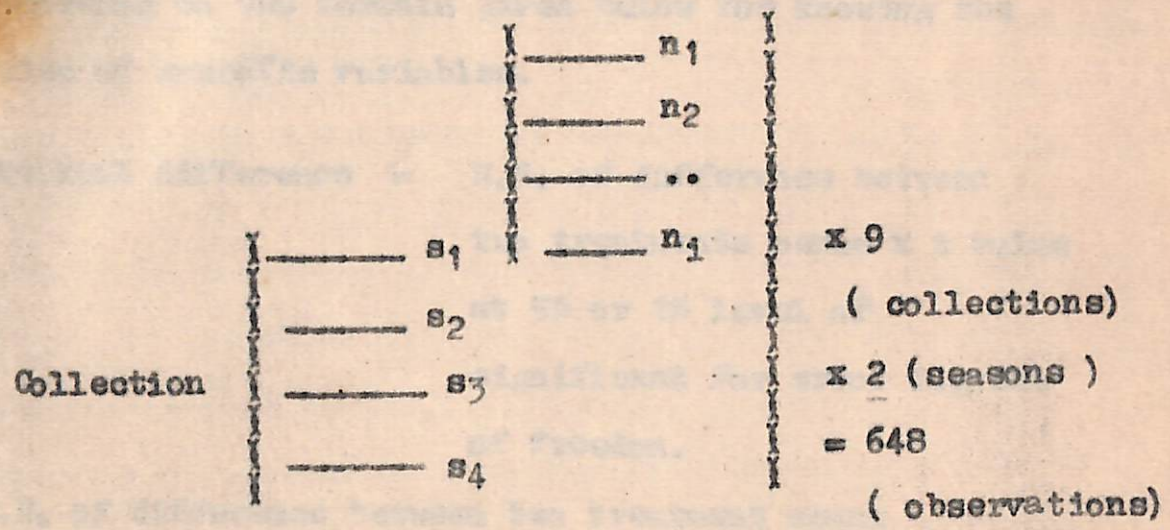


Where s_1 = Sample (1 to 4)

n_1 = Observations (1 to 9)

Similarly, for buffalo- semen, the number of observations would be as follows:

There were 6 buffalo-bulls and in each season there were 9 collections (each collection divided in to 4 equal sub-samples as in case of bull-semen). 9 ovservations were taken from one sub-sample in respect of each character (i e. 36 observations for each character from all the 4 sub-sample of one character). Thus, the total number of observations in respect to each character under study was 648 (9 collections x 2 seasons x 36 obsevations). Number of observations may easily be visualised from the following presentation indicating the experimental design.



Where s_1 = Sample (1 to 4)

n_1 = Observation number(1 to 9)

Statistical Analysis

All the mean values obtained in percentage for live spermatozoa, total motility and active motility for bull and buffalo bull-semen were transformed by Sin $\sqrt{p}$ transformation before analysis by using Bliss-table (Snedecor, 1957). The transformed values were subjected to analysis of variance (Snedecor 1957).

For the sake of statistical analysis, 0.1 was put in place of zero value.

After drawing the necessary conclusions the observations were re-transformed into percentages for presentation. In case, where mean differences were found significant by analysis of variance, they were further tested by calculating critical difference, according to the formula given below for knowing the roles of specific variables.

Critical difference = S.E. of difference between two treatments means x t value at 5% or 1% level of significant for error degrees of freedom.

S.E. of difference between two treatment means

$$\sqrt{\frac{2 \times \text{mean sum of squares for error}}{\text{Number of replications.}}}$$

Means, standard errors and coefficients of variation were calculated by using the methods suggested by Snedecor (1957).

Details about analysis of variance :

In case of bull semen analysis of variance was done in a way that in addition to main factors like seasons, bulls, modes, collection, and intervals etc., meaningful interactions among these factors were also obtained. Variance analysis was made season-wise as well as on the observations of both the seasons combined. Since seasons are usually known to affect semen quality, it was thought desirable to run this analysis season-wise to study the effects of different factors without confounding the results.

In the three modes of transport viz jeep, train, and cycle the distance were not uniform and therefore, it appeared necessary to test the effects of these modes on each of the three characters studied. Since, these three modes were found not to differ significantly either in summer or in rainy any of these three characters except the total motility of buffalo-semen, it was taken to mean that although distance was variable for each mode, this factor did not contribute to any real effect on the quality of the semen on the basis of the characters studied, except for the total motility of buffalo-semen. This led to the study of interactions also.

In the case of cow- bull semen, the number of observations was larger as compared to that of buffalo- bulls.

In the case of buffalo- bull semen, analysis of variance was made first on combined observations (summer and rainy) to see the effects of modes on all the characters. When a significant effect of modes was observed on a particular character, a season-wise analysis of variance was made assuming that a significant effect season-wise would not be observed, if the same did not exist on the observations combined for both the seasons. Out of the three characters, a significant effect of modes was observed only on the percentage of total motile spermatozoa, and therefore, a season- wise analysis was made for this character.

Only three simple sets of analysis of variance were made for buffalo-bull semen viz between seasons, between modes and between intervals. Interactions were not studied. The analysis were made character-wise on the combined observations in both the seasons.

Tables, micro-photo-graph and graphs have also been used for the sake of comparison, interpretation and drawing inferences.

RESULTS AND DISCUSSION

Three seminal characters (progressive motility, total motility and percentage of live spermatozoa) were studied in each of the four semen samples (control and those travelled by jeep, train and cycle) obtained from the same ejaculate, at 12 - hourly intervals (from 0 to 96 hours). All these samples were preserved in separate thermosflask. The distance travelled by the treatment group consisting of jee, train and cycle was 82,38 and 18 miles respectively. This is the arrangement by which the diluted semen is transported to some of the field- stations around Patna- semen bank, There is no arrangement of transporting semen by different modes to the same field-station under existing conditions, and as such, variable distances for different modes were taken. The percentage of total and progressively motile spermatozoa was recorded by the haemocytometer method (Lasley, 1951). Eosin- Nigrosin staining method was used to determine the percentage of live- spermatozoa. The experiment was conducted in summer and rainy seasons with cow and buffalo- bull semen and all the results were grouped and discussed under sections I to IV.

S E C T I O N - I

Cow and buffalo - bull semen in summer

&

rainy seasons.

S E C T I O N- I

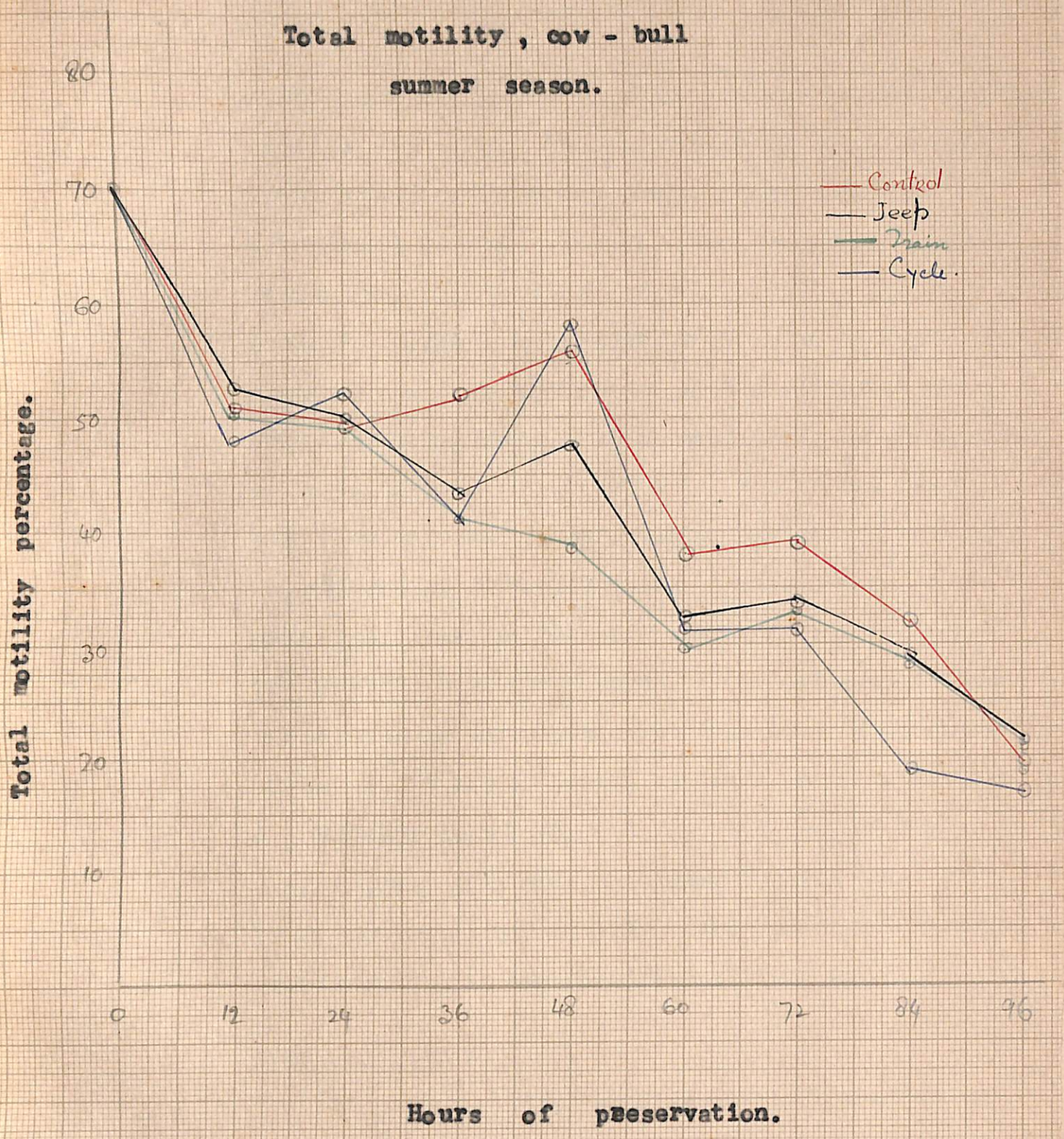
COW - BULL SEMEN

Altogether 12 ejaculates from 6 Tharparkar bulls (2 ejaculates from each bull) were collected during each season for studying the three characters viz progressive motility, total motility and percentage of live spermatozoa at 12 hours interval from 0 to 96 hours under each mode of transport.

Average percentage of progressively motile, total motile and live spermatozoa in bull semen for control and treatment group under to seasons are shown in table 4 : 1. The mean percentages are the actual average values obtained,and not the transformed ones (the number of observations for each character under each mode was 108) .

TABLE 4 : 1

	Summer			Rainy		
	Progressive motility	Total motility	Percentage live sperm	Progressive motility	Total motility	Percentage live sperm
Control.	21.5 ±.04	45.1 ±.04	70.9 ±.01	13.7 ±.06	62.6 ±.04	72.8 ±.01
Jeep.	19.1 ±.04	42.2 ±.04	66.7 ±.02	11.3 ±.07	53.4 ±.06	70.2 ±.01
Train	17.2 ±.02	40.0 ±.04	68.1 ±.01	13.2 ±.07	53.8 ±.05	70.9 ±.01
Cycle	15.5 ±.05	38.2 ±.05	67.0 ±.02	13.1 ±.07	50.9 ±.05	69.6 ±.01
Average	18.3	41.4	68.2	12.8	55.2	70.9


Total motility , cow - bull
summer season.
Control
Jeep
Train
Cycle.
Total motility percentage.
80
70
60
50
40
30
20
10
0
12
24
36
48
60
72
84
96
Hours of preservation.

