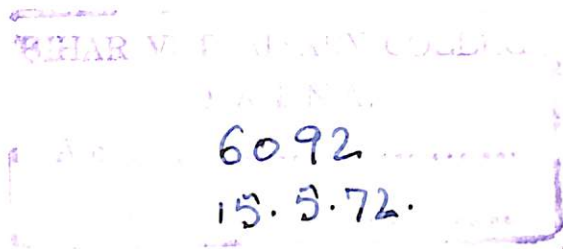


STUDIES ON

*The Incidence of Repeat Breeders among Tharparker
herd and Local Breeds of cattle with special
reference to Crystallisation Pattern, Hydrogen
ion Concentration (pH) of cervical mucus
and possible Treatments.*

A Thesis

Submitted to the Faculty of Veterinary Science
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CERTIFICATE

This is to certify that Narendra kumar Sinha has worked under my supervision and guidance for his thesis Entitled "Studies on the Incidence of Repeat Breeders among Tharparkar herd and Local breeds of cattle with special reference to crystallisation Pattern and Hydrogen ion concentration of cervical mucus and possible Treatments". For the degree of M. Sc. (Veterinary) Gynaecology, Obstetrics and Artificial Insemination of Rajendra Agricultural University. The material incorporated in this Thesis is his own work and I have checked up his results from time to time during the course of Academic year 1970-71.

PATNA the 21st February 1972

S. M. Ishaque
21-2-72
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AFFECTIONATELY DEDICATED

TO

MY PARENTS

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(N. K. SINHA)

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LIST OF ABBREVIATION USED

A.I.	-	Artificial Insemination
Av.	-	Average
c.c.	-	Cubic centimeter
C.M.	-	Cervical mucus
C.V.	-	Coefficient of variation
Max.	-	Maximum
Min.	-	Minimum
mcg.	-	Microgram
N.P.	-	Non-pregnant
N.S.	-	Non-significant
Obs.	-	observation
P.	-	Pregnant
PTS	-	Post treatment service
S.D.	-	Standard deviation
S.E.	-	Standard error

INTRODUCTION

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

The cattle is a recognised dairy animal in India and its efficiency as a milk producer depends very much on its reproductive efficiency. It is, therefore, considered that studies on various aspects of cattle breeding are of economic importance since they enable the farmers to breed their animals during the most favourable period which will help to reduce calving interval and dry periods.

India is a country where most of the people live in villages. The villagers are very much interested in cattle breeding for their maintenance. The population of cattle is the largest in India. The persons who live in towns are also very much interested as well. Five year plans are being conducted since 1952. Through the successive five year plans an effort is being made to improve our livestock with special reference to their reproduction. Experiences gained thus so far through the first and second five year plans do not indicate much satisfaction in reproduction. Milk production costs have got much influence on the consumer price. So in the milk business it is the endeavour of industry, producers and all concerned to lower the cost of production. Environment plays much greater role than the genetic influence in the performances of an individual, and it is a highly complex and variable factor differing widely from place to place. Our vast country being typically tropical with wide varying climatic conditions,

presents varied and complex environmental problems from place to place.

Repeat breeding is the problem of great economic importance in cattle breeding. The incidence of sterility, infertility and delayed conception is increasing day by day and it must be controlled successfully. The incidence of these reproductive disorders in Indian breeds of cattle is not known as reported by Luktuke (1967).

Repeat breeder results in losses which are rather difficult to estimate. They include lost production, lost replacements and increased depreciation cost, because animals frequently have to be culled before their production capacity is fully utilised.

Ranking high amongst the major causes of loss to the dairy industry brought into the public prominence by the work of Associations Survey Committee is the failure of cows to conceive readily. The anxiety of practitioners today to equip themselves fully to remedy this situation is revealed by the high degree of interest which is being aroused within the profession in the diagnosis of pregnancy and the treatment of infertility in bovines (Bhattacharya, Hammond and Day, 1941).

A great deal of literature has appeared recently on the subject of sterility and its various causes but one aspect of the condition has not been greatly stressed, this being the recognition of the sterile cows and her elimination from the herd when she ceases to be of economic value (Rowson, 1951).

Hindmarsh (1934) showed that domestication and the breeding for high milk production appears to have a definite relation to the incidence of sterility in cows. In New South Wales, diet deficiency was associated with sterility, also with a condition in which oestrus occurs but fertilization is absent even after repeated service.

Nutrition has also a great importance in repeat breeding cattle. Under feeding was found to impair reduce fertility. Over feeding of well balanced diet results in ovarian dysfunction. Protein deficiency in farm animals gives rise to reproductive disturbances.

Minerals in itself plays a great role in reproductive failures specially in farm animals. Tuff in Norway (191) and Theiler et al. in South Africa (186) were the first men to investigate the phosphorus deficiency and its effect on repeat breeder.

Christian et al. (1951) found the fertilisation rate to be somewhat lower in the Guernsey than in the Holstein repeat breeders when both were bred to bulls of percentage of cows with normal embryos at 34 days after breeding was higher than in the Holstein cows bred to Holstein bulls. Repeat breeder cows constitute a highly selected population, a population of individual each of which has failed in repeated attempts at fertility with small group of bulls.

Ulberg et al. (1952) reported that approximately 40 % of the cows bred artificially return for rebreeding after

first service. Failure of cows to settle when bred many times results in a very important loss to the dairy industries due to extended service periods, and high value per cow, increasing labour and equipment cost, farmers can not afford to fail to conceive his cow promptly. Since last decade in India wide spread use of Artificial Insemination has brought the problem of bovine sterility under control to some extent and naturally the attention of farmers, animal husbandry men and research workers is concentrated on the basic problems of reproduction of the animal. These days Artificial Insemination is being used all over the country as a major part for rapid improvement of livestock.

Hawk et al. (1955) determined the percentage of repeat breeder cows with normal embryos at 16 elapsed days of gestation and the embryonic death rate between 16 and 34 elapsed days. The estimate of embryonic death from 16 to 34 days was 51.7 %.

The most important thing in reproduction depends on the regularity of oestrus and oestrus cycle when the cow gets its maturity and detection of exact time of oestrus. Many works have been done by various scientists on the study of cervical and vaginal discharge. This mucus discharge is one of the best symptom of oestrus. This discharge differs in different stages of oestrus and in different conditions. Crystallization pattern of cervical mucus during oestrus attracts the attention of many workers.

Woodman and Hammond (1925) and Hammond (1927) were the first men who investigated accurate accounts of cyclical changes of cervical mucus and its cyclical changes have been done by Papicolaou (1945) and Rydberg (1948) in human as quoted by Abusineina (1962) and Garm and Skjerven (1952), and Bone (1954) in bovine is not of old origin. During oestrus period the cervical secretion is thin, transparent, abundant and does not readily stick to the slides. On crystallization it forms "Fern" like appearance which can also be seen with naked eyes and by microscopical examination. Gradually, as the time passes after the onset of the oestrus the mucus secretion becomes scanty, viscous and jelly like, turbid and pale in colour and crystallization pattern disappears.

Snook (1964) reported the incidence of infertility and delayed conception is common in Western Australian dairy herds.

Khan and Iuktuke (1967) published in their literature that anoestrus and repeat breeding are the problems of great economic importance in cattle throughout the country.

The hydrogen ion concentration (pH) vary in the cervical mucus of repeat breeder and in normal or pregnant cows. According to Roark and Herman (1950) the pH of difficult breeding tended to be more acidic than that of normal and pregnant cows. According to Hammond, one of the chief characteristics of the cervix is the mucus secretion, the amount and consistency of the mucus varying with the stage of oestrus cycle.

The mucus produced is very thick and viscid and relatively small amount is formed from three days after heat to three days before the next one. Just before that it becomes fluid and large quantities of it are formed, during heat it is very thin and clear becoming thicker and rather whitish due to admixture of leucocyte and debris 48 to 72 hours after the beginning of heat. Embry, Roberts quoted by Hammond found that in the cows the mucinous secretions during proestrus is derived not from the body of the uterus but from the cervix and vagina, cited by Chaudhary and Prasad (1953).

