STUDIES ON
THE INCIDENCE AND TREATMENT OF REPEAT BREEDING COWS

Thesis

Submitted to the Faculty of Veterinary Science
RAJENDRA AGRICULTURAL UNIVERSITY
in partial fulfilment of the requirements
for award of the degree of
MASTER OF SCIENCE (VETERINARY)
IN
GYNAECOLOGY AND OBSTETRICS

By
Mohammad Shoaib
B. V. Sc. & A. H. (R. A. U.)
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Post Graduate Department of Gynaecology and Obstetrics
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In the
memory of my
Late revered mother

Dr. B.K. Singh,

B.V.Sc.& A.H.(Hons., Gold Medalist),
M.V.Sc. (Madras), P.G.(Vienna),
Dr.Med.Vet.(Vienna),
Professor and Chairman,
Department of Gynaecology & Obstetrics,
Bihar Veterinary College,Patna.

P A T N A

Dated, the 28th Aug. 1975.

This is to certify that the work embodied in this Thesis entitled " STUDIES ON THE INCIDENCE AND TREATMENT OF REPEAT BREEDING COWS" is the bonafide work of Shri Mohammad Shoaib and was carried out under my guidance and supervision.

B K Singh

(B. K. SINGH)

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CERTIFICATE

Certified that the research work incorporated in this Thesis have not been published in part or in full in any other journal.

M. Shoaib

(Mohammad Shoaib)

Acknowledgement

With deep sense of gratitude I wish to owe my indebtedness to Dr. B.K.Singh, Professor and Chairman, Department of Gynaecology and Obstetrics, Bihar Veterinary College,Patna for his inspiring guidance, constant supervision and encouragement during the course of this study.

Sincere thanks are due to Dr.S.C.Biswas, Assistant Professor (Statistics) for the analysis of data and statistical interpretation of results.

Sincere thanks are also due to Dr. T.Prasad, Assistant Professor, Biochemistry and Dr. M. Murtaza, Lecturer in Biochemistry, Bihar Veterinary College, Patna for their valuable suggestions and help in the biochemical studies.

The author is also thankful to Dr. S.A.Ansari, Professor of Bacteriology, Central Disease Investigation and Livestock Production Centre,Bihar,Patna for his valuable help in the bacteriological examination of cervical mucus sample.

A word of thanks is also due to Sri A.R. Srivastava, Assistant Soil Chemist, Agricultural Research Institte, Patna and to Sri M. Akmal, Assistant Chemist, Agricultural Research Institute, Patna for their help and cooperation.

My sincere thanks are also due to Dr.S.M.Ishaque,

Ex-Principal and Dean, Veterinary and Animal Husbandry Faculty, Rajendra Agricultural University, Dr. R.N.Singh, Principal, Bihar Veterinary College, Patna, Dr. A.K.Roy, Professor and Chairman, Department of Physiology, Bihar Veterinary College, Patna, and Dr K.M.Prasad, Lecturer, Animal Husbandry, Ranchi Agriculture College, Dr. R.B.Singh, Assistant Lecturer, Gynaecology and Dr.M.H. Akhtar, Assistant Lecturer, Gynaecology, Bihar Veterinary College, Patna for their constant encouragement and help from time to time.

Thanks to all the staff of the Gynaecology and Obstetrics Department for their untiring help and cooperation during the period of study.

For the award of Junior Fellowship grateful thanks are also due to the Vice-Chancellor, Rajendra Agricultural University, Pusa, Samastipur.

Last but not the least I express my deep sense of gratitude and respect to my father and other family members for their constant help and encouragement for higher studies.

(Md. Shoaib)

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Introduction

The causes of infertility are numerous, varied and complicated, for so many factors are concerned in successful reproduction : the development of the Graafian follicle to maturity, the onset of heat, fertile coitus, the expulsion of the ovum from the follicle, its arrest by the fimbria and its meeting in the pavilion of the oviduct with a swarm of virile spermatozoa, its passage along the oviduct - there undergoing cleavage; the attachement of the blastocyst to the endometrium, the progressive development of the foetus with its membrane, and finally the expulsion of the full-term foetus with its membranes from the uterus. Should this mechanism break down at any point, perfect reproduction will fail and the animal become, if only temporarily, infertile (Arthur,1964).

Infertility among farm animals is a great economic problem which confront the veterinarians specially in a developing country like India. Though infertility occurs due to anatomical, infectious and functional factors, the latter alone causes great economic loss to the dairy industry. Deranged physiological function may lead to embryonic mortality which is a very important cause of "repeat breeding". "The repeat breeding cow is one that has normal or nearly normal estrous cycles and estrous periods and has been bred two or more times to a fertile bull yet failed

to conceive. The clinical examination of the animal may fail to reveal any definite lesion or condition to explain the failure of conception" (Roberts,1971).

Casida and his co-workers (1961) divided the causes of repeat breeding into two groups.

(i) Repeat breeding due to failure of fertilization,and

(ii)Repeat breeding due to early embryonic death.

According to Roberts (loc.cit.) repeat breeding has been classified on the basis of pathological and managerial causes as follows:

(i) Genetic or congenital anatomical defects of the genital tract,

(ii) Congenital, genetic or acquired defects of the ova, spermatozoa or early zygote,

(iii)Infectious or traumatic inflammatory process,

(iv) Endocrine disfunction and

(v) Managemental and nutritional deficiencies.

Hormonal disfunction at the time of nidation of the early embryo might interfere with its normal development. It is here that progesterone is likely to play an important role in maintaining pregnancy.

Embryonic death is attributed to be a direct cause of

lowered fertility and repeat breeding. Failure of the embryo to implant on account of insufficiency of progesterone has been reported to be one of the causes of this condition (Johnson et al.,1958).

Repeat breeding in cows is a prevalent disease and is a cause of great economic loss to the farmer. Therefore, it was decided to work on this problem and studies were made on :

(i) the incidence of repeat breeding,

(ii) the pH of cervical mucus of repeat breeding animals,

(iii) the level of sodium and potassium contents of the cervical mucus of repeat breeders,

(iv) bacteriological examination of the cervical mucus of repeat breeders, and

(v) the treatment of repeat breeding animals with Hostacyclin and progestin.

.....

Review of Literature

Incidence of repeat breeding:

Zemjanis (1963) reported the overall incidence of repeat breeding in cows to be 15.1 per cent. Lutke-Vestert(1964) reported the percentage of cows returning three times for insemination to be 18.1 for an insemination interval of 37-47 days.

In Hariana cows, Khan and Luktuke (1967) reported the percentage of repeat breeding to be variable during different periods, 27 per cent during 1955-57, 14.43 per cent during 1958-60 and 17.39 per cent during 1961-64.