SUMMER

Progressive motility : From the above table (4 : 1) it would appear that the percentage of progressively motile spermatozoa during summer season uder the 4 treatment groups (control, jeep, train and cycle) varied from 15.5 ± .05 (cycle) to 21.5 ± .04 (control), the average being 18.3. When analysis of variance was made to find out if the different modes affected the percentage of progressively motile spermatozoa differentially, (table 4 : 3) it was found that they differed significantly, meaning thereby that the effects of different modes on this character were significant at 5% level. In order to find out the specific mean differences, a test of significance was applied by calculating the " critical difference" values at 5 % and 1% levels as detailed in table 4 : 20. This test indicated that the mean of the control group differed significantly with that of train and cycle respectively. Other combinations were found stastically non-significant at 5% level.

Total motility :

Total motility of bull spermatozoa in summer varied from 38.2 ± .05 (cycle) to 45.1 ± .04 for the (control) with an average value of 41.4 four treatment groups (table 4 : 1).

Analysis of variance was conducted to find out the effect of different modes on the total motility of bull semen (table 4 : 6). It was noted that no significant difference existed between different modes at 5 % level.

Percentage of live spermatozoa :

Live sperm content of the bull semen for all the 4 treatment groups varied from 66.7 ± .02 (jeep) to 70.9 ± .01 (control) with a mid- value of 68.2. Effects of different treatments (modes) was found to be significant at 5% level after conducting analysis of variance (table 4 : 9). Test of critical difference was applied in order to detect the effects of specific modes (table 4 : 20). It was found that the mean of control differed highly significantly (at 1% level) with jeep. A significant difference was also noted between control and cycle means. Other treatment combinations had no significant effect on this character.

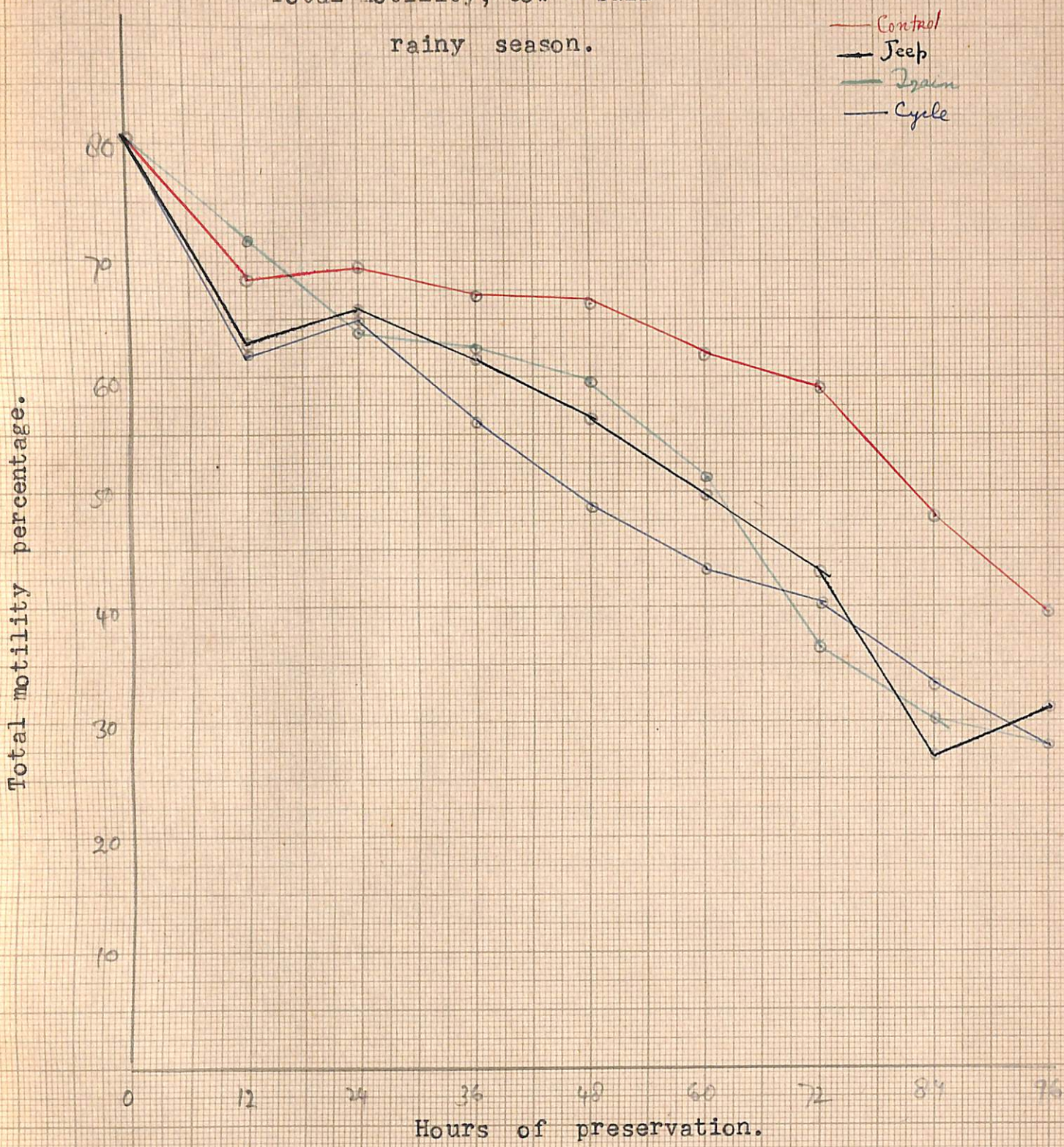
RAINY SEASON

Progressive motility ;

Progressive motility of spermatozoa in rainy season varied from 11.3 ± .07 (jeep) to 13.7 ±.06 (control) the average being 12.8 for the 4 modes of transport.

Total motility, cow - bull

rainy season.



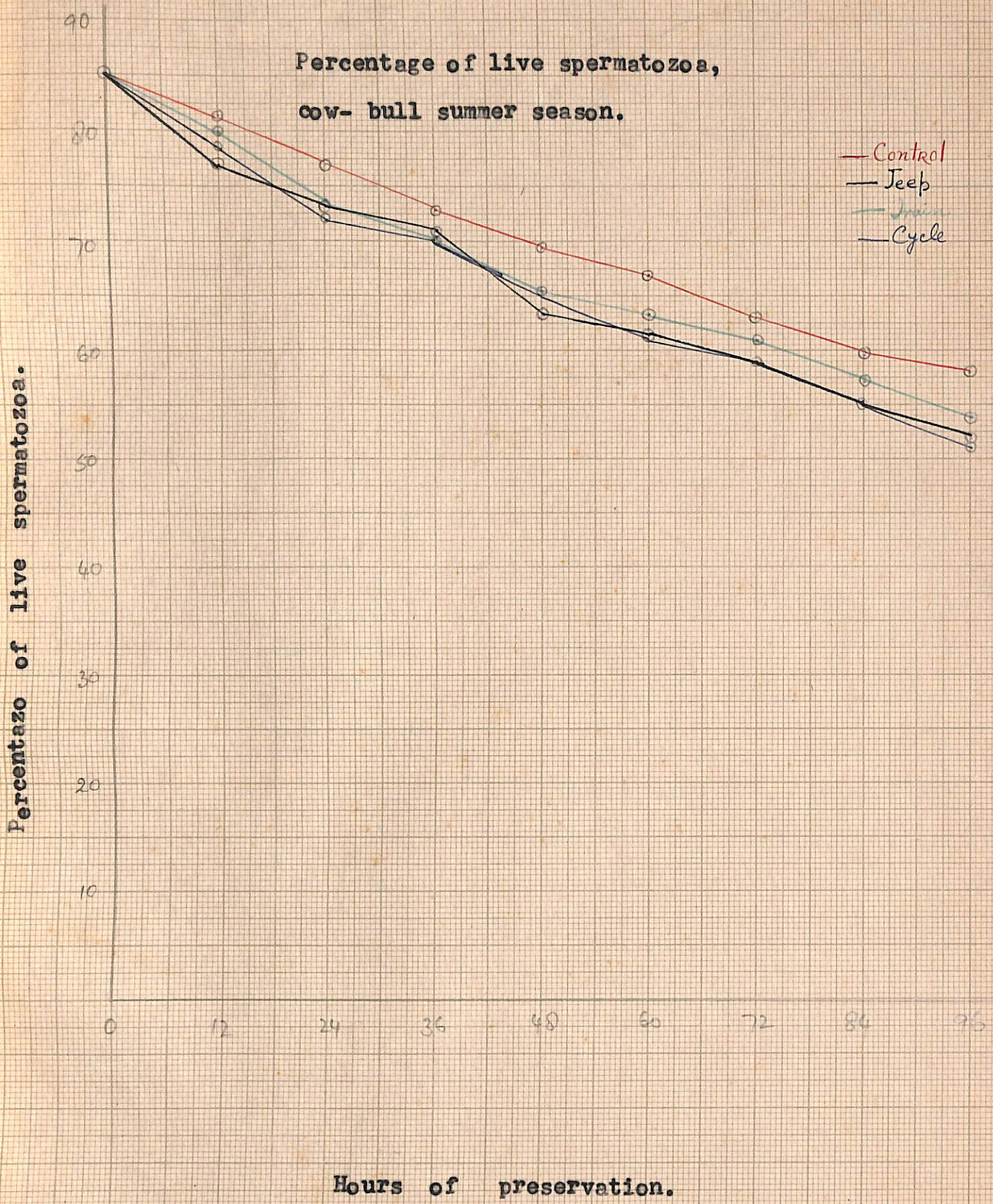
The effect of various modes of transport was found to be non- significant (table 4 : 12) meaning thereby that the effect of different modes on the variation in the progressive motile spermatozoa did not exist.

Total motility :

In the 4 modes studied in this experiment, the range of total motile spermatozoa varied from 50.9 ± .05 (cycle) to 62.6 ± .04 (control), the average being 55.2 . Analysis of variance was run to find out the effect of different modes on this seminal character. It was found that a highly significant difference existed between modes (table 4 : 15) on the total motility of the bull semen in the rainy season. In order to find out the specific significant effects of modes, a test of significance was applied (table 4 : 20). It was observed that the mean of the control group differed highly significantly (at 1% level) with that of jeep, train and cycle. The other mean combinations were found to have non- significant effect.

Live sperm percentage :

The range of the live- spermatozoa varied from 69.6 ± .01 to 72. 8 ± .01 with a mean value of 70.9 . After carrying out analysis of variance test (table 4 : 18) it was observed that different modes had no significant effect on this character.


Percentage of live spermatozoa,
cow- bull summer season.
Control
Jeep
Cycle
Percentazo of live spermatozoa.
90
80
70
60
50
40
30
20
10
0
12
24
36
48
60
72
84
96
Hours of preservation.

Following inferences could be drawn from table 4 : 1 , while comparing all the characters in both seasons for all modes:

Progressive motility :

Variation between treatments in summer in this character was significant whereas in rainy season no significant effect of modes could be observed. On the whole the average percentage of progressively motile spermatozoa was higher in summer than in rainy season in all the treatment groups. Perhaps this may be due to a greater range in enviromental variation in summer as compaired to rainy season.

Total motility :

Total motility percentage in all the groups of rainy season was higher than the respective values in summer in contrast to the progressive motility percentage. In summer no significant effect of modes was seen, but in rainy season, significant effect of all the modes (jeep, train and cycle) was evident from the test of critical difference (table 4 : 20). It may be due to higher percentage of progressively motile spermatozoa in summer which resists the effects of modes. Where as in rainy season, while the total motility is higher, the percentage of progressively motile sperm is lower,making the semen more vulnerable to the effects of modes.