The present study is meant for the treatment of repeat breeders in Tharparkar herds and local breeds of Patna with the use of different drugs to meet the difficulties of the common man, to bring their common animals in maximum reproductive periods. Tharparkar cattle is one of the important dairy breeds of India.

Crystallization pattern of the cervical mucus and chemical properties for example Hydrogen-ion concentration (pH) of cervical mucus is an attempt to know the relations of its varying characteristics to conception rates in repeat breeding cattle. In the present study the efficacy of the different drugs in cases of repeat breeding cattle have also been shown.

PLAN AND PRESENTATION

MATERIALS, PLAN AND PRESENTATION

The present study was undertaken at the Government Cattle Farm, Patna, where pure Tharparkar breed of cattle are maintained. Besides that present study was also undertaken at Bihar Veterinary College, Hospital. The total strength of cattle in the Government Cattle Farm is 818 including 225 cows, 358 heifers, 26 bulls, 39 bullocks and 2 Teaser. [Plate no.1 shows the photograph of Tharparkar herd in the Farm]. Plate no.2 shows the photograph of the cases of repeat breeders in Farm.

Feeding and management of the Farm:-

Animals were quite healthy and were kept separately according age group. Separate arrangements were made for feeding, grazing, housing and watering purposes for the milch cows and heifers. Feeds were supplied two times daily that is in morning and evening to each animal consisting of concentrate and roughage. Concentrate contained groundnut cake, wheat bran and guar meal in equal proportion along with common salt and mineral mixture is provided according to maintenance ration schedule per animal. Roughage contained green and dry grasses. Green grasses were available in the fields of farm (e.g. Berseem, Para and Napier). Concentrate was given more to milch and pregnant animals and breeding bulls as well. Sufficient fresh water was supplied in water troughs in peddocks as well as in byres. Animals (except bulls, bullocks and calves) were used to go two times for grazing between 9 A.M. to 11 A.M. and 3 P.M. to 5 P.M. daily in summer

and 10 A.M. to 12 A.M. and 2 P.M. to 4 P.M. daily in rainy and winter.

Climate:-

India is a tropical country and the climate varies from place to place and month to month. In Patna cold weather commences early in November and comes to an end in the middle of March. The hot wind then starts and lasts till about the middle of June. Then rainy season comes and continues till the end of September.

The present study has been made from June to December, 1971, that late part of summer, rainy and in middle part of winter. The monthly average of air temperature, relative humidity and rainfall for the period recorded on the basis of meteorological report of the Civil Aerodrome, Patna, which is very near to the Farm and Bihar Veterinary College, Patna.

TABLE No.1

Monthwise average air temperature, relative humidity and rain fall at Patna during the study period

Month	Av. temperature in °C		Av. relative humidity in %		Total rain fall in mm.
	Maximum	Minimum	8.30	17.30	
June	32.7	25.0	81.2	70.2	7.99
July	30.88	24.8	85.12	76.9	11.77
August	30.60	25.0	86.10	78.1	15.30
September	32.35	25.4	80.40	72.8	3.64
October	30.60	21.4	78.70	69.50	6.74
November	28.16	13.6	75.16	63.3	nil
December	24.80	8.5	74.10	62.1	nil

Handling and Sterilization:-

All animals which were repeaters, were assembled in the observation yard and detailed observations of all the reproductive genitalia were made. They were classified into several groups for the drug trial and controlled in the trevis.

Aseptic precautions were taken, while handling the animals. All metallic and glass wares to be used during examinations were sterilized in electric hot air oven at 130°C for 30 minutes and the rubber materials, gloves, cloths, cotton etc. were autoclaved at 15 lbs pressure for 30 minutes. For repeated use of speculum, it was dipped in the savlon or dettol solution. All sterilized equipments were kept in sterilized container.

In the present study the observations have been detailed in the following parts:-

PART I

- (i) Classification,
- (ii) Observation on the incidence of Repeaters,
- (iii) Incidence in different age groups of animals.

PART II

- (i) Studies on crystallization pattern of cervical mucus during oestrus cycle in repeat breeder cases:-
 - (a) Before treatment and
 - (b) After treatment.
- (ii) Determination of pH of cervical mucus during oestrus in repeat breeder cases :-
 - (a) Before treatment and
 - (b) After treatment.

PART IIITrial of different drugs:-

- (i) Penicillin G sodium + streptomycin sulphate,
 - (ii) Tonophosphan injectable,
 - (iii) Steclin granules,
 - (iv) Siolan 12 + Placentrex,
 - (v) Vitablend AD₃ + Prepaline forte,
 - (vi) Terramycin liquid.
-

CLASSIFICATION OF REPEAT BREEDERS

According to Robert "the repeat breeding cow is one that has normal or nearly normal oestrus cycles and oestrus periods and has been bred 2 or more times to a fertile Bull yet failed to conceive". In the experiments with "repeat breeder" cows (Tanabe, Casida and Co-workers) and repeat breeder heifers (Tanabe and Almquist, 1953), the animals had been bred at least four times by a fertile bull.

Robert has categorised the "repeat breeders" according to cause into two main parts. They are (i) Repeat breeder due to failure of fertilization and (ii) Repeat breeder due to early death of embryo. Further he has classified the failure of fertilization as follows due to the reasons mentioned therein:-

(a) Obstructions of the oviduct:- This includes adhesions between the ovary and bursa. Obstructions may be due to inflammation, hydrosalpinx, cysts, chronic salpingitis, pyosalpinx and developmental defects. The incidence was 2-9 % with an average of 3-4 %.

(b) Abnormalities in ovulation:- Abnormalities may include failure of ovulation, delayed ovulation or ovulation of two or more ova. From the data of Casida and others failure of ovulation occurred in three to five percentage of repeat breeders.

(c) Inability of the ova to become fertilized:- may be due to defective or pathogenic ova, aging of the ovum or other factors.

Kidder and Co-workers, Bearden, Tanabe and Almquist

reported that from 2-9.5 % of the recovered ova showed abnormalities.

(d) Inability of the sperm to fertilise:- is the most common cause for failure of fertilization in normal cows and repeat breeders. Failure of fertilization occurred for no apparent cause in 24 to 39 % of the repeat breeding cows. In virgin heifers this figure was only 12-13 %. Improper handling of the sperm by allowing sperm to become too warm, too freeze, to be shaken severely, the use of improperly mixed diluters, the addition of excessive amounts of antibiotics may injure spermatozoa and lower the fertilization rate.

(ii) Early embryonic death:-

Early embryonic death may be due to following reasons:-

- (a) Congenital or genetic defective or to abnormal ova or embryos.
- (b) Diseased or infected fertilized ova or embryos.
- (c) Due to an abnormal lethal environment due to inflammatory, hormonal, nutritional or genetic causes. In repeat breeders embryonic death is the cause of about 20 to 30 % loss of embryos and a 50 % loss of fertilized ova. In normal heifers, figures are halved.

(a) Congenital or Genetic defects of the fertilized ova or embryos:-

Congenital or Genetic defects have been less difficult to study in mammals such as mouse, rat, rabbit and pig than in the cows because the former are multiparous and therefore if a few ova or embryos die the rest will alive.

Casida stated that there was only an 8-11 % difference

between fertile and repeat breeding cows, indicating that repeat breeders might be somewhat heritable. Christian, Ulberg and Casida showed that cross breeding or using bulls of another breed on repeat breeding cows did not increase conception rates.

Foote and Bratton demonstrated that 112, 312 first service cows bred artificially before the addition of antibiotics to semen, had non-return rates of 79.1, 64.1 and 60.03 % at 28-35 days, 60-90 days and 150-180 days respectively. They further reported in 233, 354 insemination after the addition of antibiotics the respective non-return rates were 82.5, 73 and 69.7 %.