The incidence of repeat breeding cows was reported to be 10 per cent in Sweden by Hewett (1968). Autumn incidence (11.4 %) was higher than in Spring (8.6 %). Repeat breeding incidence was lowest (5.2 %) in heifers that calved for the first time. In a year old or older cows it rose to 13.3 per cent.

Sinha (1971) reported the incidence of repeat breeding in Tharparkar cows to be 8.29 per cent and in nondescript cattle to be nearly 10 per cent. In Red Sindhi cattle Namboothiripad and Raja (1972) reported the incidence of repeat breeding to be from 5.5 to 33.3 per cent.

In Red Sindhi, Sahiwal, Tharparkar and cross bred

females, Bhosrekar (1973) reported repeat breeding to be more common in heifers than in cows and more common in Sahiwals than in other breeds and cross breds.

Hydrogen ion concentration (pH) of cervical mucus:

Herman and Horton (1948) reported mean pH of bovine cervical mucus in vivo to be 7.23 during oestrus and 7.25 to 7.39 during nonoestrus. Gupta et al.(1962) reported the average pH of cervical mucus to be 7.91 and 7.90 in normal and repeat breeding cows respectively.

Kultis (1963) correlated the hydrogen ion concentration of cervical mucus and fertility and observed that when the pH was between 6.84 to 6.94 or 7.67 to 8.30, the cows did not conceive. The conception percentage progressively decreased from 55.6 to 9.5 with corresponding increase in pH from 7.0 to 7.6.

Cseh (1963) examined 293 cows and found the pH of cervico-vaginal mucus to be ranging between 6.7 to 7.0. At that instant, the mucus was clear, and conception rate was between 35 to 82 per cent. But below the above pH, mucus contained hazy granules, pus or epithelial cells and conception rate was never above 40 per cent.

Hukeri (1965) reported average pH of cervical mucus of oestrus cows (Tharparkar) to be alkaline (8.35 $\pm$ 0.04).

Peterson (1965) examined 85 uterine lavage samples from repeat breeding, sterile and easy breeding cattle and reported the pH to be as follows:

Repeat breeder	6.75 ± 0.25
Sterile	6.78 ± 0.17
Easy breeder	6.70 ± 0.18

Pattabiraman et al.(1967) examined 58 healthy and regular breeding cows and reported the pH of cervical mucus to be 8.09 ± 1.32. El Naggar and Baksai-Horvath (1972) found the pH of the cervico-vaginal mucus of 51 repeat breeding cows to be 6.1 to 7.2. Akhtar (1973) examined the cervical mucus of 10 repeat breeding cows (Tharparkar) and reported the mean pH to be 7.73 ± 0.153.

Sodium and potassium content in the cervical mucus:

Gupta et al.(1962) reported that the average concentration of sodium and potassium in cervical mucus of repeat breeding cows was 315.8 mg./100 ml. and 75.6 mg./100 ml. respectively.

Guay (1966a - 1966b) reported the mean sodium and potassium levels in cervical mucus during the normal oestrous cycle to be 2.773 mg./gm. and 0.799 mg./gm. respectively.

Guay and Lamothe (1970) reported the mean level of sodium and potassium in the cervical mucus of normal cows to be 543.12 mg./ 100 ml. and 61.63 mg./ 100 ml. respectively whereas in the case of infertile ones with no obvious lesions the mean sodium level to be 494.13 mg./ 100 ml. and mean potassium level to be 77.41 mg./ 100 ml.

Reddy (1973) reported the mean concentration of sodium in cervical mucus of normal and repeat breeding cows to be 361.82 ± 11.8 mg./ 100 ml. and 300.75 ± 28.74 mg./ 100 ml. respectively whereas potassium to be 53.83 ± 4.32 mg./100 ml.and 59.80 ± 0.20 mg./ 100 ml. respectively.

Bacteriological examination of cervical mucus of repeat breeding animals:

Vlček (1969) on bacteriological examination of reproductive tract of 19 cases of repeat breeders reported that 16 per cent yielded pathogenic or facultative pathogenic bacteria.

Nunn (1970) on examination of genital tract of 133 cows with a history of infertility but with no clinical abnormality and a normal oestrous cycle, reported the presence of various organisms in 41 per cent of the cervical mucus samples, the predominent genera being saprophytic bacilli, Staphylococci (coagulase positive and negative) and Streptococci.

Studying with 80 repeat breeding cows, Hartigan et al. (1972) isolated bacteria (Streptococcus, Staphylococcus, micro-aerophilic Streptococcus only) from 27 cows (34%).

Bacteriological examination of cervical mucus sample from 106 repeat breeding bovines revealed presence of different species of bacteria in 86 animals (81.87%) and in 20 animals (18.13%), the cervical mucus samples were sterile (Krishna Murthy et al.,1974).

Treatment with progesterone:

Herrick (1953) reported 65 per cent conceptions in repeat breeding heifers when injected 500 mg. of progesterone at the time of service, while 20 per cent in nontreated controls.

Wiltbank et al.(1956) treated 36 repeat breeding cows with 50 mg. progesterone in oil subcutaneously per day beginning from 3rd day after insemination and continued until the time of slaughter. Sixteen repeat breeding cows (44.4%) had normal embryos when slaughtered at 34th day as compared with 12 (33.3%) in an equal number of uninjected control. Thirtyone additional repeat breeding cows were injected subcutaneously with 200 mg.of progesterone per day beginning from 3rd day after insemination and continued until the time of slaughter. Twelve repeat breeding cows

(38.7%) had normal embryos at 34th day as compared with 8 (25.8%) in 31 control animals.

Johnson et al.(1958) treated normal cows and heifers with various doses of progesterone. One group of cows was given intramuscular injection of 100 mg. progesterone in oil on the 2nd,3rd,4th,6th and 9th day after insemination. Second group was given a single dose of 500 mg. progesterone intramuscularly on the 2nd day after insemination, and the 3rd group was left as untreated control. In the combined treated groups the average conception rate was 72.2 per cent and of untreated control group was 53.9 per cent.

Jeganathan (1959) reported that 61 per cent of dairy cows with a history of infertility due to suspected early embryonic mortality were successfully treated with intramuscular injection of 25 to 100 mg. of progesterone (Progestin-organon laboratories) given between the 3rd and 5th day after mating.

Danieli (1967) reported higher conception rates in repeat breeder cows free of clinically detectable lesions of the genital tract following treatment with a single intramuscular injection of 125 mg. progesterone in oil 6 to 10 days after insemination. Of 45 treated cows 73.2 per cent conceived on first service and another 6.7 per cent after two inseminations. In other

63 treated cows 57.3 per cent and 11 per cent conceived after one and two inseminations, respectively, while 54 untreated controls had a conception rate of 20.3 per cent after several services.

Risley (1968) reported good results in relation to maintenance of pregnancy in repeat breeders when given 500 mg. repository progesterone every 10 days from day 10 to day 200.