Live spermatozoa :

A significant difference between modes (control - jeep and control- cycle), was noted in summer, but no significant effect of modes was observed in rainy season. The average live sperm percentage in rainy season was 70.9, the corresponding figure for summer being 68.2. The percentage of live spermatozoa appear to be more sensitive to the environmental variables of summer as compaired to those of rainy season.

BUFFALO - BULL SEMEN :

The average percentage of progressively motile, total motile and live - spermatozoa in buffalo- bull semen for control and treatment groups under two seasons have been obtained in table 4 : 1 B (number of observations for each character in each season being 81).

TABLE 4 : 1 B

Treatments	Summer			Rainy		
	Progressive motility	Total motility	Live spermatozoa	Progressive motility	Total motility	Live spermatozoa
Control	23.1 ±.04	35.1 ±.04	66.0 ±.03	19.7 ±.07	42.2 ±.05	68.6 ±.03
Jeep	23.1 ±.04	35.2 ±.03	63.3 ±.03	15.8 ± .08	36.6 ±.04	65.8 ±.3
Train	22.7 ±.04	34.8 ±.04	63.8 ±.04	18.0 ±.06	42.0 ±.05	66.8 ±.03
Cycle	20.6 ±.04	29.9 ±.04	63.1 ±.03	13.8 ±.07	32.4 ±.05	63.8 ±.02
Average	22.4	33.7	64.0	16.8	38.3	62.2

SUMMER

Progressive motility :

From the above table (4 : 1 B) it would appear that the progressive motility in buffalo- bull semen in summer was on an average 22.4 percent the range being 20.6 ± .04 to 23.1 ± .04 in the 4 treatment groups.

No significant difference on progressive motility due to modes could be observed when an analysis of variance test was run (table 4 : 29).

Total motility :

In summer the total motility percentage varied from 29.9 ± .04 (cycle) to 35.2 ± .03 (jeep) in the 4 treatment groups; the average being 33.7 (table 4 : 1 B). When analysis of variance was made to find out if the different modes affected the percentage of total motile spermatozoa differencially, it was found that modes affected this character significantly (table 4 : 34 and 4 : 35), meaning thereby that the effects of different modes on this character were significant. In order to find out the specific mean differences, a test of significance was applied by calculating critical differences as detailed in table 4 : 42. It was observed that the mean of the control group differed significantly with that of cycle. Similarly highly significant differences were observed between means of jeep -cycle and train - cycle. Mean differences for other combinations were found to be non- significant.

Live sperm percentage :

From table 4:1 B it would appear that the average live sperm percentage in buffalo semen in summer was 64.0, the range varied from 63.1 ± .03

to 66.0 ± .03 in the 4 modes studied. No significant effect of modes could be seen on this character after an analysis of variance test. (table 4 : 40).

RAINY SEASON

Progressive motility :

The percentage of progressively motile spermatozoa varied from 13.8 ± .06 (jeep) to 19.7 ±.07 (control) with an average value of 16.8 (table 4 : 1 B). After analysis of variance test it was inferred that modes had no effect on the progressive motility of buffalo semen (table 4 : 29).

Total motility :

Total motile sperm percentage of buffalo semen varied from 32.4 ±.05 to 42.2 ± .05 in the 4 modes with average of 38.3. After analysis of variance it became evident that the modes affected the total motility significantly (table 4 :34). Therefore a season- wise analysis was made (table 4:36). A test of critical difference was done to see the specific mean differences (table 4:42) for this season. It was found that in this season, means of control and cycle, and train and cycle differed significantly.

Live spermatozoa :

From table 4 : 1B , it would appear that the percentage of live spermatozoa during rainy season

under the four treatment groups (modes) i.e. control, jeep, train and cycle varied from 63.8 ± .02 to 68.6 ± .04, the average being 66.2. Analysis of variance indicated no significant effect of modes on the live- sperm content of buffalo- semen (table 4 : 40).

On the whole, following inferences could be drawn from table 4 : 1 B.

Progressive motility :

No significant effect of modes was found on progressive motility either in summer or in rainy season on buffalo- semen. The range varied from 20.6 ± .04 to 23.1 ± .04 in summer, having an average of 22.4%, The corresponding values in rainy season were 13.8 ±.03 to 19.7 ± .07 and 16.8% respectively.

Total motility :

Modes of transport affected total motility in both the seasons (summer and rainy). The average percentage of total motile spermatozoa in summer was 33.7, the range varying from 29.9 ± .04 to 35.2 ± .03. In rainy season the corresponding values were 38.3 and 32.4 ± .05 to 42.2 ± .05 respectively.

Live sperm percentage :

Modes had no significant effect on the

livesperm content of semen in either season. The average percentage of live sperm in summer was 64.0, the range being 63.1 ± .03 to 66.0 ±.03. Rainy season had on an average 66.2% live spermatozoa ranging from 63.8 ± .02 to 68.6±.04.

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S E C T I O N - II

Comparision of cow and buffalo- bull semen in summer and rainy seasons.

S E C T I O N - 2

COMPARISION OF COW AND BUFFALO - BULL SEMEN.

S U M M E R

Progressive motility :

The average percentage of progressively motile spermatozoa in summer for cow bull semen (out of all the modes studied) was 18.3 , having a range of 15.5 ± .05 to 21.5 ± .04 (table 4 : 1). Corresponding values for buffalo- bull semen were 22.4 and 20.6 ± .04 to 23.1 ±.04 respectively (table 4 :1 B). Control mean for progressive motility differed significantly with that of train and cycle in bull semen (table 4 : 20). Modes were not found to differ significantly from each other in case of buffalo- semen (table 4 : 29). Progressive motility of semen was significantly affected by the hours of preservation in both the species.(tables 4:4 & 4:28)

Total motility :

Bull semen had, on an average (for the 4 modes studied) 41.4% total motile spermatozoa, varying from 38.2 ± .05 to 45.1 ± .04 (table 4 :1). Buffalo- semen, on the other hand had only 33.7% motile spermatozoa varying from 29.9 ± .04 to 35.2 ±.03 (table 4 : 1 B).

Total motility in buffalo- semen was found to be affected by different- modes of transport (table 4 : 34 and 4 : 35).

Control- mean differed significantly with that of cycle, and significant mean differences were also noted between jeep and cycle and train and cycle (table 4 : 42). Mean differences for other combination were found to be non- significant. The average percentage values for the three characters under study for cow and buffalo- bull semen at different hours of preservation, in thermosflasks have been compared in both the seasons (tables 4:44, 4 : 45 and 4 : 46). Thus the total motility percentage of bull semen at 0 hour in summer was 70.0 ± .24. This came to 80.5 ± .21 in rainy season. These values for buffalo semen were 52.3±.15 and 63.0 ± .47 respectively. Similarly at 96 hour the average total motility percentage in bull-semen was 19.8 ± .28 and 39. 1 ± .53 in summer and rainy seasons respectively. For buffalo- bull semen these values were 16.8 ± .32 and 22.8 ± .17 respectively.

For Bull semen the total motility percentage values at all the intervals of examination (0 to 96 hours) were higher in rainy season than the respective values for summer. In buffalo- bull semen also, the values obtained in rainy season were higher than the respective values of summer except at 72 and 84 hours. But no statistical test was applied to test the difference in mean values. Total motility in bull-semen was significantly affected by hours of preservations (table 4 : 7 and 4 : 23).

Total motility of buffalo- bull semen was affected highly significantly by the hours of preservation (table 4 : 33).

Percent live- spermatozoa :

The average percentage of live spermatozoa in bull- semen was 68.2 in summer (for the 4 modes) range being 66.7 ± .02 to 70.9 ± .01 (table 4 : 1). In case of buffalo- semen, the mean value obtained was 64.0 under the 4 modes and the range varied from 63.1 ± .03 to 66.0 ± .03 (table 4 : 1 B).

In bull semen, the effects of modes on the percentage of live spermatozoa were found significant (table 4 : 9), and finally it was noted from table 4 : 20 that the control mean differed significantly from the jeep and cycle means. No significant difference due to modes could be observed on the percentage of live spermatozoa in summer in buffalo- semen (table 4 : 40). It may be that bull sperm are subject to greater variation under the transporting system studied as compared to the sperms of buffalo- bulls.

Hours of storage had highly significant effect on the percentage live spermatozoa (table 4 :24). In buffalo- semen also,highly significant difference was observed in percent live spermatozoa due to the effect of hours of preservation (table 4 : 41).

RAINY SEASON :

Progressive motility :

The average percentag of progressively motile spermatozoa in bull- semen in rainy season was 12.8 under the 4 modes studied. The range varied from 11.3 ± .07 to 13.7 ± .06 (table 4 : 1). Buffalo- semen, had on an average 16.8 % progressively motile spermatozoa, the range being 15.8 ± .08 to 19.7 ± .07 (table 4 : 1 B).

Modes of transport had no effect on the progressive motility of either bull or buffalo semen (table 4 : 12 and 4 : 29).

Storage time or hours of preservation had highly significant effect on the progressive motility of semen from both the species (table 4 :4, 4 : 22 and 4 : 28).

Total motility :

The average total motility percentage in rainy season in bull semen was 55.2% (for the 4 modes studied), the range varied from 50.9 ± .05 to 62.6 ±.04 (table 4 : 1) . The corresponding values in buffalo semen in rainy season were 38.3 and 32.4 ± .05 to 42.2 ± .05 (table 4 : 1 B) . In bull semen, highly significant differences existed between modes (table 4 :15 and 4 :23) on total motility and it was observed

that the control group mean differed significantly from those of the jeep, train and cycle (table 4:20). Total motility of buffalo semen was also affected significantly by modes (table 4 : 34 and 4 : 36), and a significant difference was noted between the means of control and cycle and train and cycle (table 4 : 42). The effect of hours of preservation was highly significant on the total motility of bull-semen (table 4 : 7 and 4 : 23). Total motility of buffalo- semen was also highly significantly affected by the hours of preservation (table 4 : 33).

Live spermatozoa :

In bull semen, the average percentage of live spermatozoa in rainy season was 70.9 with range varying from 69.6 ± .01 to 72.8 ± .01 (table 4 : 1). In buffalo semen the average percentage of live spermatozoa was 66.2, the range varied from 63.8 ± .02 to 68.6 ± .04 (table 4 : 1 B). Modes had no significant effect on the live sperm percentage of bull semen (table 4 : 18). Live sperm percentage of buffalo- semen was also not significantly affected by the modes used (4 : 40).

Hours of preservation had highly significant effect on the total motility of cow and buffalo- bull semen (table 4 : 19 , 4 : 24 and 4 : 41).

It was clear from tables 4 : 22, 4 : 23 , 4 : 24 , 4 : 27, 4 : 32 and 4 : 39 that there was significant seasonal variation in all the characters studied that is progressive motility, total motility and percentage of live spermatozoa in the semen of both the species .

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S E C T I O N - III

Role of specific variables and their meaningful interactions.

TABLE - 4 : 2

Showing the means, standard errors and coefficients of variation for progressive motile spermatozoa of different samples at different hours of preservation in summer for bull-semen.

Treatments.		Hours of preservations								
		0hr.	12 hr.	24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96hr
Control	$\bar{x}$	54.9	33.4	27.0	28.4	21.6	15.3	12.4	9.8	4.5
	SE.	.06	.07	.07	.05	.06	.06	.16	.03	0.11
	CV.	2.8	6.5	8.5	5.7	9.2	13.7	38.6	16.8	64.0
Jeep	$\bar{x}$	54.9	26.1	22.6	18.4	18.9	12.0	16.0	10.2	4.4
	SE.	.06	.26	.07	0.10	.09	.07	0.12	.03	0.03
	CV.	2.8	29.2	11.2	17.7	15.3	18.6	23.1	11.0	21.1
Train	$\bar{x}$	54.9	27.5	25.1	14.3	8.7	8.9	14.7	8.0	6.8
	SE.	.06	.13	.04	.14	.13	0.16	.06	.19	0.05
	CV.	2.8	13.9	4.3	29.4	54.5	49.5	13.5	63.2	23.8
Cycle	$\bar{x}$	54.9	25.3	25.0	15.3	10.7	9.1	9.5	4.5	3.3
	SE.	.06	.14	0.11	.08	.11	0.11	.05	.12	.05
	CV.	2.8	16.8	13.7	17.6	3.29	37.3	16.7	68.3	45.3

n = 12

TABLE 4 : 3

Analysis of variance showing the affects of modes on progressive motility of bull semen in summer.