(b) Diseased or infection of the fertilized ova or embryos:- is the most common specific cause for early embryonic death e.g. vibriosis, trichomoniasis and brucellosis.

Hawk and Co-workers reported that in repeat breeding cows most embryonic deaths occurred between 16 and 34 days. Some workers believe that hormonal disturbances such as progesterone deficiency making an improper environment in the uterus for nidation might be an etiologic factor in early embryonic death.

Laing (1952) indicated the possibility that isoimmunization may possibly be a factor in early embryonic death. The relation of nutrition to early embryonic death in cattle appears very tenuous. Casida has cited data showing that in swine a lack of vit. B₁₂ and animal protein and full feed for gilts during their breeding period resulted in a higher embryonic death rate

than if sows and gilts were fed vit. B₁₂ and kept on a low plane nutrition during breeding period and gestation. Blood, organ and tissue chemistry in repeat breeding cows has revealed nothing of significance (Asdell and Coworkers, 1942) and Bentley and Coworkers.

According to Andrews (1949) 1.8 services per conception is generally accepted as the average breeding efficiency for dairy cattle. The number of services per conception as listed by other workers are 2.02 by Bowling et al. (1940); 1.7 to 2.0 by Asdell (1948); 1.8 to 2.5 by Lush (1949) and 1.72 by Gilmore (1952).

According to Salisbury and VanDemark the following are the causes of repeat breeders:-

(i) Failure of ovulation:- In some cows and heifers typical physiological and psychological symptoms are shown for a time e.g. split oestrus, are discontinued, and again are expressed with ovulation probably following the second manifestation. In this case if insemination were done during first manifestation it would be too early and spermatozoa might not live until ovulation occurred.

(ii) Failure of ovum and spermatozoa to meet after ovulation because of :-

- (a) Anatomical abnormalities,
- (b) Infectious and /or adhesions of the Cervix, Uterus, Oviducts and Ovarian bursae.
- (c) Improper oxytocin controlled ascent of spermatozoa.
- (d) Improper timing of insemination in relation to ovulation.

(iii) Failure of fertilization though gametes meet, because of:-

- (a) Sperm-cell failure for whatever cause,
- (b) Ovum failure.

(iv) Failure of implantation though fertilization occurs because of :-

- (a) Lethal recessive genes,
 - (b) Bacteria or viral invasion of the uterus.
- (v) Early embryonic death.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

REVIEW OF LITERATURES

INCIDENCE OF REPEAT BREEDERS:

A repeat breeder is that which shows a reduced probability of conception. Several informations about the incidence were obtained from several places.

Olds and Seath (1941) reported that 10 % of the herds or individual cow in Kentucky affected per year by an infertility or sterility problem, requiring more than an average of two services per pregnancy, continued to be affected the following year. They reported that the number of problem herds remained quite constant from year to year at 6.5 %.

Stuhlenmiller (1941) found a large number of cases of repeat breeder in a farm and he treated all the affected cows. Yet 64.3 % returned to oestrus every three weeks.

Tanabe and Casida (1947) calculated the data of repeat breeders and the author observed that there was a higher occurrence in Holstein (18.2%) than in the Guernsey (2.0%). The average incidence was 10.1 % and he categorised all these animals into three categories on the basis of reproductive performance during 1st 34 days after breeding i.e.

(i) Failure of fertilization (39.7%)

(ii) Embryonic mortality before 34 days (39.2%)

(iii) Embryos still normal at 34 days (21.1%).

Tanabe and Casida (1949) reported that 66 % of the repeat breeder cows with no clinical detectable abnormality and fertilized ova were found. At 34 days those having normal embryo had decreased to 23 %.

Casida (1950) reported that a repeat breeder is arbitrarily defined as a cow requiring more than one service to produce a calf. From statistics compiled by American A.I. organisation it appeared that 50 % of the cows are repeat breeder.

Ulberg et al. (1952) found that 40 % of the cows when bred artificially, returned for rebreeding after first service.

Herrick (1953) studied the incidence of repeat breeding in a normal clinical examination but the exact data of percentage was not available in the paper.

Bhattacharya et al. (1953) in a countrywide survey on bovine infertility examined over 20,000 cattle and estimated on an average that 3 % were sterile and about 8 % were sub-fertile.

Flerchinger and Erb (1953) calculated repeat service interval in repeat breeding and found that 57.4 % of repeat service intervals reoccurred from 17 to 26 days compared with 55.5 % in the closed observed herd.

Bellani (1961) surveyed the incidence of repeat breeder on the basis of late and early returns to heat after

artificial insemination in some districts of Mantua. He found the highest incidence from March to July. He got 3.75 % as early and 27.52 % as late returns to oestrus.

Lutke-Vestert (1964) investigated the oestrus interval of repeat breeders based on the records of a West German A.I. centre. According to the report of the author the percentage of cows returning to once or 2-3 times for inseminations were 17.1 and 14.9-18.1 % respectively for an insemination interval of 37-47 days and 6.5 and 3.6-6.4 % respectively for an interval of 57-67 days. About 50 % of the animals returning to service were reinseminated after an interval of more than 24 days.

Chung et al. (1966) examined 71 farms and out of 523 cows they revealed 124 (23.70%) as sterile. Of the infertile cows 72.7 % had ovarian and 12.5% had uterine abnormalities, 6.4 % had vaginitis.

Khan and Luktuke (1967) studied on the incidence of repeat breeders in Hariana breed and reported that the incidence of repeat breeders varied between 14 and 27 percent.

Hewett (1968) surveyed the incidence of the repeat breeder cow in Sweden with reference to herd size, season, age and milk yield. According to the report of the author records were taken at three A.I. centres on more than 16,000 cows on 1500 farms, the incidence was found 10 % and more than 50% of the farms were affected by such conditions. It increased with farm size and was affected by season and

age. It was higher in autumn than in spring.

Kodagali (1968) investigated Jaffarbadi buffalocows under field conditions of Saurashtra for sexual health and found the incidence of physiological, infections and anatomical forms of infertility to be 76.32, 13.60 and 10.08 % of 228 animals respectively.

Singal and Arora (1970) demonstrated that reproductive failure in cattle is recognised today as one of the most serious problems affecting the economy of cattle industry. They used to call 'repeater' to those cows or heifers which repeated more than two times and could not conceive.

CRYSTALLISATION PATTERN OF THE CERVICAL MUCUS:

When a thin smear of cervical mucus is prepared on a glass or slide and left for drying crystal like appearance is seen, hence the name crystallisation was given. It is seen through naked eye and when seen microscopically (under low power) crystals become more apparent alongwith venations and sub-venations.

The formation of crystals in cervical mucus is due to three factors under the influence of folliculin:-

- (i) Presence of mucoprotein,
- (ii) Sodium and Potassium chlorides, and
- (iii) an increase in the water contents.

When the mucus is spread on slide crystallisation of salts occurs as water evaporates behind the large protein molecules. These protein molecules play the part of the crystalliser remaining some solution by surface tension phenomenon, concentration of salts increases as water evaporates which provoke extension of crystallisation and form a typical fern like structure.

Papanicolaou (1945) was the first scientist who drew the attention on cervical mucus under microscope for the fern like configuration of cervical mucus and described how the phenomenon might be emphasised to detect the time of ovulation.

Rydberg (1948) investigated that the pattern was due to sodium chloride and mucin content of the cervical mucus. The author observed the absence of crystallization pattern in subsequent phases of the oestrus cycle.

Garm and Skjerven (1952) observed the typical crystallisation pattern at the time of oestrus and advanced pregnancy and according to them the absence of fern like structure after 21 days of insemination that is in early pregnancy or in luteal phase.

Higaki and Awai (1953) concluded that pregnancy could be diagnosed with 95.1 % accuracy from 35 days after service by the "Frizzy hair like pattern" of the cervical mucus.