Ustinov (1970) gave 100 mg. progesterone to 72 cows on the 3rd, 4th, 6th, 8th and 10th day after insemination and found the conception rate after first insemination to be 61.1 per cent. In an equal number of untreated control the conception rate was 40.3 per cent.

Treatment with antibiotics:

Lindley (1954) reported good results with intrauterine infusion of streptomycin sulphate 1 gm. and penicillin G sodium 2 to 5 lacs units dissolved in 50 ml. of sterile distilled water on second day following breeding.

Sacchi et al.(1958) reported 70 per cent conception rate by antibiotic douches with 10 ml. oxytetracycline (Terramycin) solution at insemination time or 24-72 hours later in 219 repeat breeding cows.

Jeganathan and Maheswaram (1961) treated 9 repeat breeding cows by intrauterine infusion with 100 mg. of oxytetracycline and 1,00,000 units of polymixin B sulphate, 15 minutes after insemination and found that 6 conceived.

Hjerpe (1961) found no difference in conception rate in tetracycline treated and nontreated repeat breeders (harbouring pathogens).

Khan and Luktuke (1967) treated 16 repeat breeding cows with intrauterine injection post service with penicillin 4 lacs I.U. and streptomycin 1 gm. dissolved in 40 ml. of distilled water; 8 repeat breeding cows were kept as control. No post service treatment was given in these animals. 69 per cent of the treated and 25 per cent of the nontreated cows conceived. Most of the treated animals conceived with a single treatment only but for some cows two or three treatments were required for conception.

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Materials and Methods

Experiments were conducted on nondescript cows and heifers brought for Gynaecological check up at the department of Gynaecology and Obstetrics, Bihar Veterinary College, Patna. In each case history of the animal was recorded in relation to age of the animal, number of calvings, number of infertile services and oestrous cycle whether regular or irregular. Then the animals were subjected to Gynaecological examinations. Only those animals were included in the study which were normal on clinical examination, had oestrous cycles of normal duration but had failed to conceive even after three inseminations. Those with short oestrous cycles or cystic ovaries were excluded from the experiment.

The experimental animals were divided into the following groups:

Group - A : 15 animals which were found to be positive for micro organisms on bacteriological examination. They were treated with Hostacyclin water soluble vet.

Group - B : 25 animals which were found to be negative for micro organisms on bacteriological examination. They were treated with Progestin.

Group - C : 10 animals which were kept as nontreated controls.

Technique of collection of cervical mucus:

The animals were properly secured in a service crate. Before collection, the vulva and the hind quarters of the animal were wiped with savlon solution and lastly the vulva was washed with sterile glass distilled water. Speculum alongwith covering was sterilized in an autoclave at 120°C under 15 pounds pressure for half an hour. The thick glass tube (having a length of 40 cm. and inner diameter 1 cm.), glass tubes, glass catheters were sterilized in a hot air oven for one hour at 160°C. The tail of the animal was held on to a side and a sterile metallic vaginal speculum was introduced into the vagina after separating the two vulvar lips. After locating the cervix the sterile thick glass tube was introduced in the cervix and the mucus was sucked orally through a sterilized rubber tube attached to one end of the glass tube. The mucus thus collected was transferred into sterile tubes for further investigation.

Determination of hydrogen-ion concentration (pH) in the cervical mucus:

pH was obtained with Philips pH meter having a single glass electrode. The instrument was stabilized and standardized before use. The electrode was dipped in buffer solution of pH 7

for accuracy and then the electrode bulb was washed with double glass distilled water, dried with filter paper and then dipped in the mucus. Care was taken to see that no air bubble remained in the bulb. The glass electrode when not in use, was always kept in vertical position dipped in glass distilled water.

Determination of sodium and potassium in cervical mucus:

The collected cervical mucus samples were preserved in refrigerator for further analysis. The mucus was diluted with double glass distilled water. Sodium and potassium were estimated by using 'Systronic' flame photometer.

0.1 ml. cervical mucus was mixed with 9.9 ml. of double glass distilled water to make 1:100 dilution of the cervical mucus. The dilution rate of cervical mucus was the same for both sodium and potassium.

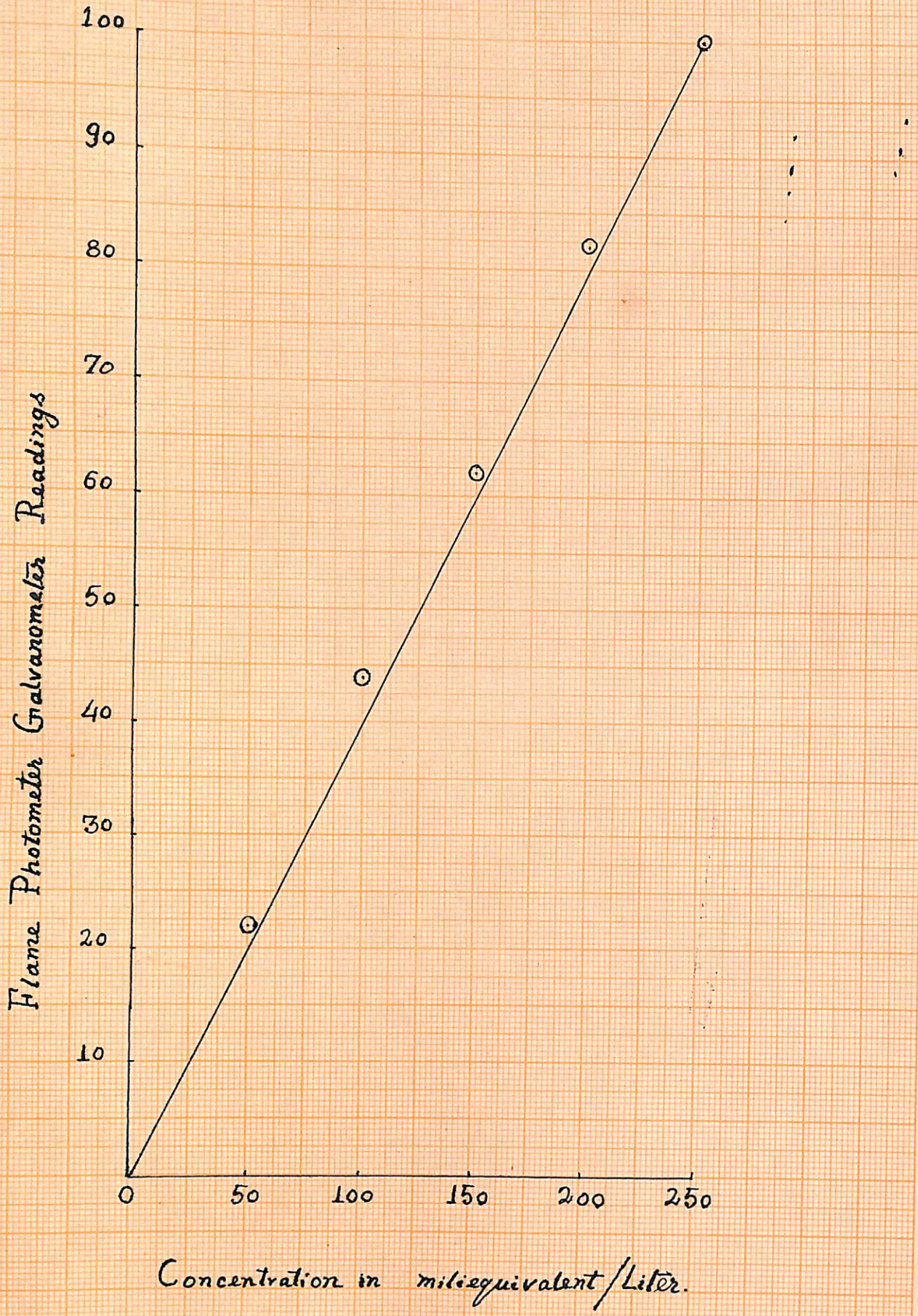
Sodium standards.- Standard solutions covering the range from 50 to 250 milliequivalent/liter of sodium at a dilution of 1:100 are usually adequate for calibration. Stock standard containing 100 milliequivalent of sodium per liter was prepared by dissolving 5.85 gms. of NaCl in glass distilled water and diluting to one liter. Working standards were prepared from this stock standard by measuring out 0.5, 1.0, 1.5, 2.0 and 2.5 ml.