Sources of variation	D.F.	S.S.	M.S.	F.
Between modes.	3	1161.14	387.04	2.85*
Within modes.	428	58080.67	135.70	
Total	431	59241.81		

*- Significant at 5% level.

TABLE 4 : 4

Analysis of variance showing the effects of hours of preservation on progressive motility of bull semen in rainy season.

Sources of variation	D.F.	S.S.	M.S.	F.
Between hours	8	42305.91	5288.24	132.10 **
Within hours	423	16935.90	40.03	
Total	431	59241.81		

** - Significant at 1% level of probability.

TABLE 4 : 5

Showing the means, standard errors, and coefficients of variation for percentage of total motile spermatozoa of different samples at different hours of preservation in the summer season for bull-semen.

Treatments.		Hour of preservations								
		0 hrs	12 hr.	24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96 hr
Control	$\bar{x}$	70.0	50.8	49.2	52.0	56.0	38.6	38.9	32.1	19.8
	SE.	0.24	0.10	.08	.19	.18	.15	.23	.05	.28
	CV.	8.7	5.6	4.9	10.7	9.2	11.5	17.0	21.2	41.0
Jeep	$\bar{x}$	70.0	52.9	39.8	43.8	47.6	32.8	33.6	28.9	21.4
	SE.	.24	.11	.12	.15	.19	.14	.15	.11	.28
	CV.	8.7	5.7	7.2	10.0	11.4	13.0	13.7	11.7	37.5
Train	$\bar{x}$	70.0	50.7	39.7	41.7	38.8	29.3	33.1	28.6	20.2
	SE.	.24	.13	.7	.15	.13	.10	.21	.22	.21
	CV.	8.7	7.2	4.0	11.4	10.1	10.5	19.7	23.2	31.4
Cycle	$\bar{x}$	70.0	48.0	52.0	41.4	37.3	32.0	31.1	19.1	17.8
	SE.	.24	.12	.19	.15	.22	.28	.19	0.12	12.1
	CV.	8.7	7.2	10.1	10.4	17.5	25.9	19.1	19.8	27.3

n = 12

TABLE 4 : 6

Analysis of variance showing the effects of modes on total motility of bull semen in summer season.

Sources of variation	D.F.	S.S.	M.S.	F.
Between modes	3	946.37	315.45	2.26
Within modes	428	59627.16	139.31	N.S.
Total	431	60573.43		

N.S. = Non-significant.

TABLE 4 : 7

Analysis of variance showing the effects of hours of preservation on total motility of bull semen in summer.

Sources of variation	D.F.	S.S.	M.S.	F.
Between hours	8	31052.36	3881.54	55.61 **
Within hours	423	29521.17	69.79	
Total	431	60573.53		

** = Significant at 1% level.

TABLE 4 : 8

Showing the averages, standard errors, and coefficients of variation for the percentage of live spermatozoa in control and treatment groups, in bull semen preserved in summer.

Treatments.	Hour of preservations	0 hr	12 hr	24 hr	36 hr	48 hr	60 hr	72 hr	85 hr	96 hr
Control	$\bar{x}$	85.3	81.4	77.1	73.0	69.4	66.9	62.9	59.7	58.0
	SE.	.01	.01	.01	.01	.03	.02	.02	.02	.02
	CV.	.26	.33	4.7	.37	.92	.68	.89	.96	.92
Jeep	$\bar{x}$	85.3	77.7	73.0	71.0	63.6	61.8	58.8	54.9	52.3
	SE.	.01	.08	.02	.01	.02	.02	.02	.02	.02
	CV.	.26	2.4	.57	.42	.79	.68	.82	.73	1.1
Train	$\bar{x}$	85.3	80.0	73.5	70.4	65.6	63.6	61.3	57.2	54.2
	SE.	.01	.01	.02	.01	.02	.02	.02	.02	.05
	CV.	.26	.38	.57	.44	.86	.86	1.1	1.1	2.4
Cycle	$\bar{x}$	85.3	78.4	72.7	70.5	65.3	62.5	58.8	54.9	51.0
	SE.	.01	.02	.02	.02	.03	.03	.02	.01	.03
	CV.	.26	.51	.73	.62	1.6	1.1	.96	.54	1.9

n = 12

TABLE 4 : 9

Analysis of variance showing the effect of modes on the percentage of live spermatozoa of bull semen in summer.

Sources of variation	D.F.	S.S.	M.S.	F.
Between modes	3	441.11	147.03	3.18 *
Within modes	428	19735.43	46.11	
Total	431	20176.54		

* Significant at 5% level.

TABLE 4 : 10

Analysis of variance showing the effects of 4 hours of preservation on percentage of live spermatozoa of bull semen in summer.

Sources of variation	D.F.	S.S.	M.S.	F.
Between hours	8	16789.95	2098.74	262.34 **
Within hours	423	3386.59	8.00	
Total	431	20176.54		

** Significant at 1% level of probability.

TABLE 4 : 11

Showing the means, standard errors, and coefficients of variation for percentage of progressively motile spermatozoa of the control and treatment samples of bull-semen in rainy season.

Treatments		Hours of preservation								
		0 hr	12 hr	24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96 hr
Control	$\bar{x}$	56.2	34.4	26.3	13.0	10.0	5.7	4.6	1.7	1.6
	SE.	.15	.23	.12	.15	.17	.10	.23	.11	.08
	CV.	7.5	20.2	14.1	34.4	47.4	49.5	93.8	99.4	98.0
Jeep	$\bar{x}$	56.2	25.9	21.8	12.7	5.9	4.0	2.4	2.1	1.3
	SE.	.15	.11	.35	.26	.23	.21	.10	.09	.10
	CV.	7.5	13.2	44.3	55.3	86.1	96.6	62.1	91.2	98.2
Train	$\bar{x}$	56.2	30.5	24.0	12.4	12.6	6.3	2.6	1.9	1.7
	SE.	.15	.13	.36	.21	.42	.17	.06	.07	.12
	CV.	7.5	13.3	42.6	48.4	77.7	70.5	62.6	87.1	98.2
Cycle	$\bar{x}$	56.2	30.5	24.0	12.4	12.6	6.3	2.6	1.9	1.7
	SE.	.15	.13	.36	.21	.42	.17	.06	.07	.04
	CV.	7.5	13.3	42.6	48.3	77.4	70.5	63.0	87.1	93.8

n = 12

TABLE 4 : 12

Analysis of variance showing the effects of modes on the progressive motility of bull-semen in rainy season:

Sources of variation	D.F.	S.S.	M.S.	F.
Between modes	3	277.95	92.65	0.390
Within modes	428	101486.62	237.11	NS.
Total	431	101764.57		

N S. = Non- significant.

TABLE 4 : 13

Analysis of variance showing the effects of hours of preservation on progressive motility of bull- semen in rainy season:

Sources of variation	D.F.	S.S	M.S.	F.
Between hours	8	73519.00	9189.87	137.63**
Within hours	423	28245.57	66.77	
Total	431	101764.57		

** = Significant at 1% level of probability.

TABLE 4 : 14

Showing the means, standard- errors and coefficients of variation, of percentage of total motile spermatozoa of the control and treatment groups of bull-semen in rainy season :

Treatments		Hours of preservation								
		0 hr	12 hr	24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96 hr
Control	$\bar{x}$	80.5	69.4	68.4	67.3	66.5	62.5	58.9	47.9	39.1
	SE.	.21	.24	.01	.23	.26	.19	.17	.15	.53
	CV.	6.1	8.8	3.5	9.0	10.0	7.9	7.7	9.3	36.4
Jeep	$\bar{x}$	80.5	63.0	66.6	61.6	56.5	49.2	43.1	27.3	31.1
	SE.	.21	.22	.17	.19	.22	.19	.19	.63	.34
	CV.	6.1	9.2	6.6	8.6	10.7	11.2	18.2	58.7	32.3
Train	$\bar{x}$	80.5	70.2	64.0	62.6	59.4	51.0	36.6	30.2	28.2
	SE.	.21	.13	.26	.10	.19	.22	.19	.23	.34
	CV.	6.1	4.7	10.8	4.4	8.8	12.1	21.8	22.3	34.5
Cycle	$\bar{x}$	80.5	62.3	64.6	56.0	48.3	43.6	40.3	33.1	28.1
	SE.	.21	.18	.16	.17	.12	.13	.38	.56	.30
	CV.	6.1	7.7	6.6	8.5	7.3	8.6	28.8	45.3	30.9

n = 12

TABLE 4 : 15

Analysis of variance showing the effects of modes on total motility of bull semen in rainy season:

Sources of variation	D.F.	S.S.	M.S.	F.
Between modes	3	2846.04	948.64	5.47 **
Within modes	428	74095.77	173.12	
Total	431	76941.81		

** = Significant at 1% level of probability.

TABLE 4 : 16

Analysis of variance showing the effects of hours of preservation on total motility of bull-semen in rainy season :

Sources of variation	D.F.	S.S.	M.S.	F
Between hours	8	34819.06	4352.38	43.71 **
Within hours	423	42122.75	99.58	
Total	431	76941.81		

** = Significant at 1% level of probability.

TABLE 4 : 17

Showing the means, standard errors and coefficients of variation for the percentage of live spermatozoa in the treatment and control groups in rainy season in bull- semen :

Treatments		Hours of preservation								
		0 hr	12 hr	24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96 hr
Control	$\bar{x}$	84.0	81.5	77.4	76.1	72.9	70.2	66.1	63.0	59.6
	SE.	.01	.01	.01	.01	.01	.01	.02	.01	.01
	CV.	.16	.15	.10	.16	.38	.26	.47	.40	.01
Jeep	$\bar{x}$	84.0	79.7	76.7	74.6	71.2	67.7	63.1	59.2	51.6
	SE.	.01	.01	.01	.01	.06	.01	.01	.04	.04
	CV.	.16	.11	.10	.24	.38	.49	1.0	1.5	2.1
Train	$\bar{x}$	84.0	80.6	76.7	73.8	72.7	67.7	63.7	59.1	55.6
	SE.	.01	.01	.01	.01	.02	.02	.01	.02	.02
	CV.	.16	.18	.10	.36	2.1	.49	.30	.28	.49
Cycle	$\bar{x}$	84.0	79.2	76.1	72.9	69.6	65.8	63.1	57.8	53.8
	SE.	.01	.01	.01	.01	.01	.01	.01	.01	.01
	CV.	.16	.14	.22	.34	.59	.89	.36	.76	.79

n = 12

TABLE 4 : 18

Analysis of variance showing the effects of modes on the percentage of live spermatozoa in the bull-semen in rainy season :

Sources of variation	D.F.	S.S.	M.S.	F.
Between modes	3	236.73	78.91	2.08 N.S.
Within modes	428	16193.21	37.83	
Total	431	16429.94		

N.S. = Non- significant.