Bone (1954) recorded the crystallization pattern

of the cervical mucus from 183 cows. According to the author crystallisation is related to progesterone activity. In his report cervical mucus smear is not recommended at present as a substitute for accepted methods of determining pregnancy and reproductive activity. However, the value of cervical smears as a test is much greater if accurate records of the cows were available.

Coluzzi and Battistacci (1954) reported the value of papanicolaou test of the fern like appearance of cervical mucus for various stages of oestrus cycle and found that this appearance was seen in pregnant cows and further concluded in the year 1956 that crystallisation pattern of cervical mucus was not a practicable method for pregnancy diagnosis.

Fedrigo (1955) confirmed the work of Papanicolaou and others by finding typical crystallization pattern of cervical mucus during various stages of oestrus cycles in cows.

Pozolora (1955) reported the technique of collecting cervical mucus and classified the crystalline structure according to differences in microscopic appearance.

Mc Donald and Raeside (1956) used Papanicolaou's technique to study the crystallization pattern of cervical mucus in pregnant ovariectomised ewes and also from ewes receiving oestrogen, progesterone and gonadotrophins. Fern like patterns were present.

Scott Blair and Glover (1957a) studied the

crystallization pattern for early pregnancy diagnosis in short horn, Friesian and Gurnsey cows in which 81 % diagnosed correctly at 18-19 days after conception, 88 % at 24-25 days after conception, 90 % at 27-29 days after conception and 97 % at 35 days after conception. They classified the fern like structure as (i) very marked fern structure, (ii) marked fern structure, (iii) some ferns, (iv) traces of ferns, and (v) no ferns, traces of cellular structure, (vi) definitely cellular, and (vii) highly cellular. In nonpregnant cows the pattern was like (i) to (iv) and in pregnant cows (v) to (vii).

Alliston et al. (1958) described that crystallization pattern in beef heifers was maximum at the time of oestrus and began to subside before ovulation.

Fallon and Crofts (1960) studied that the cervical mucus fern pattern during oestrus cycle is strongly related with the oestrogenic activity within five days of oestrus.

Horrath (1960) reported the diagnosis of pregnancy in 36 cows, 69 mares and mating time in 18 bitches with the help of cervical mucus crystallization pattern.

Makkaveev (1961) advocated that fern pattern of cervical mucus should be taken as a positive test of oestrus if symptoms of heat were not apparent and insemination should be done when os of the uterus was opened.

Abusineina (1962) studied the fern like crystallization pattern of the cervical and vaginal mucus of cattle. The author classified the fern patterns into three parts

according to the physical properties and formation of the ferns of mucus. In type 'A', cervical mucus was translucent, acellular elastic and easily obtainable from the cervix. The stems were long and thin, wavy or curved with well developed clear venation and tiny subvenation. In type 'B' mucus was semi clear, elastic. Patterns were like fern shape. The venation and subvenation are clear and well developed. Crystals cover 50 % of the film.

In type 'C' mucus was opaque, cellular and difficult to aspirate. The stems were short, with or without irregular stellate, cruciform and linear patterns. The formations are not dense and cover 2-5 % of the film and no pattern was found for 7-9 days in dioestrus. The author emphasised that fern like patterns should not be used in the diagnosis of pregnancy as the pattern was similar to pattern of dioestrus in non-pregnant and it should be used to diagnose the stages of oestrus cycle.

Irregular venations scattered over the film due to the lowest oestrogen level between last 4 or 5 days of the oestrus cycle and no pattern for 7 to 9 days in dioestrus. The author concluded that crystallization pattern should be used to diagnose the stages of oestrus cycle experimentally but not recommended to diagnose pregnancy as the pattern was similar to pattern of dioestrus in non-pregnant.

Chaurasia (1962) observed the crystallization pattern of vaginal mucus in 86 Haryana cows and found definite patterns between 5 to 9 hours of oestrus. The author concluded

this method to be useful for knowing optimal stage of oestrus for insemination.

Ganic and Sevcik (1962) reported the crystallization pattern of cervical mucus from 2 to 3 days before oestrus and found that fern structure was more pronounced during oestrus and at about the time of ovulation. They concluded that the fern pattern test was suitable to diagnose various phases of oestrus cycle and proved to be the secondary method for diagnosing pregnancy.

Senze and Zebracki (1962) estimated the value of crystallization of cervical mucus and glycogen test to correct the hormonal level, feeding and management of the animals for better conception rates.

Quayam and Venkataswami (1964) observed the crystallization pattern of cervical mucus in Murrah buffaloes. They found fern pattern at oestrus and gradually declined at dioestrus with the lowest value of 5 % at 12th and 13th day post-oestrus and concluded that patterns were affected during pathological conditions of reproductive organs which increased more when ovary involved.

Roy Choudhary (1964) reported that the crystallization pattern of cervical mucus of Tharparkar, Sahiwal and Red Sindhi cows and heifers at Karnal and noted that there was marked difference in crystallization pattern at different stages, oestrus characterised by typical fern like appearance, during dioestrus it has straighted appearance whereas during

pregnancy it was frizzy hair like with cellular infiltration and in advanced pregnancy the matrix appeared just like lipid droplets with slight cellular infiltration. He further studied the fern pattern of cervical mucus in 81 oestruses of 31 Hariana heifers and found typical, atypical and no pattern in 35.80 %, 29.62 % and 34.58 % respectively and ovulation percentage as per pattern was 88.88 %, 41.66 % and 3.33 % respectively.

Hukeri (1965) observed the crystallization pattern of cervical mucus in Tharparkar cows during the oestrus cycle, pregnancy and anoestrus conditions. The author found no pattern during pregnancy and anoestrus conditions but typical fern pattern was recorded in 95 % cases during oestrus cycle which gradually disappeared in dioestrus stage. The author concluded that with crystallization technique the stage of oestrus cycle could be ascertained.

Luktuke and Roy (1967) collected the samples of cervical mucus from 95 Hariana cows and 33 Murrah buffaloes during oestrus and classified as typical, atypical or nil pattern. In cattle the percentage was found 63 %, 17.7 % and 19.2 % respectively; in buffaloes 72.7 %, 18.2 % and 9.1 %. The cows showing gestational heat, 25 % showed typical fern pattern and of those 66.7 % calved normally.

Roy Choudhury and Rajdan (1968) studied the crystallization pattern in 170 Tharparkar, Sahiwal and Red Sindhi cows. The authors found typical fern pattern during oestrus and no definite fern pattern during dioestrus. They

considered the crystallization pattern as a suitable index for ascertaining the different stages of the oestrus cycle in cattle.

Rosanowaski and Koefoedjohmen (1968) conducted daily examination of crystallisation pattern of cervical mucus during one or more sexual cycles in cows and heifers. They showed that the pattern had a tendency to change from short, coarse and broken fern leaves during growth of follicles into long, fine, winding leaves during the period of complete maturation and rupture of the follicles took place.

Sharma et al. (1968) studied on Haryana females at Mathura and found that the stages of oestrus could be recognised as early, middle and late with shape and size of fern pattern observed in dried smears of cervico-vaginal secretions.

Sinha (1969) studied the crystallisation pattern in heifers, normal breeders and repeat breeder cows of Tharparkar breed. The author did not find any significant difference between repeat and normal breeders.

HYDROGEN ION CONCENTRATION (pH) OF CERVICAL MUCUS:

Hydrogen ion concentration (pH) of the cervical mucus seems to have an important role to play with repeat breeders in conception rate, because the spermatozoa has to swim enroute in this medium and the contents and qualities of which should be favourable to sperm to increase its motility and viability for fertilization of the ovum. Spermatozoa comes under the influence of cervical mucus but it is not known to what extent and what manner (Henrit and Veryvack, 1963). It has been proved that spermatozoa remains alive for eight hours in cervical mucus during oestrus of all the luminal fluids (Olds and VanDemark, 1957a). After knowing the various properties of cervical mucus it seems that pH may be a responsible factor in cervical mucus in repeat breeders.