aliquots into 100 ml. volumetric flasks and diluting to the mark with glass distilled water. These standards represent respectively 50, 100, 150, 200 and 250 milliequivalents of sodium per liter at 1:100 dilution. From these working standards a calibrated curve was prepared by plotting Flame Photometer Galvanometer readings against milliequivalent sodium content in milliequivalent per liter.

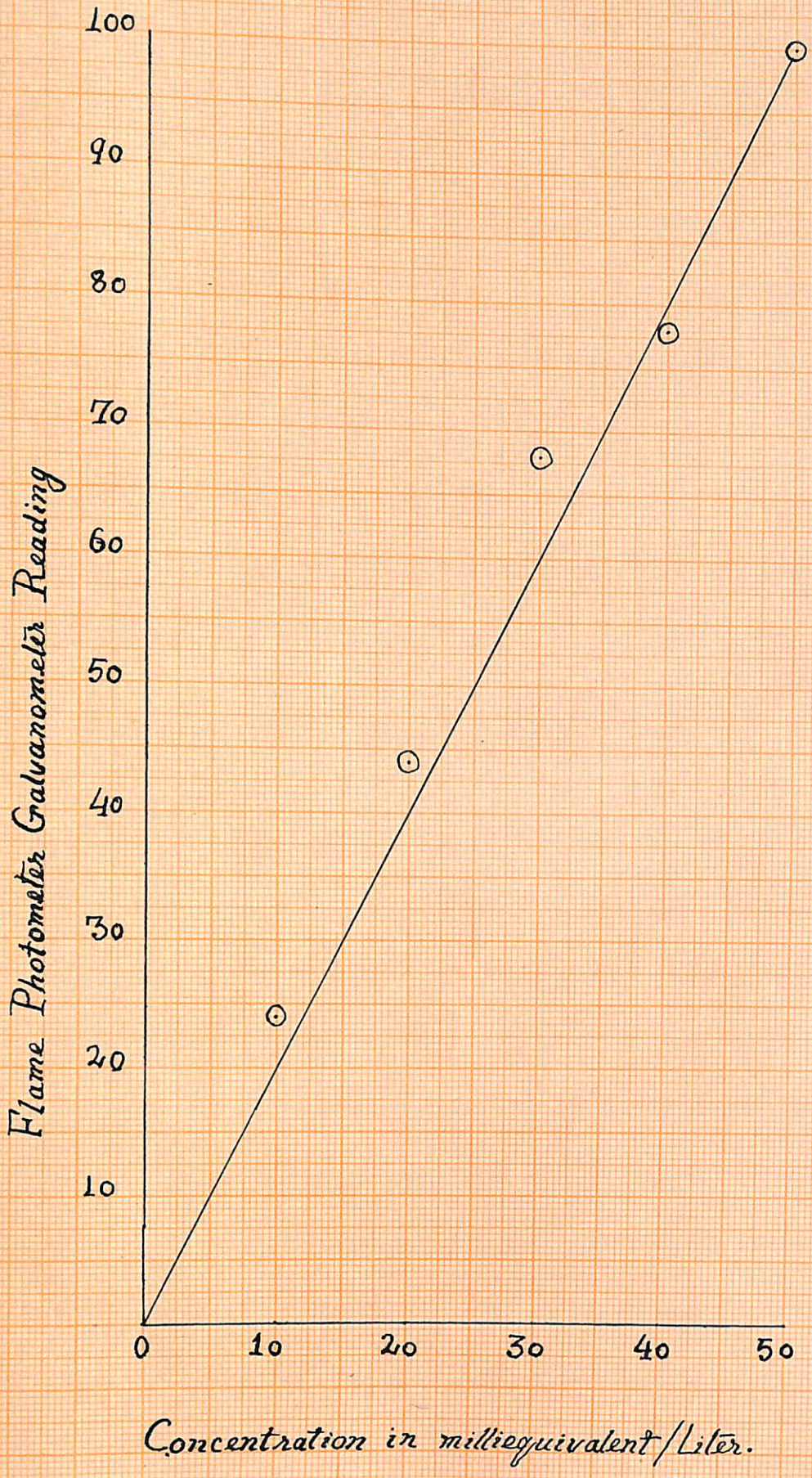
Potassium standards.- Standard solutions covering a range from 10 to 50 milliequivalent of potassium per liter, at a dilution of 1: 100 are usually adequate for calibration. Stock standard containing 10 milliequivalent of potassium per liter was prepared by dissolving 0.746 gms. of Kcl in glass distilled water and diluting to one liter. Working standards equivalent to 10, 20, 30, 40 and 50 milliequivalent per liter at a dilution of 1:100 were prepared by diluting 1.0, 2.0, 3.0, 4.0 and 5.0 ml. of stock solution respectively to 100 ml. with glass distilled water. Calibration curve from these working standards were prepared as described in the determination of sodium.

Estimation of sodium.- The Flame Photometer was calibrated by sodium standard diluted solution of 50, 100, 150, 200 and 250 milliequivalent per liter. The standard curve was

Calibration Curve For Sodium



Calibration Curve For Potassium



prepared. The reading in Galvanometer for unknown sample diluted at 1:100 was noted and quantity found out from the standard curve.