TABLE 4 : 19

Analysis of variance showing the effects of hours of preservation on the percentage of live spermatozoa in bull semen in rainy season :

Sources of variation	D.F.	S.S.	M.S.	F.
Between hours	8	14194.29	1774.28	336.03 **
Within hours	423	2235.65	5.28	
Total	431	16429.95		

** = Significant at 1% level of probability.

TABLE 4 : 20

Showing the critical-differences between control and treatment means for three seminal characters of bull semen in summer and rainy seasons :

Comparison	Differences					
	Summer			Rainy		
	Progressive motility	Total motility	% of live spermatozoa	Progressive motility	Total motility	% of live spermatozoa
Control-jeep	1.73	1.75	2.56**	2.08	5.36**	1.61
Control-train	3.11*	2.94	1.77	0.04	5.10**	1.16
Control-cycle	4.41**	3.98	2.37*	0.50	6.76**	1.96
Jeep - train	1.38	1.19	0.79	1.68	0.26	0.45
Jeep - cycle	2.68	2.23	.19	1.58	1.40	0.35
Train -cycle	1.30	1.04	.60	0.10	1.66	.80
C.D. at 1% level	4.08		2.38		3.825	
C.D. at 5% level	3.106		1.81		2.91	

** = Significant at 1% level.

* = Significant at 5% level.

TABLE 4 : 21

Showing the average percentages of various types of spermatozoa at 0 hour in cow and buffalo bull semen respectively in summer and rainy seasons :

	Percentage live spermatozoa	Percentage motile spermatozoa	Percentage live - non motile spermatozoa	Percentage weakly motile spermatozoa
Bull semen				
Summer	85.3	70.0	15.3	15.1
Rainy	84.0	80.5	3.5	24.3
Buffalo semen				
Summer	83.5	52.3	31.2	3.5
Rainy	84.5	63.0	21.5	11.9

TABLE 4 : 22

Analysis of variance table showing the effects of some important factors alongwith some meaningful interactions on the progressive motility of bull semen (observations combined for both the seasons) :

Sources of variation	D.F.	S.S.	M.S.	F.
Season	1	3980.95	3980.95	117.01 **
Collection	1	865.60	865.60	25.44 **
Bulls	5	3351.11	670.22	19.70 **
Mode of transport	3	713.47	237.82	6.99 **
No. of intervals(hours)	8	112458.28	14057.28	413.20 *
Season x collection	1	0.59	0.59	0.01 N.S.
Season x bull	5	6719.07	1343.81	39.50 **
Season x mode	3	725.62	241.87	7.10 **
Season x interval	8	3366.68	420.83	12.37 **
Collection x bull	5	1635.79	327.16	9.61 **
Collection x mode	3	17.63	5.87	0.17 N.S.
Collection x interval	8	1443.51	180.43	5.30 **
Bull x mode	15	757.01	50.46	1.48 N.S.
Bull x interval	40	3225.32	80.63	2.37 **
Mode x interval	24	788.99	32.87	0.96 N.S.
Error	733	24937.71	34.02	
Total	863	164987.33		

* = Significant at 5% level.

** = Indicates significant at 1% level of probablity.
N.S. = Non- significant.

TABLE 4 : 23

Analysis of variance showing the effects of some important factors along with some meaningful interactions on the total motility of bull-semen (observation combined for both the seasons) :

Sources of variation	D.F.	S.S.	M.S.	F.
Season	1	13797.61	13797.61	418.23 *
Collection	1	416.25	416.25	12.61 *
Bull	5	3221.03	644.26	19.52 **
Mode of transport	3	3406.52	1135.33	34.41 **
No. of intervals (hours)	8	65137.67	8142.20	246.80 **
Season x collection	1	1461.98	1461.98	44.31 **
Season x bull	5	9732.13	1946.42	59.00 **
Season x mode	3	385.81	128.60	3.89 **
Season x hour	8	733.67	91.70	2.77 **
Collection x bull	5	6146.10	1229.22	37.26 **
Collection x mode	3	267.93	89.31	2.70 *
Collection x hour	8	1352.14	169.01	5.12 **
Bull x mode	15	1559.95	103.99	3.15 **
Bull x interval	40	2863.56	71.58	2.16 **
Mode x interval	24	16649.18	693.71	21.02 **
Error	733	24181.33	32.99	
Total	863	151312.86		

* = Significant at 5% level.

** = Significant at 1% level.

TABLE 4 : 24

Analysis of variance showing the effect of some important factors and relavant interactions on the live spermatozoa of bull semen (both seasons combined) :

Sources of variation	D.F.	S.S.	M.S.	F
Season	1	582.81	582.81	171.92 **
Collection	1	51.78	51.78	15.27 **
Bulls	5	1139.25	227.85	67.21 **
Mode of transport	3	652.58	217.53	64.16 **
Intervals (hours)	8	30693.81	3836.73	1131.77 **
Season x collection	1	65.66	65.66	16.70 **
Season x bull	5	186.75	37.35	11.01 **
Season x mode	3	25.91	8.64	2.54 N.S.
Season x hour	8	290.21	36.28	10.70 **
Collection x bull	5	447.77	89.55	26.41 **
Collection x mode	3	0.79	0.26	0.076 N.S.
Collection x hour	8	50.26	6.28	1.852 N.S.
Bull x mode	15	21.01	1.40	0.41 N.S.
Bull x interval	40	338.97	8.47	2.49 **
Mode x interval	24	152.46	6.35	1.87 **
Error	733	2489.91	3.39	
Total	863	39073.93		

* = Significant at 5% level.

** = Significant at 1% level.

N.S.= Non significant.

From the above three analysis of variance of tables (4 : 22 , 4 : 23 and 4 : 24), it was evident that the effects of bulls, seasons, collections and hours of preservation were significant on all the three seminal characters of bull-semen.

" Within season between modes " analysis and a test of " critical difference " (table - 4 : 20) indicated significant differences between control and treatment means. But no significant difference could be found out between the means of the modes (when control was excluded) meaning thereby that between mode differences did not exist at the respective distance of particular modes. Range of variation for different bulls of cow and buffalo species is shown in table 4 : 21.

As would be evident from table numbers 4 : 22, 4:23 and 4 : 24, most of the interactions are significant showing that the combined effects in the simple interaction (1st degree) of two variables together have produced a real effect on these three characters.

TABLE 4 : 25

Showing the means, standard errors and coefficients of variation in the percentage of progressively motile spermatozoa in the buffalo-semen in summer in control and treatment samples at different hours of preservation :

Treatments		Preservation hours								
		0 hr	12 hr	24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96 hr
Control	$\bar{x}$	48.5	37.8	29.2	22.8	19.5	21.3	13.1	16.3	8.4
	SE.	.05	.07	.38	.11	.14	.20	.20	.29	.20
	CV.	2.4	4.5	27.9	11.8	17.1	22.2	34.2	38.7	51.9
Jeep	$\bar{x}$	48.5	35.8	28.9	24.3	19.1	19.9	16.3	17.8	6.6
	SE.	.05	.06	.03	.30	.30	.29	.07	.27	.12
	CV.	2.4	4.1	2.5	27.5	35.2	32.7	10.5	21.9	40.3
Train	$\bar{x}$	48.5	31.3	29.4	27.2	20.8	20.8	17.9	9.6	8.1
	SE.	.05	.14	.12	.15	1.3	.21	.13	.20	.17
	CV.	2.4	10.4	9.5	13.2	44.1	23.2	18.0	45.3	46.6
Cycle	$\bar{x}$	48.5	32.0	26.3	20.1	24.1	16.3	13.2	10.7	5.8
	SE.	.05	.09	.18	.14	.34	.39	.19	.13	.07
	CV.	2.4	6.5	16.3	16.6	5.5	49.8	32.6	27.8	28.9

n = 9

TABLE 4 : 26

Showing the means, standard error and coefficient of variation, in progressively motile spermatozoa in the buffalo- semen at different hours of preservation in control and treatment samples in rainy season.

Treatment		Hours of preservation								
		0 hr	12 hr	24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96 hr
Control	$\bar{x}$	51.1	42.6	29.5	19.1	18.6	15.2	16.2	3.6	2.4
	SE.	.04	.21	.17	.15	.18	.31	.38	.35	.21
	CV.	1.6	10.7	13.8	18.6	22.9	44.2	49.7	80.1	95.7
Jeep	$\bar{x}$	51.1	38.5	25.3	15.8	14.7	11.2	7.4	1.7	1.8
	SE.	.04	.56	.24	.17	.12	.19	.23	.07	.39
	CV.	1.6	.33	21.3	25.5	19.8	39.2	62.3	78.7	97.3
Train	$\bar{x}$	51.1	30.2	21.5	18.0	21.9	19.1	12.5	4.8	1.8
	SE.	.04	.28	.05	.20	.30	.21	.30	.36	.14
	CV.	1.6	20.7	5.5	25.8	30.8	45.0	50.0	97.4	95.1
Cycle	$\bar{x}$	51.1	25.3	24.3	13.6	15.2	12.6	6.0	2.1	1.9
	SE.	.04	.20	.46	.32	.25	.21	.28	.1	.04
	CV.	1.6	18.7	40.0	50.4	33.7	38.3	80.1	80.4	95.2

n = 9

TABLE 4 : 27

Analysis of variance showing the effects of seasons on the progressive motility of buffalo- semen in (both the seasons combined) :

Sources of variation	D.F.	S.S.	M.S.	F.
Between seasons	1	2621.28	2621.28	17.45 **
Within seasons	646	96999.50	150.15	
Total	647	99620.78		

** = Significant at 1% level.

TABLE 4 : 28

Analysis of variance showing the effects of hours of preservation on progressive motility in buffalo- semen (for both the seasons):

Sources of variation	D.F.	S.S.	M.S.	F.
Between hours	8	59871.97	7483.99	113.05 **
Within hours	639	39748.81	6620	
Total	647	99620.78		

** = Significant at 1% level.

TABLE 4 : 30

Showing the averages, standard errors and coefficients of variation for total motility in the buffalo- semen at different intervals in the control and treatment groups, preserved in summer :

Treatment		Hours of preservation								
		0 hr	12 hr	24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96 hr
Control	$\bar{x}$	52.3	46.7	40.7	34.1	34.4	36.4	28.9	28.7	16.8
	SE.	.15	.05	.23	.09	.05	.20	.19	.23	.32
	CV.	6.2	2.5	12.4	6.5	3.1	12.6	12.0	18.3	40.6
Jeep	$\bar{x}$	52.3	43.4	42.9	39.6	34.9	35.6	25.5	30.8	15.3
	SE.	.15	.04	.04	.06	.19	.21	.15	.09	.36
	CV.	6.2	1.7	2.1	3.6	12.8	13.2	13.9	6.9	48.8
Train	$\bar{x}$	52.3	43.4	42.9	39.6	39.1	35.6	30.2	21.3	13.8
	SE.	.15	.09	.07	.08	.13	.19	.08	.23	.36
	CV.	6.2	4.8	3.7	4.8	7.3	12.2	6.5	24.3	53.3
Cycle	$\bar{x}$	52.3	40.4	38.9	29.4	38.1	26.4	26.4	14.3	8.6
	SE.	.15	.04	.12	.05	.04	.30	.21	.05	.17
	CV.	6.2	2.3	7.3	4.1	2.6	25.3	18.8	5.2	42.9

n = 9

TABLE 4 :31

Showing the averages, standard errors and coefficients of variation in buffalo- semen for total motility in rainy season for control and treatment groups :

Treatment		Hours of preservation								
		0 hr	12 hr	24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96 hr
Control	$\bar{x}$	63.0	64.8	54.6	43.8	49.2	36.6	26.1	23.4	22.8
	SE.	.47	.12	.46	.13	.08	.26	.29	.27	.17
	CV.	14.7	3.6	17.2	6.5	3.8	15.8	25.2	26.6	17.1
Jeep	$\bar{x}$	63.0	60.1	49.5	35.3	37.4	27.6	30.0	15.9	16.4
	SE.	.47	.15	.13	.15	.06	.14	.04	.05	.10
	CV.	14.7	5.0	5.7	12.6	4.0	11.9	4.1	8.4	15.7
Train	$\bar{x}$	63.0	51.4	50.7	44.1	51.1	43.1	35.8	24.9	18.7
	SE.	.47	.05	.16	.30	.32	.34	.18	.24	.23
	CV.	14.7	2.0	6.8	18.0	13.2	16.8	11.7	22.5	28.1
Cycle	$\bar{x}$	63.0	47.9	50.2	36.3	32.9	28.7	17.4	13.9	11.3
	SE.	.47	.26	.32	.15	.08	.06	.15	.14	.08
	CV.	14.7	11.8	13.2	11.8	5.9	4.9	20.9	24.0	17.0

n = 9

TABLE 4 : 29

Analysis of variance showing the effects of modes of transport on the progressive motility in buffalo semen (both seasons) :

Sources of variation	D.F.	S.S.	M.S.	F.	
Between modes	3	839.27	279.96	1.82	
Within modes	644	98781.51	153.38		N.S.
Total	647	99620.78			

N.S. = Non significant.