Some workers have reported that acid condition in cervico-vaginal passage may be responsible for temporary sterility which can be corrected by alkaline douchings at the time of service which insists to know something more about the effect of pH of the cervical mucus.

Shipley (1924) reported that when the litmus paper in cervical canal is introduced in those cows which were ~~are~~ suffering from repeat breeder, pH was found acidic which accounted considerably for the sterility.

McNutt et al. (1939) observed the reaction of vaginal secretions of 8 virgin heifers and 3 cows in oestrus.

The pH was found alkaline during oestrus and that might had been due to the copious cervical secretion during oestrus which was more alkaline than that of vaginal pH varying between 6 to 7.

Smith and Asdell (1941) found the pH of vaginal mucus as alkaline and a drop in pH (8.0 to 7.0) a short period during oestrus and just after oestrus and concluded that large doses of oestrogen did not cause pH acidic and sterility is not due to acid condition of the vagina.

Calisti (1946) studied the isoelectric pH values of cervical mucus during $4\frac{1}{2}$, 7, 8, $9\frac{1}{3}$ and $9\frac{2}{3}$ months of pregnancy as 3.96, 3.92, 4.28, 3.04 and 2.88 respectively.

Filatova (1952) reported the pH of vaginal mucus 7.0 - 7.8 and found that there was no relation between conception and pH within these limits.

Chaudhary and Prasad (1954) recorded the pH values of vaginal washings in 135 normal Tharparkar cows which were between 6.5 to 7.0 with a range of 6.0 - 8.5 and slightly below 7.0 from 4th month onwards of pregnancy and during oestrus the pH value appeared to be near 7.0. They concluded that age and stage of lactation does not appear to have any effect on pH values and no abnormalities in pH values could be found in cases taking three or more services before conceiving. Embrys, Roberts quoted by Hammond found that in the cows the mucinous secretions during proestrus is derived not from body of the uterus but from cervix and

vagina. They have quoted the work of Roark and Herman(1950) who did pH determination of bovine mucus during oestrus cycle with range of 6.18 to 8.30 which varied more between 6.90 to 7.29. They found that the vaginal pH of the difficult breeding tended to be more acidic than that of normal and pregnant cows. They recorded that the pH of vaginal mucus was more alkaline during pregnancy than normal and difficult breeding cows.

Olds and VanDemark (1957a) reported the average pH of cervical vaginal mucus as 7.8 in bovine females and concluded further (1957c) that the vaginal and uterine pH rises during oestrus and infection in the cows.

Shalash (1958) studied the pH of vaginal secretion in 50 buffalo-cows during different stages of oestrus cycle. The average pH was 7.3 during oestrus, 8.1 during dioestrus and 8.3 during pregnancy and concluded that though these differences were statistically significant they are of no diagnostic value.

Higaki et al. (1959) reported the pH of cervical mucus in 10 Holstein cows as 7.5 just after parturition and thereafter gradually falling to 6.7-6.6.

Hartwig (1959) observed pH in 269 cases of cervical mucus by electrical apparatus which varied from 6.12 to 8.5. The average pH being 7.24 and found 40.67 % and 45.36 % conception rate per pH below average and above average respectively.

Gupta (1962) studied on 40 samples of cervical mucus in cows recorded the average pH 7.8 and concluded that it varied among successive samples from the same cow while hormonal treatment influence the pH as oestrogen shifted it towards alkalinity while chorionic gonadotropin to acidity.

Cseh (1963) examined the pH of cervico-vaginal mucus in 293 cows and found the conception rate 35.82 % when the cervico-vaginal mucus had a pH of 6.7 to 7.0. According to the author below or above the mentioned pH value the mucus contained hazy granules, pus and/or epithelial cells. He suggested that the change in mucus pH value can be brought about by external influences such as feeding and therefore it is of great diagnostic value in repeat breeder cases (cows or heifers) when clinical examination of the cervix and microscopic inspection of the mucus failed.

Hukeri (1965) showed, on analysis of variance that the difference of pH between and within groups of estrus and oestrus cycle was not significant. Correlation between pH of cervical mucus of 8 oestrus groups with the corresponding groups of conception rate was not significant.

Toubrich (1959) described the pH from cervical portion of vaginal secretion 179 times in 96 cattle with a range of 7.0 to 8.2 and 34 % of the samples showed 7.7 to 7.9 pH value while pH was 0.6 - 1.0 higher in the initial portion of the vaginal than the cervical portion and during oestrus pH was lowered by 0.5 and concluded that there was no difference in pH between pregnant and non-pregnant animals and the age and bacterial content of the vagina appeared to have no influence on pH.

Salisbury and VanDemark (1961) cited the work of Swedish workers (Thygesen, 1947) that infection in reproductive tract cause an increase rather than decrease in the pH between infected part and the exterior and donot cause an acid condition. They quoted the pH of cervical mucus in vitro as alkaline 9.0-9.2, 8.33 and 6.95 as per work of Woodman and Hammond (1925), Miller et al. (1931), Lardy et al. (1940) and Sergin et al. (1941) respectively.

Ahuja et al. (1961) reported the average pH of vaginal mucus in Hariana cows as 7.33 ± 0.04 during oestrus.

Chaurasia (1962) studied the pH of vaginal secretion in Hariana cows at 4, 5, 6, 7, 8, 9, 10, 12 and above 12 hours from onset of oestrus and found the results as 8.0, 7.8, 7.9, 7.9, 8.0, 8.1, 8.0, 8.1 and 8.1 respectively with overall average as 7.98 and concluded that pH was more towards alkalinity with advancing stage of oestrus but is not of any significance in identifying the stage of heat.

Gupta (1962) studied on 40 samples of cervical mucus in cows recorded the average pH 7.8 and concluded that pH varied among successive samples from the same cow while hormonal treatment influence the pH as oestrogen shifted it to alkalinity while chorionic gonadotropin to acidity.

Cseh (1963) examined the pH of cervico-vaginal mucus in 293 cows and found the conception rate 35.82 % when the cervico-vaginal mucus had a pH of 6.7 to 7.0. According to the author below or above the mentioned pH value the mucus contained hazy granules, pus and/or epithelial cells. He suggested that the change in mucus pH value can be brought about by external influences such as feeding and therefore it is of great diagnostic value in repeat breeder cases (cows or heifers) when clinical examination of the cow and microscopic inspection of the mucus failed.

Hukeri (1965) showed, on analysis of variance that the difference of pH between and within groups of oestrus and oestrus cycle was not significant. Correlation of pH of cervical mucus of 8 oestrus groups with the corresponding groups of conception rate was not significant.

TREATMENT OF REPEAT BREEDERS:

In recent years research workers have published data that tend to minimise the importance of repeat breeders. Repeat breeding is a disease of unknown etiology with no definite remedial measures. No satisfactory results have been obtained till now, although various medications have been tried to eliminate the condition.

In infection whether nonspecific or specific would have to be subclinical in nature as determined by definition of the animal. There are numerous reports that indicate an improvement in breeding performance following treatment with antibiotics but in most of these instances either there were no control or there was clinical evidence of inflammation ahead of time which is also ruled out in present discussion.

Vogt-Moller (1931) reported the importance of vitamin E, which is lacking in the food. He used wheat germoil (vit.E) for the treatment of repeat breeding. The results obtained after a single dose of wheat germoil administered I/m must be termed so satisfactory, that they encourage further experiments with the said therapy. When the cow is in heat 10 to 20 c.c. of wheat germoil is injected I/m was covered on the same day and has been quite since.

Frank (1934) used 80 c.c. of extract of ovary after removal of corpora lutea was found to be efficacious in influencing conception in animals which previously failed to conceive after a number of services. Approximately 90 %

of the cows conceived after the first service.