Estimation of potassium.- The standard curve was prepared similarly from standard diluted solution of 10, 20, 30, 40 and 50 milliequivalent/liter. The reading in Galvanometer for unknown sample diluted at 1:100 was noted and the quantity found out from the standard curve.

The concentration of sodium in milliequivalent/liter was converted into mg per cent by multiplying the results by 2.3 and for potassium by 3.9.(Oser,1965).

Bacteriological study of cervical mucus:

Isolation of organism.- Samples of cervical mucus collected under sterile precautions, were inoculated on sheep blood agar plate and MacConkey's agar plate. They were incubated aerobically at 37°C and examined after 24 hours and then again after 48 hours. Bacterial colonies were examined by magnifying glass and by transmitted light for their colonial characteristics. Minute colonies which appeared in 24 to 48 hours were transferred on blood agar slant. Organisms showing moderate or luxurient growth were transferred in nutrient agar slant. They were incubated at 37°C for 24-48 hours and then tested for purity.

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Test for purity of isolated strains.- The slant cultures were tested for purity after staining by Gram's method. Morphology was studied for each of them. Cultures of minute colonies were grown in glucose broth and blood agar plate whereas others were subcultured in nutrient broth and nutrient agar plate. The pure cultures were then subjected to primary and secondary tests. In general, the methods suggested by Cowan and Steel (1970) were followed throughout the study.

Under primary tests motility, catalase activity, oxidase activity, oxidation or fermentation of glucose were carried out as suggested by Cowan and Steel (loc.cit.).

Treatment:

Group A.- The animals in this group were treated with Hostacyclin water soluble vet. of Hoechst Pharmaceutical Ltd., Bombay. 100 gms.of the powder is equivalent to 5 gms. of tetracycline hydrochloride I.P.

Fifteen gms. of the powder was dissolved in 30 ml. of sterile distilled water and administered through uterine route. Five such injections were given on alternate day beginning from the next day of oestrus, when the animals were declared to be positive for micro organisms in the cervical mucus.

When the animals next came in heat, were examined per vaginum and per rectum. When it was seen that the os was open, vagina hyperaemic and uterine tone normal with development of follicle in either ovary, the animals were advised for artificial insemination. All inseminations were done at Semen Bank,Patna. Cervical mucus was collected before the animals were inseminated. All the cervical mucus samples of the animals treated with Hostacyclin were found to be free from micro organisms. Twelve animals of this group became pregnant. These 12 animals were considered as normal and pH values of cervical mucus and sodium and potassium contents of cervical mucus was determined.

Group B.- The animals in this group were treated with progestin (Progesterone I.P.) of Organon (India) Ltd.,Calcutta.

Those animals whose cervical mucus samples were free from bacteria were included in this group. The animals were advised artificial insemination and the owners were asked to bring the animals for further examination 10 days after insemination. Rectal examination was done to ascertain the presence of corpus luteum in the ovary. Then a single dose of 125 mg. progestin was injected intramuscularly in each animal.

Collection, compilation and statistical analysis of data:-

Statistical analysis was done according to the methods suggested by Snedecor and Cochran (1967).

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Results and Discussion

Incidence of repeat breeding:

TABLE - I

Table showing the monthwise incidence of repeat breeding in cattle.

Month	Total no.of cases	No. of repeat breeding animals.	Monthwise percentage of incidence.	Overall percentage of repeat breeding animals.
May	54	4	7.4	
June	61	8	13.11	
July	60	7	11.66	
August	74	8	10.81	
September	63	4	6.34	
October	54	5	9.25	12.36
November	48	4	8.33	
December	51	6	11.76	
January	68	9	13.23	
February	96	12	12.50	
March	55	14	25.25	
April	84	14	16.66	
TOTAL	768	95		

Monthwise Incidence Of Repeat Breeding In Cattle

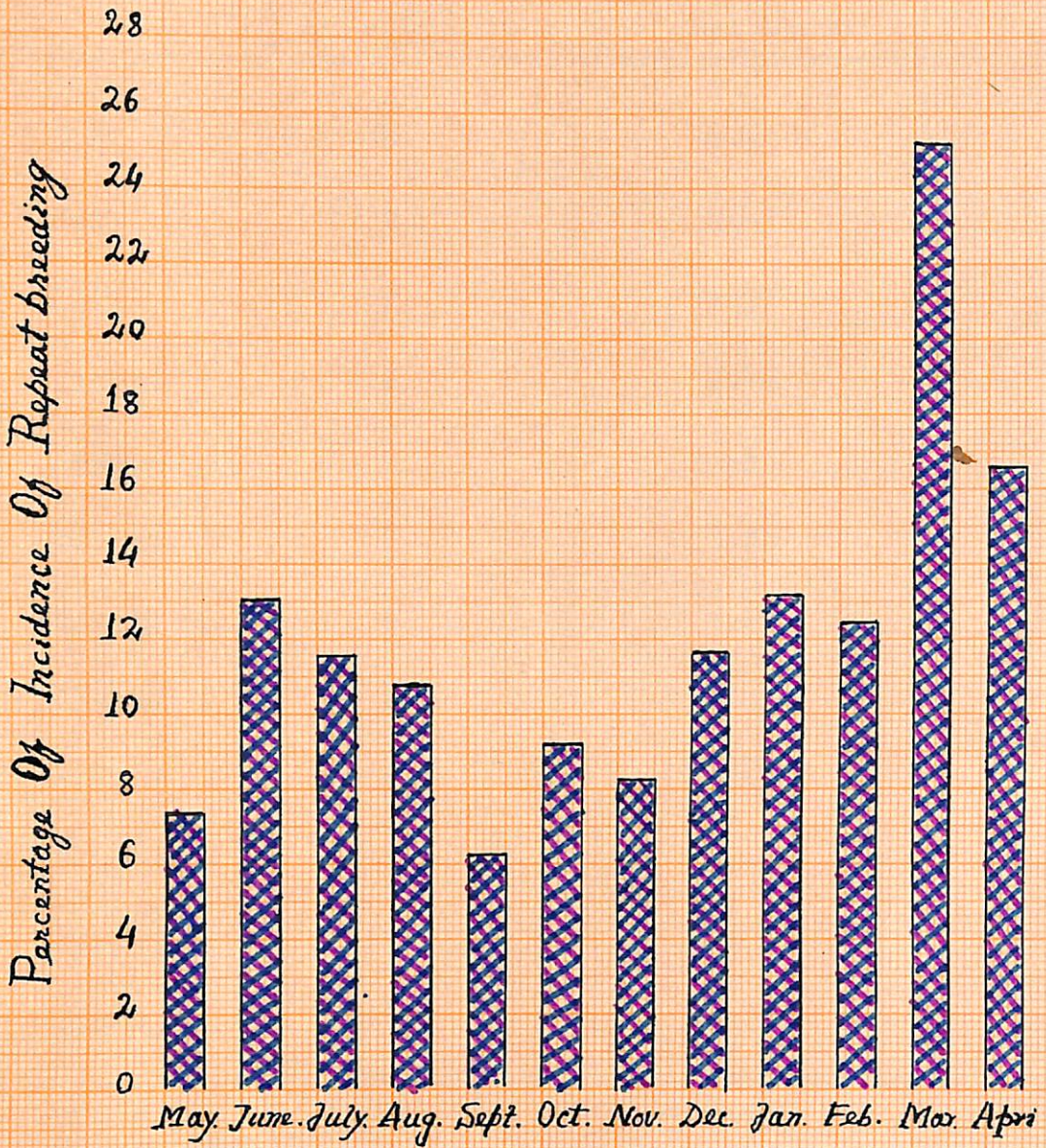


TABLE II

Table showing monthwise distribution of repeat breeding cases.