TABLE 4 : 32

Analysis of variance showing the effects of seasons on the total motility of buffalo - semen (both seasons) :

Sources of variation	D.F.	S.S.	M.S.	F.	
Between seasons	1	1198.30	1198.30	9.90	
Within seasons	646	78179.60	121.02		**
Total	647	79377.90			

** = Significant at 1% level.

TABLE 4 : 33

Analysis of variance showing the effects of hours of preservation on the total motility of buffalo-semen (both seasons) :

Sources of variation	D.F.	S.S.	M.S.	F.
Between hours	8	42826.40	5103.30	84.58 **
Within hours	639	38551.50	60.33	
Total	647	79377.90		

** = Significant at 1% level.

TABLE 4 : 34

Analysis of variance showing the effects of modes of transport on the total motility of buffalo- semen (both season combined) :

Sources of variation	D.F.	S.S.	M.S.	F.
Between modes	3	2195.73	731.91	6.10 **
Within modes	644	77182.17	119.84	
Total	647	79377.90		

** = Significant at 1 % level.

From above table it was indicated that the modes of transport used in this experiment had highly significant effect on the total motility of buffalo semen.

For determining the specific effects of modes and season, this analysis was done season-wise.

TABLE 4 : 35

The analysis of variance showing the effects of various modes in summer season on the total motility of buffalo- semen :

Sources of variation	D.F.	S.S.	M.S.	F.
Between modes	3	628.50	209.16	2.36 *
Within modes	320	28406.33	88.77	
Total	323	29034.83		

* = Significant at 5% level.

A further analysis was done to ascertain the effects of modes in rainy season.

TABLE 4 : 36

Showing the effects of modes on the total motility of buffalo semen in rainy season:

Sources of variation	D.F.	S.S.	M.S.	F.
Between modes	3	1969.80	656.60	4.45 **
Within modes	320	47174.99	147.42	
Total	323	49144.79		

** = Significant at 1% level.

To test the specific effect of modes on the total motility test of significance was calculated in both the seasons as detailed in table 4 : 42 .

TABLE 4 : 37

Showing the averages, standard errors and coefficient of variation for the live sperm percentage in the buffalo- semen in summer for control and treatment groups :

Treatment		Hours of preservation								
		0 hr	12 hr	24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96 hr
Control	$\bar{x}$	83.5	78.0	74.4	71.0	66.3	60.4	57.3	51.8	48.5
	SE.	.01	.01	.03	.04	.06	.09	.16	.07	.60
	CV.	.29	.23	.65	.93	1.3	3.0	5.6	2.9	2.7
Jeep	$\bar{x}$	83.5	75.5	73.2	68.1	62.1	59.1	54.2	45.7	45.8
	SE.	.01	.01	.04	.03	.06	.06	.13	.06	.17
	CV.	.29	.15	.79	.76	1.9	2.3	4.8	2.9	8.3
Train	$\bar{x}$	83.5	76.4	72.7	69.3	63.8	59.4	54.9	46.7	43.2
	SE.	.01	.02	.03	.05	.07	.09	.10	.04	.04
	CV.	.29	.36	.65	1.3	2.1	3.2	4.1	2.1	1.7
Jeep	$\bar{x}$	83.5	76.4	71.6	67.5	62.8	58.6	53.2	47.8	43.6
	SE.	.01	.01	.04	.03	.06	.08	.14	.04	.06
	CV.	.29	.29	.99	.86	2.0	2.9	5.5	2.1	3.2

n = 9

TABLE 4 : 38

Showing the means, standard errors and coefficients of variation for live sperm percentage in the buffalo-semen in rainy season for control and treatment groups.

Treatment		Hour of preservation								
		0 hr	12 hr	24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96 hr
Control	$\bar{x}$	84.5	80.7	76.6	71.8	68.9	65.2	61.0	58.6	49.5
	SE.	.001	.01	.02	.02	.04	.05	.05	.07	.05
	CV.	.01	.21	.54	.52	.96	1.5	1.8	2.5	2.2
Jeep	$\bar{x}$	84.5	77.8	72.6	67.1	64.5	63.5	57.9	54.8	45.7
	SE.	.001	.01	.02	.49	.06	.06	.06	.06	.05
	CV.	.01	.30	.40	13.8	2.0	2.0	2.1	2.4	2.4
Train	$\bar{x}$	84.5	78.4	74.1	69.9	66.8	62.8	59.2	54.4	46.7
	SE.	.001	.09	.01	.04	.05	.04	.06	.12	.07
	CV.	.01	2.1	.30	1.2	1.3	1.3	2.1	5.7	3.2
Cycle	$\bar{x}$	84.5	77.3	70.9	67.0	62.8	59.6	56.0	49.9	44.1
	SE.	.001	.01	.01	.04	.04	.02	.03	.05	.03
	CV.	.01	.08	.15	.99	1.2	.75	1.1	2.1	1.5

n = 9

TABLE 4 : 39

Analysis of variance showing the effects of seasons on the live spermatozoa of buffalo semen :

Sources of variation	D.F.	S.S.	M.S.	F.
Between seasons	1	268.22	268.22	3.97 *
Within season	646	43595.43	67.48	
Total	647	43863.65		

* = Significant at 5 % level.

TABLE 4 : 40

Analysis of variance showing the effects of modes of transport on the live spermatozoa of buffalo-semen.

Sources of variation	D.F.	S.S.	M.S.	F.
Between modes	3	456.12	152.03	2.25
Within modes	644	43407.53	67.40	N.S.
Total	647	43863.65		

N.S. = Non significant.

TABLE 4 : 41

Analysis of variance showing the effects of hours of preservation on the live spermatozoa of buffalo semen:

Sources of variation	D.F.	S.S.	M.S.	F.
Between hours	8	33788.83	4223.60	267.99 **
Within hours	639	10074.82	15.76	
Total	647	43863.65		

** = Significant at 1% level.

TABLE 4 : 42

Showing the significance or otherwise of the group means comparision for total motility in buffalo semen for both the seasons :

Comparisdn	Difference	
	Summer	Rainy
Control - Jeep	0.1	3.3
Control - Train	0.2	0.1
Control - Cycle	3.2 **	5.8 **
Jeep - Train	0.3	3.2
Jeep - Cycle	3.3**	2.5
Train - Cycle	3.0 **	5.7 **
C.D. at 1% level	2.81	4.91
C.D. at 5 5% level	2.14	3.74

TABLE 4 : 43

Showing the average percentage of the three characters in cow and buffalo bull in both the season:

		SUMMER			RAINY		
		Progressive motility	Total motility	Live spermatozoa	Progressive motility	Total motility	Live spermatozoa
Bull semen	Control	21.5 ±.04	45.1 ±.04	70.0 ±.01	13.7 ±.06	62.6 ±.04	72.8 ±.01
	Jeep	19.1 ±.04	42.2 ±.04	66.7 ±.02	11.3 ±.07	53.4 ±.06	70.2 ±.01
	Train	17.2 ±.02	40.0 ±.04	68.1 ±.01	13.2 ±.07	53.8 ±.05	70.9 ±.01
	Cycle	15.5 ±.05	38.2 ±.05	67.1 ±.02	13.1 ±.07	50.9 ±.05	69.6 ±.01
	Average	18.3	41.4	68.2	12.8	55.2	70.9
Buffalo Semen.	Control	23.1 ±.04	35.1 ±.04	66.0 ±.03	19.7 ±.07	42.2 ±.05	68.6 ±.04
	Jeep	23.1 ±.04	35.2 ±.03	63.3 ±.03	15.8 ±.08	36.6 ±.04	65.8 ±.03
	Train	22.7 ±.04	34.8 ±.04	63.8 ±.04	18.0 ±.06	42.0 ±.05	66.8 ±.03
	Cycle	20.6 ±.04	29.9 ±.04	63.1 ±.03	13.8 ±.07	32.4 ±.05	63.8 ±.02
	Average	22.4	33.7	64.0	16.8	38.3	66.2

TABLE 4 : 44

Showing the averages with standard errors and of progressive motility of cow and buffalo bull semen in thermosflask (control group only) at dirrerent hours of preservation (interval) :

		0 hr	12 hr	24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96 hr
Bull semen	Summer	54.9 ±.06	33.4 ±.07	27.0 ±.07	28.4 ±.05	21.6 ±.06	15.3 ±.06	12.4 ±.16	9.8 ±.03	4.5 ±.11
	Rainy	56.2 ±.15	34.4 ±.23	26.3 ±.12	13.0 ±.15	10.0 ±.17	5.7 ±.10	4.6 ±.23	1.7 ±.11	1.6 ±.08
Buffalo semen	Summer	58.5 ±.05	37.8 ±.07	29.2 ±.38	22.8 ±.11	19.5 ±.14	21.3 ±.20	13.1 ±.20	16.3 ±.29	8.4 ±.20
	Rainy	51.1 ±.04	42.6 ±.21	29.5 ±.17	19.1 ±.17	18.6 ±.18	15.2 ±.31	16.2 ±.38	3.6 ±.35	2.4 ±.21

TABLE 4 : 45

Showing the averages with their standard error for total motility of cow and buffalo bull semen in thermosflask (Control group only) at different hours of preservation (intervals) :

		0 hr	12 hr	24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96 hr
Bull semen	Summer	70.0 ±.24	50.8 ±.10	49.2 ±.08	52.0 ±.19	56.0 ±.18	38.6 ±.15	38.9 ±.23	32.1 ±.05	19.8 ±.28
	Rainy	80.5 ±.21	68.5 ±.24	69.4 ±.01	67.3 ±.23	66.5 ±.26	62.5 ±.19	58.9 ±.17	57.9 ±.15	39.1 ±.53
Buffalo semen	Summer	52.3 ±.15	46.7 ±.05	40.7 ±.23	34.1 ±.09	34.4 ±.05	36.4 ±.02	28.9 ±.19	28.7 ±.23	16.8 ±.32
	Rainy	63.0 ±.47	64.8 ±.12	54.6 ±.46	43.8 ±.13	49.2 ±.08	36.6 ±.26	26.1 ±.29	23.4 ±.27	22.8 ±.17

TABLE 4 :46

Showing the averages with standard errors for the percentage of live spermatozoa of cow and buffalo-bull semen in thermosflask (control group only) at different hours of preservation (intervals) :