Stuhlen (1942) reported those cases of cows which returned to heat at three weeks interval. According to him in 687 cases 64.3 % ~~the~~ cows returned to the bull every three weeks, in rather more than half of those, the ovaries functionally normal, while the rest were characterised by false heat. So repeat breeding may be due to cystic ovaries and the best treatment of it is the injection of oestrogen and removal of persistent corpus luteum.

Asdell and Coworkers (1942) showed that blood, tissue and organ chemistry in repeat breeding cows have revealed nothing of significance. Bentley and coworkers also found the same results.

Labatut (1945) claimed that in a returning group the persistent corpus luteum was found in each case, but there were no cases of cyst formation. Enucleation was accompanied by wheat germoil injections, for treatment of all the cows returning to service, 93 % subsequently conceived to the 1st or 2nd service.

Hingerford (1946) did a routine treatment of repeat breeders. The ovaries were examined per rectum and any cyst or corpora lutea found were expressed. An aqueous solution of iodine was injected into the uterus and 30 mg of stilboestrol injected intramuscularly. It is claimed that after one treatment 70 % of cows became pregnant after service.

Durrell (1947) observed in thirtynine cases varying between the age of one and half to twelve years old cows which had failed to conceive after at least three services, Conception resulted in 11 out of 23 cows treated with P.M.S. (Pregnant mare's serum), in 4 out of 9 injected with APL, in one out of 4 injected with progesterone and in one out of 3 injected with P.M.S. early in oestrus with progesterone late in oestrus. Neither of two cows conceived to service after the application of hyaluronidase to the cervix in early oestrus.

Chambers (1948) treated to those cows which had oestrus at regular intervals after failed to conceive following repeated service. Rectal examination revealed a slightly enlarged uterus lacking in tone which is believed to indicate low grade uterine infection. He used intra-uterine infusion of 200000 units of penicillin in 15 ml of water in 5 cases. All the treated cows bred at oestrus subsequent to the treatment.

Kingman (1948) suggested that too little attention had been given to the physiology and pathology of the placenta, in such conditions as retained placenta, abortion and failure of implantation. The subcutaneous injection of 125 mg. progesterone over 25 days produced marked increase in the size of uterine horn.

Tanabe and Casida (1949) observed that the initial fertilisation ratio in 104 repeat breeding cows of Holstein and Guernsey breeds was as high as 66.1 % three days after

service. But at 34 days the percentage having normal embryos had dropped to 23.1 % and there was an embryonic death rate of 65.11 %. Casida (1950) listed uterine infections among others as a major cause of embryonic mortality leading to repeat breeding.

Easterbrooks and Plastridge (1950) recommended the use of Streptomycin and Penicillin for vibronic infertility in dairy cows. They observed 83.33 % cows and 75 % heifers settled as a result of intra-uterine treatment with antibiotic in present investigation.

Herrick (1951) gave intra-uterine injection of penicillin and Streptomycin to 78 repeat breeders of which 67 % became pregnant at first service.

Christian et al. (1951) treated 116 cows, each of which had been bred 4 or more times previously without apparent conception. 5 gm of Chlorobutanol fed daily increased the ascorbic acid concentration of the blood plasma on the day of and two days after heat and also increased the ascorbic acid concentration of the anterior pituitary. 3 gm of ascorbic acid injected subcutaneously every other day from the day of heat until slaughtered failed to maintain an increased ascorbic acid content of the blood plasma. When the results of Chlorobutanol and ascorbic acid were combined, the percentage of cows with normal embryos at 34 days after breeding was 26 in 43 control cows and 30 in 45 treated cows. There is no evidence from these studies that ascorbic acid stimulation is beneficial in increasing conception rate in repeaters.

Bentley et al. (1951) was of opinion that trace minerals have little if any direct relationship to the repeat breeding. In the same year Bentley and Philips investigated the effect of the level of manganese fed on the calves. A diet low in manganese with or without supplements was fed to 18 grade Holstein-Friesians for 1, 2 or 3 lactations. There was no appreciable difference in growth due to level of manganese fed. Heifers on the low manganese diet first came on heat about 2 months later and required more services per conception than those fed manganese supplements.

Flynn (1951) presumed all cows to have been mated atleast oestral period, uterine irrigation will rather dislodge those calves, which may have been conceived than affect any improvement.

Ulberg et al. (1952) treated 57 cows which had been inseminated four or more times without apparent conception. Treatment consisted of a mixture of 1 gm. of dihydrostreptomycin; 0.25 gm. of aureomycin and 100000 units of penicillin in 125 ml of sterile distilled water placed in the uterine lumen. Those animals that had not returned to oestrus by 34 days after a breeding were slaughtered and for the presence of normal embryo. The percentage of cows with normal embryos was 34.8 for the treated animals and 56.0 for the control animals. The difference was not regarded as statistically significant.

Casida (1953) cited his works with Ulberg showing that progesterone administered during the early stages of pregnancy in the rabbit had no effect on reducing embryonic deaths. Laing stated that histological evidence is against a lack of progesterone as a cause of embryonic death.

Asdell et al. (1953) found that the treatment of repeat breeding using various hormone preparations has not been very successful.

Herrick (1953) reported that when 500 mg. of progesterone was given in 20 heifers at the time of service and 20 were kept as control, pregnancy resulted in 65 % and 20 % of the cows respectively.

Bonfert (1953) treated with Enoulan forte and Evion found to be efficacious in cases of sterility due to various ovarian disturbances and in cases of sterility without symptoms. Of 65 treated females, 39 conceived to first insemination and 5 to a subsequent one.

Mackintosh (1954) treated the apparently normal cows which regularly returned to service by expressing of the corpus luteum between 5 and 14 days after oestrus. Of cows so treated and not already in calf to the previous service 61.5 % conceived to the service which ensued in 2-9 days, 30.2 % to the next and 1.7 % to the 3rd service. A total of 93.4 % became pregnant within 7 or 8 weeks.

Merk (1954) incriminated vitamin E deficiency as the cause of repeat breeding in cow. So the author administered

vitamin E preparation, Enoulan forte to 144 cows which had failed to conceive after 1-4 services, 131 conceived when served at the next oestrus, the remainder conceived after a further one to two services.

Dawson (1954) worked on 47 heifers or cows with prolonged histories of repeat breeding were treated with 100 mg. progesterone by implantation and thereafter 36 held to first or second service. Of 18 kept as untreated controls from the same herd, 15 failed to at least 5 services, each while the treated animals were settling.

Lindley (1954) stated that a cow becomes a candidate for intra-uterine therapy if bred unsuccessfully two or more times. The intra-uterine infusion is given on the first or second day following service, whichever is most convenient. In the majority of cases treatment was administered on the second day following breeding.

Preparation:-

Streptomycin sulfate 1 gm.

Penicillin sodium or Potassium 2 to 5 lacs units dissolved in (a) 50 c.c. of sterile saline soln. or (b) 50 c.c. of sterile distilled water, or (c) 12 % of sulfapyridine solution.

The intra-uterine infusions were given with a Chambers or Woelffer type of uterine catheter to which a 50 c.c. glass syringe was attached. This gave a good result.

Flerchinger and Erb (1954) recommended that repeat service cycles reoccurred at intervals near the mode of 21 days

with non-falling outside the modal length of 18 to 24 days. He used penicillin, penicillin streptomycin and penicillin-streptomycin sulfanilamide but found that it did not materially influence the average length of return to service interval. This was caused by a higher percentage of repeat services ($p < 0.05$) recurring from 17 to 26 days in the low fertility group.

Johnson (1955) treated 20 females having had from 4 to 9 infertile services to a male of known fertility with a reposital progesterone. 16 conceived on first breeding, 3 on second and 1 on 3rd breeding after treatment giving a breeding efficiency of 80.0 %.