Month	No.of repeat breeding animals.	Percentage of incidence
May	4	4.21
June	8	8.42
July	7	7.37
August	8	8.42
September	4	4.21
October	5	5.26
November	4	4.21
December	6	6.32
January	9	9.47
February	12	12.63
March	14	14.74
April	14	14.74
TOTAL	95	100.00

From table above it was evident that the highest incidence (14.74%) of repeat breeding animals was in March and April and the lowest (4.21%) in May,September and November.

Monthwise Distribution Of Repeat Breeding Cases

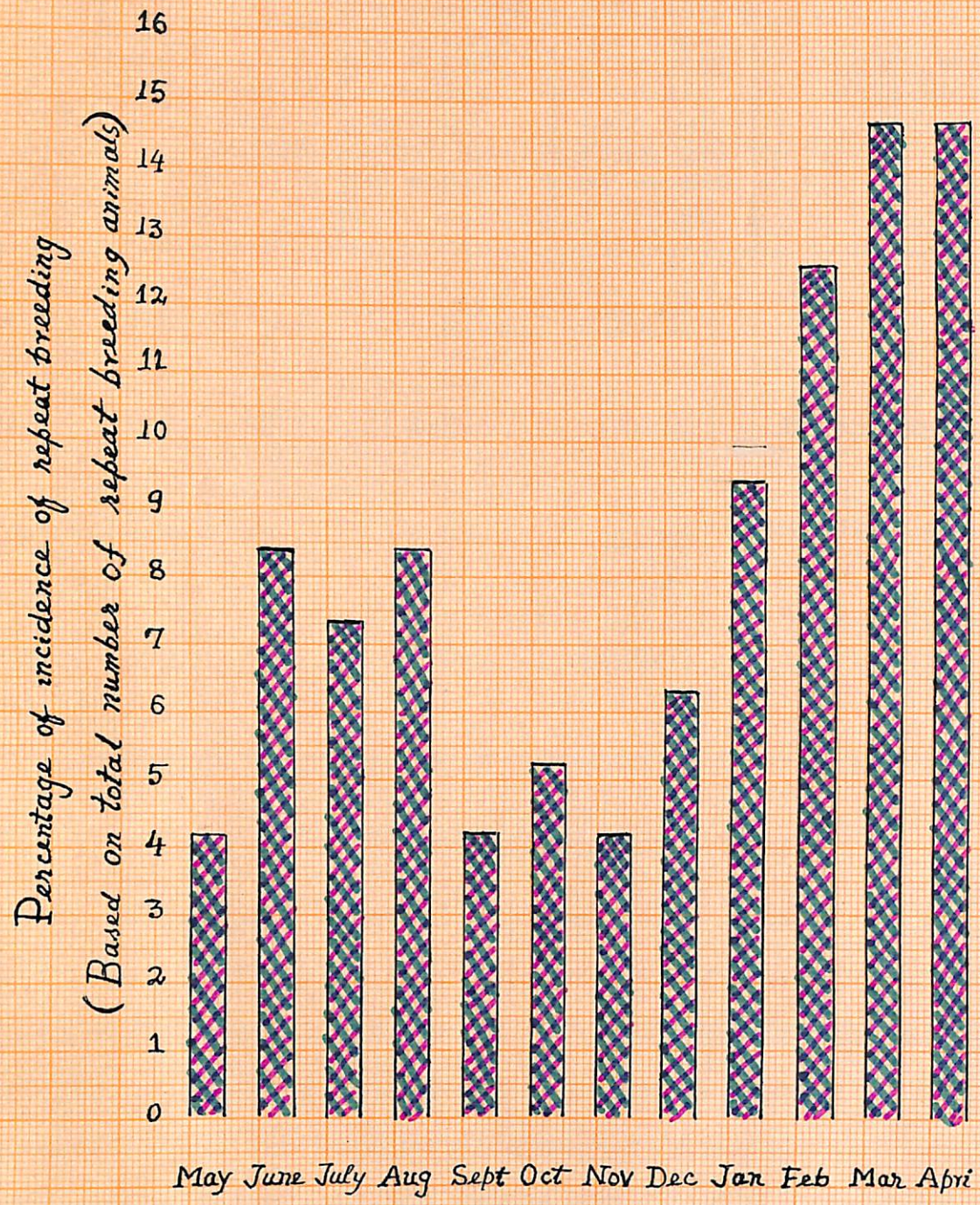


TABLE III

Table showing the seasonal incidence of repeat breeding.

Season	Total no.of cases	No.of repeat breeding animals	Percentage incidence of repeat breeding (out of total no. of cases.	Percentage incidence of repeat breeding (out of total no. of repeat breeding cases).	Calculated chisquare.
Summer (May-July)	175	19	10.85	20.00	
Autumn (Aug-Oct.)	191	17	8.90	17.90	Chisquare = 14.94** 3 df
Winter (Nov-Jan.)	167	19	11.37	20.00	(Tab.chisquare at 3df 1% = 11.34).
Spring (Feb-April)	235	40	17.02	42.10	