		0hr	12 hr	24 hr	36 hr	48 hr	60 hr	72 hr	84 hr	96 hr
Bull semen	Summer	85.3 ±.01	81.4 ±.01	77.1 ±.01	73.0 ±.03	69.4 ±.02	66.9 ±.02	62.9 ±.02	59.7 ±.02	58.0 ±.02
	Rainy	84.0 ±.01	81.5 ±.01	77.4 ±.01	76.1 ±.01	72.9 ±.01	70.2 ±.01	66.1 ±.02	63.0 ±.01	59.6 ±.01
Buffalo semen	Summer	83.5 ±.01	78.0 ±.01	74.4 ±.03	71.0 ±.04	66.3 ±.06	60.4 ±.16	57.3 ±.09	51.8 ±.07	48.5 ±.06
	Rainy	84.5 ±.001	80.7 ±.01	76.6 ±.02	71.8 ±.02	68.9 ±.04	65.2 ±.05	61.0 ±.05	58.6 ±.07	59.5 ±.05

TABLE 4 : 47

Showing the decline in various characters after 96 hours of preservation at room temperature in thermos-flask (control) for bull- semen in summer and rainy seasons :

Character	Summer			Rainy		
	values at 0 hr	96 hr	Difference	Values at 0 hr	96 hr	Difference
Progressive motility	54.9	4.5	50.4	56.2	1.6	54.6
Total motility	70.0	19.8	50.2	80.5	39.1	40.4
Percentage of live spermatozoa	85.3	58.0	27.3	84.0	59.6	24.4

TABLE 4 : 48

Showing the decline in various characters after 96 hour of preservations at room temperature in thermosflask (Control) for buffalo-semen in summer and rainy seaso

Name of Characters	Summer			Rainy		
	0 hr	96 hr	Diff.	0 hr	96 hr	Diff.
Progressive motility	48.5	8. 4	40.1	51.1	2.4	48.7
Total motility	52.3	16.8	35.5	63.0	22.8	40.2
Percentage live spermatozoa	83.5	48.5	35.0	84.5	49.5	35.0

TABLE 4 : 49

A condensed table showing the significance or otherwise of the effects of various factors on percentage of progressively motile, total motile and live spermatozoa of the bull and buffalo-bull semen :

Sources of variation, characters etc.	BULL			BUFFALO		
	Significant	Non-Significant.	Remark	Significant	Non-significant.	Remarks.
Progressive motility						
Between season	**		Both seasons combined	**		Both seasons combined
Between modes	*	n.s.	Summer Rainy		n.s.	-do-
Between hours	**		Rainy	**		-do-
	**		Summer			
Between bulls	**		Both seasons combined			Not done
Between collection	**		-do-			
Total motility						
Between seasons	**		-do-	**		Both seasons combined.
Between modes		n.s.	summer	**		-do-
-do-	**		Rainy	*		Summer
-do-				**		Rainy
Between hours	**		Summer & rainy combined	**		Both seasons combined.
Between bulls	**		-do-			Not done
Between collection	**					
Percent live spermatozoa						
Between seasons	**		combined	*		combined data
Between mode	*		Summer		n.s.	-do-
-do-		n.s.	Rainy			
Between hours	**		Summer & rainy combined	**		summer & rainy
Between bulls	**					
Between collection	**		data			not done

* = Significant at 5% level. ** = Significant at 1% level
n.s. = Non significant. Combined = Data of rainy & summer seasons analysed together.

TABLE 4 : 50

Showing the records on temperature, humidity and rainfall during the period of this study :

Season	Months	Maximum temperature		Minimum temperature		Relative humidity (%)	Rainfall (inches)
		Ambient	Room	Ambient	Room		
Summer							
	May	38.8°c	36.4°c	25.1°c	34.4°c	64.3	.32"
	June	38.7°c	36.8°c	27.2°c	34. 8°c	70.0	3.78"
	Average	38.7	36.6	26.1	34.6	67.1	2.05
Rainy							
	July	34.7°c	33.8°c	26.7°c	33.1°c	85.3	2.59"
	August	32.5°c	33.3°c	25.5°c	32.8°c	87.1	9.12"
	Average	33.6	33.5	26.1	32.9	86.2	5.85

S E C T I O N - IV

APPENDIX

Results and their interpretations.

S E C T I O N - IV

RESULTS AND THEIR INTERPRETATIONS.

In bull semen, kept in thermosflask at room temperature, the average percentage of progressively motile spermatozoa was higher (18.3 %) in summer than in rainy season (12.8 %, Table 4 : 43). But the average percentage of total motile spermatozoa in rainy season was considerably higher (55.2 %) in comparision to that of summer (41.4%). Though the average percentage of live spermatozoa was higher in rainy season (70.9), the difference was not much marked than that of summer (68.2%).

In buffalo semen also the percentage of progressively motile spermatozoa was considerably higher in summer (22.4) as compared to its corresponding value in rainy season (16.8), but the average total motility percentage here also was found to have a higher value in rainy season (38.3) in comparision to its corresponding value in summer (33.7). Here also the average value of live sperm percentage was though higher in rainy season (66.2) than that of summer (64.0), the difference was not much marked.

These values indicate that the semen characteristics of both the species follow the similar trend with respect to the effects of summer and rainy seasons.

In summer, a decline of 50.4,50.2 and 27.3 in percentage was observed after 96 hours of preservation for progressive motility, total motility and live sperm content respectively in respect of bull semen kept in thermosflask at room temperature (table 4 : 47).

From the table 4 : 48 it is clear that a decline of 40.1, 35.5 and 35.0 percent in progressive motility, total motility and live sperm percentage respectively was caused by 96 hours' preservation of buffalo semen in thermosflask at room temperature in summer season. Corresponding values in rainy season were 48.7, 40.2 and 35.0 % respectively.

The average percentage of various types of spermatozoa at 0 hour in bull and buffalo bull semen respectively in summer and rainy season has been obtained in table 4 : 21. It was observed from the above table that at 0 hour, the percentage of live non- motile spermatozoa was minimum (3.5) in bull semen in rainy season and maximum in buffalo semen in summer season (31 : 2).

A comparision of all the seminal characters, studied in this experiment for bull and buffalo semen samples kept in thermosflask at room temperature has been made in tables 4 : 44, 4: 45

and 4 : 46. It is clear from the above table that in thermosflask the total motility of bull semen is better maintained till 96 hours of preservation in both rainy and summer seasons than that of buffalo semen.

The characteristic progressive motility of spermatozoa is now regarded to be not solely responsible for fertilisation. The transport of spermatozoa from cervix to the fallopian tube has been reported to be mainly dub to the rhythmic muscular contraction of wall of the uterus and the currents produced by the cillia, lining the fallopian tubes (cited by Bhattacharya 1958). Total motility and percentage of live spermatozoa can be taken as guide line for the semen q quality (Lasley(1951) observed that percentage of motile spermatozoa was significantly correlated (r = 0.314) with the fertilising capacity. Taking these two characters as basis, it was found that the semen of bulls was better in rainy than that of summer. This observation is corroborated by the findings of kodagali(1962) who found that the semen obtained in the cold season was of highest quality, followed by wet and hot seasons. Seasonal variation in the semen quality has also been reported by Mukherjee and Bhatacharya (1952), Anderson (1945), Swanson et. al.(1945), Erb et al . (1944) and Brown (1959).

As regards seasonal variation, the findings of this study differ with those earlier reported by Phillips et al (1943), Mercier and Salisbury (1946) and Tripathi (1965), who found no seasonal variation in intial motility of bull semen. Sharma and Saha (1962) and Patric et al (1959) also did not find any seasonal variation in semen quality of bull .

It is observed that sensonalnveriation exists in the buffalo semen also, when total motility and live sperm content are considered as basis of semen quality. Semen in rainy season is of better quality than that of summer. These findings are in agreement with those earlier reported by Sengupta et al. (1963) who observed that the semen of buffalo bull was worst in summer and considerably imporved with the onset of rainy season. Kushwaha et al.(1955) also observed seasonal variation in the semen quality, (initial motility being lower in summer than that of rainy). Mishra et al. (1965) also reported a seasonal variation in semen- quality, being very poor in summer. Poor semen quality in summer could possibly be due to :

(i) Physical stress resulting from excessive heat,
(ii) Poorly developed heat- controlling centre of buffaloes (Kaleff 1962),

(iii) Black colour of the buffalo skin .

This stress resulted in the lowest percentage of buffalo bulls donating semen in summer (47.3 % Singh et al. 1958). The quality of buffalo semen improved considerably with the onset of rains , Mishra et al. (1965). Along with the improvement in the semen quality , the percentage of buffalo bulls capable of donating semen also increased (61.6 % Singh et al. 1958). Baširov (1960) has also reported that increased heat (45°c to 50°c) resulted in the inhibition of libido and ejaculation in the buffalo- bulls. These findings conform with the results obtained in this study.

Though, the effect of different modes of transport with their respective distances,used in this experiment were significant in comparision with control (table 4 : 20), no significant difference existed between the means of two modes in either season for any character in bull semen, when control as a mode was excluded. The sample that travelled least distance (18 miles) i.e. by cycle, showed significant difference with control mean for 3 character, 2 in summer that is progressive motility and live sperm percentage , and one in rainy i.e. total motility , taking both seasons into consideration. This means that though the sample

has travelled least distance, still it showed the lowest quality of semen for all the three characters, indicating that at this particular distance, the semen travelled by cycle is of the same quality, if not inferior, but never superior to the samples travelled by jeep and train to a distance of 32 and 38 miles respectively. This indicated that out of all the four modes used, cycle has the maximum deletarious effect. (table 4 : 20) .

In the case of buffalo semen, significant difference between means of control and cycle were found in both the seasons where as a significant difference was found between jeep and cycle means in summer season only.

In the case of sample transported by cycle, the explanation for its poor quality may be that there were more bumps and jerk than that of train or jeep. The effects of jerk was direct on the thermosflask in cycle-samples. But the intensity of jerk was considerably minimised on thermosflask in the case of jeep because in this case 4 to 5 thermos-flasks were kept in a wooden box. The results obtained here are in conformity with those of Lysak (1965) who reported that sperm survival was greater after transport over a smooth road in vehicle fitted with shock- absorber than over a rough road by a moter cycle.

SUMMARY

A study of keeping quality of cow and buffalo bull semen in thermosflask, at room temperature under laboratory and field conditions was made in summer and rainy seasons till 96 hours of preservation. For despatching semen to field stations , 3 modes of transport (viz train, jeep and cycle) for variable distances were employed. The semen samples were then brought to the laboratory and examined at regular intervals for their quality, which was evaluated on the basis of the following characters :

(i) Percentage of progressively motile spermatozoa.

(ii) Percentage of total motile spermatozoa, and

(iii) Percentage of live spermatozoa.

Reliable informations on keeping quality of cow- bull and buffalo bull semen using the usual modes of transport under field conditions in the tropics are virtually lacking and hence this study.

It was observed that bull semen was better maintained in thermosflask in rainy season than in summer. Buffalo semen quality was also better in rainy than that of summer season.

In both the seasons, the quality and

keeping quality of bull semen was better than that of buffalo semen.

Semen from cow and buffalo bull was found to follow a semilar trend for the three characters studied in rainy and summer seasons, for example , in both the species, the average percentage of progressively motile spermatozoa was found to be higher in summer whereas the average percentage of total motile and live spermatozoa was found higher in rainy than in summer.

Significant seasonal variations were recorded in all the seminal characters in both the species.

Seasons, bulls, collections, hours of preservation and their interactions had significant effects on the three seminal characters of bull semen. In case of buffalo - bull semen, seasons and hours of preservation were found to affect significantly all the three seminal characters studied.

So far as semen from cow- bull is concerned , the three modes of transport (cycle, train and jeep) did not differ significantly in respect of their effects on all the three seminal characters in both the seasons.

In case of buffalo bull semen, these three modes of transport did not differ significantly in their effects on the seminal characters , except on total motility, on which the modes differed significantly in their effects.