Wiltbank et al. (1956) observed after injecting in 36 repeat breeder cows with 50 mg of progesterone per day beginning 3 days after heat had normal embryos at 34 days as compared with 12 (33.3 %) in an equal number of uninjected control animals. 31 additional animals were injected daily with 200 mg. of progesterone, amongst them 12 animals (38.7%) had normal embryos at 34 days as compared with 8 (25.8%) in 31 control animals.

Jaskowerki and Walkowski (1957) investigated the use of gonadotropic hormones on repeat breeding with the removal of corpus luteum. The percentage of conception within 60 days varied from 30.6 % in a group of 75 cows receiving no gonadotropin after corpus luteum removal to 68.7% in 16 cows receiving 500 I.U. gonadotropin plus 25 mg progesterone

after the removal of corpus luteum. Of 55 cows receiving 500 I.U. gonadotropin after corpus luteum removal plus 25 mg. progesterone, 17 (30.9 %) conceived and of 28 cows with inactive corpora lutea that received 1000 I.U. gonadotropin, 12 (42.8 %) conceived. In a control group of 67 cows the conception rate within 60 days was 28.5 %.

McClure et al. (1957) mentioned the treatment of repeat breeders by expressing corpora lutea in 35 repeatedly returning cows. It reduced the 63 %. It did not, however, increase fertility at the next oestrus when compared with the fertility of cows mated at the oestrus after ineffective treatment.

Rosson (1957) evaluated the effect of enucleation of the corpus luteum on the fertility of the cows that failed to conceive. He treated 52 cows that failed to conceive after 3 or 4 inseminations although suffering from no obvious defect or disease, had the corpus luteum removed on the 11th or 12th day after oestrus. 23 (44.2 %) animals became pregnant after a single insemination. In a group of 52 untreated cows the conception rate after a single insemination was 42.3 % (21 animals). The difference in fertility was insignificant and indicates that corpus luteum enucleation does not necessarily improve the conception rate to any great extent while Mackintosh (1954) found a good result by enucleation of the corpus luteum and he observed 93.8 % conception in 7 or 8 weeks.

Johnson (1958) reported the effect of progesterone on reproductive efficiency which was studied in 166 cows. Progesterone (100 mg of 17- α -hydroxy progesterone-17-n-caproate) was injected intramuscular on the second, fourth, sixth and ninth day after first service in 82 animals. In treated 70.7 % conceived while the 84 controls had 46.4 %.

McKay and Thomson (1958) treated 312 repeat breeder cows with an organic iodine compound, 201 conceived after the first service, of 215 cows treated by expressing the corpus luteum 95 conceived after first service.

Sacchi et al. (1958) got good result by irrigating at insemination time or 24-72 hours later, with 10 ml. oxytetracycline (terramycin) solution containing equivalent 100 mg. bioactivity in 219 clinically normal cows after a mean of over 3 services. Conception rates of about 70 % to first insemination resulted and most of the remainder were settled at a second insemination. 67 untreated controls required about twice as many inseminations. The simultaneous treatment appeared less satisfactory than that given 24-72 hours after insemination.

Janzen (1959) in a survey of sterile cows treated 415 repeater cows. Astrom infused very dilute Lugol's solution into the uterus 2-4 days after service. He adopted this method by infusing 100-150 ml. of a solution of the Sulphone "Lotagen" 24-48 hours after insemination in which 73.5 % of the above cows became pregnant.

Luktuke et al. (1959) mentioned the preliminary investigations on intra-uterine treatment with antibiotics in repeat breeders cattle. According to their report, 36 cows and 4 heifers belonging to experimental Haryana herd at Izatnagar were used in this investigation. In 11 cows, 2 services did not result in pregnancy and they were taken for treatment on returning to the 3rd service. 25 cows had received more than three services each and had not settled. The heifers were 4-5 years old and did not conceive after two services. A proprietary drug (Strepto-penicillin-Dumex) containing - Penicillin G procain - 3,00000 I.U.

Penicillin G sodium - 1,00000 I.U.

Streptomycin - 0.5 gm.

Dihydrostreptomycin - 0.5 gm.

was dissolved in 40 ml. of sterile distilled water and the solution was injected in both the horns of the uterus through catheter with the help of 50 ml. record syringe. Of the treated cows 20 conceived after a single treatment and 10 after two treatments. Out of 4 heifers, three conceived after a single treatment.

Moller (1959) used the calciferol injections on bovine fertility. 1545 cows were treated and 1571 matched for age, yield and calving time left as controls. 32 normal herds and 7 with herd breeding problems were involved. Each treated cow received 3 millions units of calciferol oil subcutaneously, 6-10 weeks before commencement of the serving season. 7 problem herds, free from the usual genital

infections, showed an increased difference in conception rate about 6 percent in favour of the treated animals.

Tobler (1959) did special experiments with vitamin E on the treatment of 34 cows and 13 hiefers which had returned to service 2 or 3 times and had no pathological changes in the uterus or ovaries, were given an intramuscular injection of Enoulan forte at the same time as the next insemination. Out of the above treated animals 33 immediately became pregnant. After a further insemination without vitamin E injection, 5 more became pregnant.

Hansel et al. (1960) compared the conception rate in three groups of repeat breeder cows treated early in oestrus and bred at the normal time. The authors experimented 28 control cows injected with physiological saline, 38 cows treated with 20 mg. of progesterone and 40 cows treated with 2000 I.U. of Chorionic gonadotrophin. All of these cows had previously been bred 2 or more times artificially and had failed to conceive. The conception rate was lowered in the Chorionic gonadotrophin treated cows and was the same in control and progesterone treated cows. This indicates that Chorionic gonadotrophin should not be used in this way and suggests that little is to be gained by late breeding.

Becze (1961) used progesterone in the treatment of repeat breeder cows and in the increase of the litter size of the sow. The trials were based on assumption that progesterone would prevent the early death and reabsorption of the

embryo through its influence on the secretory phase of the oestrus cycle.

According to Becze, progesterone was of great value in the treatment of repeat breeder cows. A dose of 100 mg. progesterone was administered intramuscular daily to cows of three groups showing various irregularities in their breeding cycle, for 5 consecutive days. From first group of 11 treated cows, 5 conceived while of 11 such untreated cows, three conceived. Of a group of 28, most of which showed abnormal secretion during oestrus, 14 animals were treated and 7 of them conceived in contrast with 4 of the remaining untreated 14 animals. So it was concluded that progesterone is only valuable in the treatment of infertility of cows, when the oestral secretion is normal, or of a low viscosity, but is not likely to be so when the secretion is purulent.

Jeganathan and Maheswaram (1961) treated the repeat breeders in bovine by intra-uterine infusion with oxytetracycline hydrochloride and polymyxin B sulphate (Terramycin vaginal tablets). The infusions contained 100 mg. oxytetracycline + 1,00000 units of polymyxin B sulphate. The authors treated 9 animals 15 minutes after insemination, and out of 9, 6 conceived.

Luktuke and Joshi (1961) observed a satisfactory result when they treated 67 cows and 8 heifers with a solution containing penicillin 4,00000 I.U. + Streptomycin 1 gm. dissolved in 40 ml. of sterile distilled water, 24 hours after service. Of these, 45 conceived after one and 15 after two treatments.

The response of the above medicine was satisfactory in cows which have had 5 or less infertile services but poor in those which had 6 or more services.

Casida (1961) published a paper on present status of repeat breeder cows. Wiltbank et al.(43) used 50 mg. progesterone daily in one group and 200 mg. daily in other group, had better embryo survival by 11 % in the other. Recently Johnson et al.(20) have studied the ability of a similar treatment to improve the conception rate of 1st service and they found to have shown an advantage of the progesterone treatment.

Hjerpe (1961) evaluated the use of uterine infusion with antibiotics as a treatment for repeat breeding in dairy cows. The author divided into two groups of animals. One group cultured cervical mucus from 207 cows. Cows harboring pathogens were allotted into control and tetracycline treated groups. There was no difference in conception rates among control, harboring pathogens treated cows and bacteriologically negative cows.