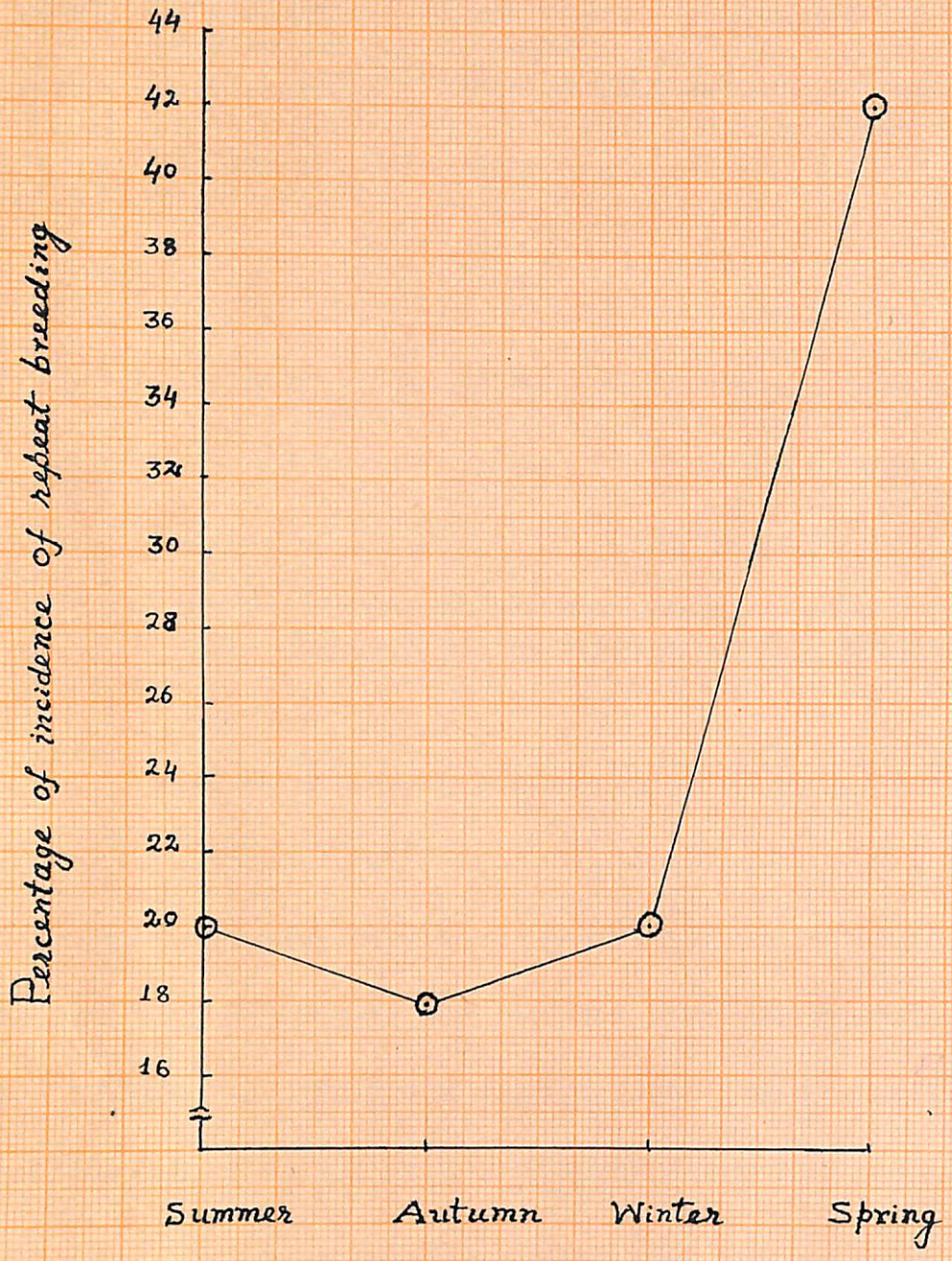
** indicate significant at 1% level.

From table above it was clear that seasons highly significantly influenced the incidence of repeat breeding cases,the maximum being recorded in Spring (42.10%) and the minimum in Autumn (17.90%). The present report did not seem to agree with the findings of Hewett (1968) who reported the highest incidence in Autumn and Winter months (10.2 - 13.7%) and lowest in Spring and Summer months (7.1 - 9.4%).Variation in climatic and managemental conditions and differences in breed might be the causes for the differences in the findings.

Seasonal Incidence Of Repeat Breeding

Scale

1 0 small square = 2%



(Based on total number of repeat breeding animals)

TABLE IV

Table showing the incidence of repeat breeding in relation to sequence of calving.

Calving sequence	No.of repeat breeding cases.	Percentage of incidence (out of total no.of repeat breeding cases)	Calculated chisquare
Heifer (no calving)	13	26.53	
Ist calving	7	14.29	Chisquare $_{3df}$ = 8.06*
2nd calving	20	40.82	(Tab.chisquare $_{3df}$ at
3rd calving	9	18.36	5 % = 7.82).
TOTAL	49	100.00	

* indicates significant at 5 % level.

Calving sequence seemed to influence the frequency of repeat breeding significantly, the maximum incidence being observed in the 2nd calving (40.82%) and the minimum in the first calving (14.29%). The present findings seemed to confirm those of Hewett (1968) who reported the lowest incidence of repeat breeding in heifers that calved for the first time (5.2%).

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pH of the cervical mucus:

TABLE V

Table showing mean pH of cervical mucus in treatment group of repeat breeding cases,(the pH was taken before starting the treatment).

Group	n	pH mean ± S.E.	C.V. %
Nontreated control	8	7.80 ± 0.22	8.08
Progestin	17	8.09 ± 0.16	7.91
Hostacyclin	10	7.62 ± 0.13	6.82
Overall	35	7.89 ± 0.10	8.11

The overall mean pH of cervical mucus of repeat breeding animals during oestrus was recorded to be 7.89 ± 0.10. Gupta et al.(1962) and Akhtar (1973) who reported the mean pH of cervical mucus of repeat breeding cows to be 7.90 and 7.73 ± 0.153 respectively, seemed to agree with the present results. On the contrary Roark (1953) reported acidic pH of cervical mucus in repeat breeding cows.

The alkalinity of the cervical mucus might be due to copious cervical secretion during oestrus (Mc Nutt et al.,1939). Breed and nutritional variations might also be the causes of pH variation (Bacic,1962).

The difference in pH might also be due to electrolytes fluctuations. The fluctuations in sodium and potassium contents of cervical mucus which has been reported to affect the pH might also be one of the reasons for pH variation.

TABLE VI

Analysis of variance showing the effects of grouping on pH of the cervical mucus of repeat breeding animals.

Sources of variation	d.f.	M.S.	
Between groups	2	0.47	N.S.
Within groups	32	0.40	
TOTAL	34		

N.S. indicates non-significant.

No significant differences in the pH of cervical mucus in the three treatment groups were found (Table VI).

TABLE VII

Table showing the comparison of pH of cervical mucus of normal and repeat breeding animals.

Group	n	pH mean ± S.E.	C.V.%	Differences in mean
Normal	11	7.74 ± 0.08	3.61	0.15**
Repeat breeding animals.	35	7.89 ± 0.10	8.11	(Calculated $t_{44\ df}$ = 3.0 as against 3.690 for 44 df.)

** indicate nm significant at 1% level.

The mean pH of cervical mucus of normal animals during oestrus was found to be 7.74 ± 0.08 (Table VII). The mean pH difference between normal and repeat breeding animals was found to be statistically significant (Table VII).