Out of these three modes of transport, the semen sample sent by cycle was found worst in both the species for its quality, although the distance travelled by this mode was the shortest.

Following informations can profitably be utilised in the practice of Artificial Insemination under field conditions :

Since considerable variation among bulls for the fitness of their semen quality for transport has been found to exist in this work, supported by the findings of Herman and Swanson (1944), it would be advisible to isolate superior bulls, whose semen could be used for long range transhipment.

Since deterioration in semen quality was found to be maximum when semen was transported by cycle (although the distance travelled by this mode was the shortest), it would be advisible to provide some sort of shock- absorber,when semen is being transported by cycle, because semen so transported is likely to have maximum jerk effect as is also corroborated by the work of Lysak (1965).

As the quality and keeping quality of semen was found to be inferior in summer, efforts should be made to protect bulls as far as practicable from the rigors of heat and strong wind of summer , in view of informations that these factors affect semen quality adversely.

Although, the informations collected and inferences drawn there from, in this study, are expected to be useful in planning the transport of semen under field condition using the usual modes of transport, further studies on these lines in all the seasons, using different tests of semen evaluation for uniform and variable distances on the basis of larger number of observations, would be necessary to obtain adequate informations on this problem.

REFERENCES CITED

Aamdal, J. 1952 .

A.B.A., 21,No 716 cited from Maule, J.P. The semen of animal and artificial insemination." Commenwealth Agricultural Bureaux. Farnham, Royal, Bucks; England.

Adler, H.C. 1960. A.B.A; 30,(2) : 191

Anderson, J. 1945 . J. agric. Sci., 35, 184-196 .

Aslanjan, M.M. 1952. 21 , (2) : 148 (Abs. 718).

Ayyar, R.V. 1944. Ind. Vet. Jour. 20 ,:253-260.

Basirov, E.B. 1960. A.B.A., 28 , Abs. 1294.

Bhattacharya, P. and Prabhu, S.S. 1953. A.B.A., 21,(4) Abs. 1701.

Bhattacharya, P. 1958. Porc. Indi. Sci congress, 45th session 138.

Bonadonna. T. 1949. XII Inter. Dairy Congr. 5; 195-198.

Bonadonna, T. 1951. A.B.A., 20 , (3) : 233. Abs.1089.

Brown, M.A. 1959 . A.B.A., 28 : 399.

Burgess, T.D. 1953. Canad. J. agric. sci.33 : 396- 398.

Casady, R.B., Myers, R.M., and Legates, J.E. 1953. J. Dairy Sci., 36 : 14 - 23.

Chang, M.C. and Walton, A. 1940.

Cited by Maule J.P. 1962. " The semen of animals and artificial insemination" Commenwealth Agricultural Bureaux Farnham Royal, Bucks ; England.

Chaudhari, S.C. 1963. A.B.A.,31,(3) Abs. 1797.

Campbell, R.C. 1953. J. agric. Sci., 43, 256-259.

De Groot, T. and Bekedam, M.1957. A.B.A., 26 (1) Abs.201

Dharampal, 1961. Ind. Vet. Jour. 38 (2) :94-99.

Dunn, H.O. and Welker, R.M.1957. J. Dairy. Sci.,40 : 588.

Dzilinski, J.1958. A.B.A., 26 Abs. 1937.

Edwards, J., Walton, A. and Siebenga, J. 1938.
J.agric. Sci. 28 : 503.

Erb, R.E., Andrews, F.N. and Hilton, J.H. 1942.
J. Dairy Sci., 25 : 815-826.

Erb, R.E., Wilber, J.W. and Hilton, J.H. 1940.
J. Dairy Sci., 23 : 549.

Fryer, H.C., Marion, G.B., and Farmer, E.L.1958.
J. Dairy Sci., 41 : 987-993.

Gokhale, D.R. 1958. Ind. Vet. Jour. 35 : 573-581.

Gosamvardhan Seminar - 1963.

Herman, H.A. and Swanson, Eric. 1944. J. Dairy Sci.27 :662.

Hewetson, R.W. 1955. A.B.A., 24 : Abs 166

Horie, T. and Ishikura, F.1964. A.B.A., 33 ,(4):566.

Hronopulo, N.P.1940. A.B.A., 13 , (1) : 22.

Hudopisk, A.1948. A.B.A., 19 , (3) :340.

Jaiskowski, L. and Romaniuk, J. 1957. A.B.A., 26 , (3) : 281.Abs.1343.

Johnston, J.E. and Branton, C. 1953. J.Dairy Sci.,36 ; 934- 942.

Jurgens, J.1951. A.B.A.,21,(2) : 150 Abs. 730.

Kaleff, B.1942. A.B.A., 10 : 148.

Kampschmidt, R.F., Mayer, D.T. and Herman, H.A. 1953 b. A.B.A., 22 , Abs. 147.

Kampschmidt, R.F., Mayer, D.T. , Herman, H.A. and Dickerson, G.E. 1951. J. Dairy Sci. 34 : 45- 51.

Kelly, J.W. and Hurst, V.1963. J. Am. Vet. Med. Ass. 143 : 40- 43.

Kodagali, S.B. 1962. Ind. Vet. Jour., 39 : 593-599.

Kodagali, S.B. 1963. Ind. Jour. Vet. Sci.& A.H. 33 : 26-28.

Kouser, M. 1965. M. Sc. thesis , Magadh University.

Kumar,B.B. 1965 . M.Sc. thesis, Agra University.

Kushwaha, N.S., Mukherji, D.P. and Bhattacharya, P. 1955. Ind. Jour. Vet. Sci. & A.H. 25 : 317 -328.

Lambert, W.W. and Mckenzie, Fred. 1940.
Cited from Prabhu, et al.1953. Ind. Jour. Vet. Sci. & A.H.22 , (4) : 289-281.

Lardy, H.A. and Phillips, P.H. 1939. Proc. Amer. Soc.Anim. Prod. 32 nd. Ann. Meet, 219-221.

Lasley, J.F. 1944. J. Anim. Sci., 3 : 43.

Lasley, J.F. 1951. J. Anim. Sci., 10 : 211-218.

Lasley, J.F. and Bogart, R. 1943.

Cited from Lasley, J.F. 1951. J.Anim.Sci., 10 : 211-218.

Latard, E., Szumowski, P. and Arruti, F. 1949. A.B.A.,19, (1) : 66- 67 Abs. 68.

Lysak, L.A. 1965. A.B.A. 33 , (4) : 567. Abs. 3378.

Mahajan, S.C. 1960. M.V.Sc. thesis , Agra University.

Mahajan, S.C. and Sharma U.D. 1960.

Cited from Prabhu,S.S., 1966. " A note submitted to the 1966 achievement audit committee."

Malkani, M. 1954.

Cited from Maule, J.P. 1962. " The semen of animals and artificial insemination." Commenwealth Agricultural Bureaux Farnham Royal Bucks ; England.

Mann, T. 1954.

Cited from Maule, J.P. 1962."The semen of animals and artificial insemination."

Maule, J.P. 1962.

" The semen of animals and artificial insemination". Commenwealth Agricultural Bureaux Farnham Royal Bucks ; England.

Melrose, D.R. 1952 b. A.B.A., 20 Abs 1645.

Mercier, E. and Salisbury, G.W.1946. Cornell. Vet. 36 : 301-311.

Milovanov. 1932.

Cited from Prabhu, et al 1953. Ind. J. Vet. Sci; & A.H. 23 , (4) : 279-281.

Mishra, M.S. and Sengupta, B.P. 1965. Ind. Jour. Dairy Sci., 18,(4) : 130.

Milicevic, D. 1965. A.B.A., 33 , (2) : 201 Abs. 1207.

Morozov, V.A., Dupenko, O.A., and Lavro, O.B. 1961. A.B.A. 30 , (2) : 154 Abs. 773.

Mukherji, D.P. and Bhattacharya, P. 1947. Proc. Ind. Sci. Congress, 34th session.

Nishikawa, Y. 1964 A.B.A. 33 , (1) Abs. 41.

Patrick, T.E., Kellgren, H.C., Johnston, P.E., Hindery, C.A., Shelwick, J.O. and Bankston, J.1959. J. Dairy Sci., 42 : 394.

Phillips, P.H. and Lardy, H.H. 1940. J. Dairy Sci., 23: 399-404.

Phillips, R.W., Knapp, B., Heemstra, L.C. and Eaton, O.N. 1943. Cited from Tripathi, V.N. 1965. M.Sc. thesis, Agra University.

Prabhu, S.S. 1966. A note submitted to the 1966 Achievement audit committee.

Prabhu, S.S. and Bhattacharya, P. 1953. Ind. Jour. Vet. Sci., & A.H. 23, (2) : 129-133.

Prabhu, S.S.,Bhaya, K.D., and Bhattacharya, P.1953. Ind. Jour. Vet. Sci., & A.H. 23 ,(4): 279- 281.

Prince, P.W. and Almquist, J.O. 1948. J.Dairy.Sci., 31: 839-844.

Rao, D.M. 1966. M.Sc. thesis , Magadh University.

Report on Livestock census of Bihar, 1961.

Rottensten, K. 1953 II. A.B.A., 21,(1): 52 Abs. 236.

Rottensten, K. and Andersen, H. 1956. A.B.A., 25 Abs.159.

Roussel, G.1954. A.B.A., 23 , Abs. 183.

Sakala, J.and Turecer, K. 1957. A.B.A., 26, (3) : 283 Abs. 1354.

Salisbury, G.W., Fuller, H.K. and Willett, E.L. 1941. J. Dairy Sci., 24 : 905-910.

Saxena, V.B. 1965. M.Sc. thesis , Agra University.

Schindler, H. 1954. A.B.A., 23,(2) : 153 Abs. 679.

Schmidt, K. and Kroll, H.1953. A.B.A., (4): 357 Abs.1731.

Schultze, A.B.,Davis, H.P., Blunn, C.T. and Olufa, M.M. 1948. A.B.A., 17, Abs. 1336.

Sengupta, B.P., Mishra, M.S. and Roy , A. 1963. Ind. Jour. Dairy Sci; 16 : 150- 155.

Sharma, S.C. and Saha, S.K. 1962. Ind. Jour. Vet. Sci; & A.H. 32 : 50-53.

Shrivastava, P.N. and Prabhu,S.S.1956. Curr. Sci; 25 ; 58- 59.

Singh, R.B. and Hussain, K.Q. 1958. Proc. Ind. Sci. Congress 45th session : 387.

Smirnov - Egerjumov. Citation from Prince et al.1948.

Smith, A.V. and Polge, C. 1950. Vet. Rec., 62 : 115-116.

Snedecor, G.W. 1957.

" Statistical methods " The Iowa State College Press, Ames, Iowa.

Stewart, D.L. 1950. Vet. Rec. 62 : 389-395.

Swanson, E.W. and Bearden, H.J.1951. J. Anim. Sci., 10 : 981 - 987.

Swanson, E.W. and Herman, H.A. 1944. J. Dairy Sci., 27 : 303-310.

Tomer, N.S. and Desai, R.N. 1961. Ind. Vet. Jour. 38 : 89- 94.

Tomer, N.S. and Desai, R.N. 1961. Ind. Vet. Jour. 38 : 311 - 317.

Tripathi, V.N. 1965. M.Sc. thesis Agra University.

Van Demark, N. L. and Sharma, U.D. 1957. J. Dairy Sci., 40 : 438-439.

Walton, A. 1930. Brit. J. Exp. Biol. 7 : 201- 219.

Willette, E.L. 1953. J. Dairy Sci. 36 : 1182- 1185.

Wolf . 1921. J.Agric. Sci.,11 : 310- 322.