Variation in pH of cervical mucus has been reported to be associated with fertility problems. Kultis (1963) found that cows with pH of cervical mucus between 6.84 to 6.94 and 7.67 to 8.30 did not conceive. He also observed progressive decline in conception rate from 55.6 per cent to 9.5 per cent with corresponding increase in pH from 7.0 to 7.6. Cseh (1963) also never reported conception rate above 40 per cent when the pH was below 6.7.

The present findings did not seem to agree with that of Kultis(loc.cit.) that cows with pH 7.61 to 8.30 of cervical mucus did not conceive. In the present case the pH of normal animals (all of which became pregnant) ranged from 7.0 to 8.2, the mean being 7.74 ± 0.08.

Sodium and potassium contents in the cervical mucus:

TABLE VIII

Table showing the mean sodium and potassium concentration in cervical mucus of normal and repeat breeding animals.

Element	Normal animals			Repeat breeding animals			Differences between means
	n	Mean ± S.E. (mg./100 ml)	C.V.%	n	Mean ± S.E. (mg./100 ml.)	C.V.%	
Sodium	6	380.00± 11.11	7.14	14	358.98 ± 36.99	38.56	21.02^{NS}
Potassium	6	55.36± 4.54	19.11	14	67.13 ± 9.60	53.49	11.77^{NS}

NS - indicates non-significant.

The present study did not reveal any significant difference between the concentration of sodium and potassium in the cervical mucus of repeat breeding cattle and the normal ones. Gupta et al.(1962) and Reddy (1973) also found similar results. But the present finding did not agree with the findings of Guay and Lamothe (1970) who reported significant differences in sodium and potassium concentration in cervical mucus between repeat breeders and normal ones, after analysis of cervical mucus with absorption spectrophotometer.

The findings of Guay and Lamothe (loc.cit.) as against the findings of the present experiment and those of Reddy(loc.cit.) and Gupta et al.(loc.cit.) indicate some differences in sodium and potassium concentration in the cervical mucus in normal and repeat breeders on account of breed differences or some unknown

factors. Guay (1966,b) also stated that the mechanisms which determine the excretion of sodium in cervical mucus is still unknown.

Treatment of repeat breeding animals:

TABLE IX

Table showing conception rate after different treatments in repeat breeding animals.

Treatment	No.of repeat breeding animals	No. of animals conceiving at Ist insemination.	No. of animals conceiving at 2nd insemination.	Total percentage of pregnancy after I & II inseminations.	No. of animals failing to conceive
Hostacyclin	15	11(73.33%)	1(6.67%)	80	3(20.00%)
Progestin	25	13(52.00%)	3(12.00%)	64	9(36.00%)
Control (Non-treated)	10	2(20.00%)	nil	20	8(80.00%)

The cervical mucus samples from fifteen animals were found to be positive for the presence of different organisms. Three different genera of organisms such as Stephylococci, Bacilli and Alcaligenes were recorded.

Treatment of repeat breeding animals with Hostacyclin gave 80 per cent conception whereas it was 20 per cent in non-treated controls (Table IX). This finding completely disagreed

with that of Hjerpe (1961) who found no difference in conception in tetracycline-treated and non-treated repeat breeders (harbouring pathogens). But Roberts (1971) reported the possibility of recovery after antibiotic treatment in repeat breeding cows.

Sixtyfour per cent conception (Table IX) was found after progestin treatment of repeat breeding animals as against 20 per cent in non-treated controls. But Danieli (1967) found 86.3 per cent conception after progestin treatment. Though the percentage of conception in the present study was lower than that reported by Danieli (loc.cit.) it seems to hold great promise in the treatment of repeat breeding animals. Herrick (1953) and Dawson (1954) also used progesterone in the treatment of repeat breeders and reported that higher percentage of treated animals settled than did their controls.

TABLE X

Effect of treatments on conception rate in repeat breeding animals.

Treatment	Total no.of repeat breeders	Pregnant	Non-pregnant.	Calculated chisquare
Hostacyclin	15	12 (80%)	3 (20%)	
Progestin	25	16 (64%)	9 (36%)	less than 1 (Tab.chisquare 1 df at 5% = 3.841).
Control (Non-treated)	10	2 (20%)	8 (80%)	

From table X,it was clear that as the chisquare was non-significant for the progestin Vs non-treated control, progestin is not having any significant effect on the frequency of conception rate over those of the non-treated control group.

As for Hostacyclin, due to less number of observations no valid conclusions can be drawn in comparison to the non-treated control but from the data it appears that there is 80 per cent pregnancy in Hostacyclin group in comparison to 20 per cent pregnancy in the non-treated control. This seems to indicate that Hostacyclin might be having significant effect in comparison to the non-treated control as regards the frequency of conception rate but to have real valid conclusion in this aspect more exhaustive work with larger number of cases need to be taken up.

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Summary

From the cows which came to the Gynaecological clinic of Bihar Veterinary College, Patna during the period from May, 1974 to April, 1975, the incidence of repeat breeding was studied. The overall incidence of repeat breeding was recorded to be 12.36 per cent out of 768 cases referred to the clinic.

Though repeat breeding cases came throughout the year, the highest incidence was found in March (25.25%) and the lowest in September (6.34%).

Seasons were observed to influence the incidence of repeat breeding highly significantly, the maximum incidence being recorded in Spring (42.10%) out of total number of repeat breeding animals and the minimum in Autumn (17.90%) out of total number of repeat breeding animals.

Sequence of calving also had significant effect on the incidence of repeat breeding, second calvers showing the maximum incidence (40.82%) and first calvers the minimum (14.29%) incidence out of the total number of repeat breeders.

The overall mean pH of cervical mucus in repeat breeding animals was found to be 7.89 ± 0.10 as against the normal ones whose value was 7.74 ± 0.08. Highly significant statistical

difference between the mean pH of normal and repeat breeding animals was recorded.

The mean sodium content of cervical mucus of normal and repeat breeding animals was recorded to be 380.00 ± 11.11 and 358.98 ± 36.99 mg/100 ml. respectively. No significant difference between the normal and repeat breeding animals as regards the sodium concentration could be recorded. In the case of potassium concentration in cervical mucus of normal and repeat breeding animals was recorded to be 55.36 ± 4.54 and 63.13 ± 9.60 mg/100 ml. respectively. In this case also no significant difference in the potassium concentration of cervical mucus between normal and repeat breeding animals could be detected.

No significant difference in conception of repeat breeding animals could be obtained by treatment with progestin over those of non-treated controls. Conception rate in Hostacyclin treated group was 80 per cent, that of progestin group 64 per cent and that of non-treated controls was 20 per cent. Due to less number of data Hostacyclin group could not be statistically compared with that of non-treated controls.

The bacteriological survey of 40 samples of cervical

mucus of repeat breeding animals showed 37.5 per cent positive for organisms. Three different genera of bacteria - Staphylococci, Bacillus and Alcaligenes were recorded.

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