Other workers studied a group of 48 repeat breeders They divided these animals into two groups. Half were kept as control and half were treated with antibiotics. 30-40 days after insemination, the cows were slaughtered and examined for the presence of embryos. Conception rates were 34.8 % for treated cattle and 56.0 % for control. The histopathological findings in uteri from 109 repeat breeders were studied and found that the endometria of the repeat breeding cows did not differ greatly from that of apparently normal heifers.

According to the report of Hjerpe (1961), a worker treated 58 repeat breeder cows with F.S.H. and out of 58, only 12 conceived. Administration of estrogen early in heat with delay ovulation has not been proved successful in the treatment of this or any other type of repeat breeders. A group of workers reported no increase in conception rate over controls in 36 repeat breeders treated early in heat with progesterone. 40 repeat breeders treated similarly with Chorionic gonadotropin had a significant decrease in conception rate compared with controls.

A total of 45 repeat breeding cows were treated by uterine infusion with penicillin 3 millions units and dihydrostreptomycin sulfate 1 gm in 10 c.c. of 0.9 % saline solution 24 hours post service. Of these, 28 cows (62.2%) became pregnant after treatment. In the control group out of 33 cows, 20 (60.6%) became pregnant. The difference between treated and control group is not significant.

Marion (1962) described the effect of wheat germ-oil on reproductive efficiency in repeat breeder cows. According to the author's report, clinically normal cows that had failed to maintain pregnancy after 3 or more services were alternately assigned to experimental or control group. The experimental cows were given 2 oz. of wheat germ oil in their feed, once a week for 6 consecutive weeks. A good result was obtained in 79 treated cows after the first post treatment service than of the 88 untreated cows. The difference in post-treatment conception between treated and untreated cows that

had three unsuccessful services before treatment was not significant, however, there was great difference in favour of the treated cows that had been bred 4 or more times before initiation of treatment. The results obtained by Marion are the following:- out of 28 cows bred three times before treatment and after first post treatment service the conception rate was 68 % whereas for the untreated 52 % only. In the cows bred four or more times before treatment, out of 30 cows, 80 % conceived in the treatment group and out of 15 cows only 34 % conceived in untreated group.

Persson (1962) treated the cows returning to insemination two or more times with an antibiotic ointment containing penicillin 50,000 I.U. and Dihydrostreptomycin (0.025 gm.) was injected through a catheter having 2-3 m.m. diameter into the uterus after deposition of the semen. In those cows who returned to insemination for the third time the conception rate was 72 % after this treatment and 60 % in controls; in 113 cows returning for second insemination 82.5 % became pregnant whereas in 268 controls 65.7 % became pregnant, in 160 cows given the antibiotic treatment at 4th insemination the conception rate was 73.1 % as compared with 49 controls being 48% conception.

Tesink (1962) fed mineral supplements equivalent to 120 mg. copper and 720 mg. manganese during winter housing, in the middle of April and the end of May the pastures were dressed with 5 kg. manganese sulphate and 2.5 kg copper sulphate per hectare. Treatment during winter appeared to increase the proportion of cows pregnant after first insemination from 51-69%.

Hawk et al. (1963) used hormonal therapy after ovariectomy in repeat breeder cattle and observed the embryo survival in first service. The author performed ovariectomy 5-7 days after mating, followed by replacement therapy with progesterone or progesterone + oestrogen. The result was obtained that pregnancy can be maintained in ovariectomised first service cattle, they do not indicate the minimal amounts of exogenous hormones necessary during early pregnancy in normal cattle, injected estrogens was not found to be necessary. Injections of same amounts and balances of ovarian hormones into ovariectomised repeat breeders did not improve their fertility.

Petersson (1963) compared the repeat breeder cows by dividing into two groups. In one group he used intra-uterine treatment with iodoform, liquid paraffin, terramycin-polymyxin B, embryostat (an oxytetracycline preparation) and strepto-enicillin and the other group was kept as control. Iodoform, liquid paraffin and terramycin-polymyxin B tended to reduce fertility as compared with controls. Intra-uterine treatment with embryostat between calving and first insemination also seemed to reduce the fertility. Intra-uterine treatment of repeat breeder cows with germicidal or bacteriostatic drugs should be avoided if pathogenic agents do not appear to be present.

Gibbons and Kiesel (1964) treated the repeat breeder cows with the antibiotic through uterine infusion; and found a better result.

Filkins (1964) used progesterone in the treatment of repeat breeder cows. According to the author the treatment with progesterone in repeat breeding heifers can increase conception rates.

Vlachos and Tsakalop (1964) treated the repeat breeder cows suspected of wind sucking and found a good result.

Wilson (1964) used nutritional treatment for the repeat breeder cows. Bentley et al. (1951) showed average values of 23.2 ppm. as the manganese content of the hays with a range of 8-9 to 48.5 ppm. 12 farms out of 29 farms had hay below 20 ppm. which seems to be on the low side.

Zaki (1964) reported the treatment of repeat breeder cows by injecting vigantol to improve the pregnancy rate.

Brone (1965) reported the importance of suspensions CIBA 10370-Ba and 35632-Ba in Bovine and porcine in repeat breeder cases. CIBA 10370-Ba contains 5 % formosulphathiazole 5% sulphachloropyridazine and 0.002 % ethynyloestradiol. CIBA 35632-Ba contains 5% sulphachlorodazine, 5% sulphamethylphenazol, 3% iodochlorhydroxyquinoline and 0.002 % ethynyloestradiol. A dose of 30 ml. was injected into the uterus of 438 repeater cows (1) immediately after or (2) 24 hours after A.I. or (3) during heat followed by insemination or service, 3 or 6 weeks later, 330 (75.3 %) became pregnant.

Gomes and Erb (1965) used progesterone in the treatment of repeat breeder cows. They found a better result.

Gubarevich and Tereshenkov (1965) treated the repeat breeder cows by using prozerin (neostigmine) and ascorbic acid.

Maksimov et al. (1965) reported the importance of vitamin E upon repeat breeder cows. The work of Tobler(1959), and Merk (1954) is also agreeable with the work of the above author.

McClure (1965) investigated that the cows are repeaters due to nutritional deficiency. The author divided all the animals into treatment and control group. In the treatment group hay was fed ad lib from calving to 3 weeks after first service. At first insemination, supplemented cows had a body weight 96.5% of that immediately after calving verses 93.3 % in controls. The corresponding body weights for cows having fertile and infertile services were 98.1 and 92.7 %. The non-return rate to first insemination of 34 supplemented cows was 62 % Vs 13 % in 39 control and the difference is significant.

Roussel et al.(1965) treated the repeat breeders cows with pregnant mare serum. They worked on 41 repeat breeders of 19 Holstein, 12 Jersey, 4 Guernsey and 6 Brown-swiss cows. Cows were randomly assigned into a control group of 18 cows and the treated group of 23 cows. The average unsuccessful service for each animal prior to commencement of this study was 6.8 for the control and 6.4 for treated

group with a range of 4.0 to 12.0 services. The animals which were in treated group were given 500 R.U. of P.M.S. I/m on the 15th or 16th day of cycle. The percentage of conception for the control and treated cows were 44.4 and 73.9 % respectively. Finally the author concluded that the level of 500 R.U. of P.M.S. has no adverse effect on young embryo.

Hullet (1966) reported the effect of various treatments with 6-methyl 17-acetoxypregesterone (MAP) on repeat breeders in ewes. He reported that a higher proportion of ewes lambed to the first service when put to the ram at the second heat after treatment with MAP (75.9%) than when mated at the first heat (56.3%). A 60 mg. daily dose of MAP resulted in 13 % more ewes lambing to the first service than did a 50 mg. dose (73.1%) vs. 54.6 %.

Nedyalkov (1966) used some trace elements in the ovine sterility. In 3 flocks where around 12 % of the ewes had improved by dosing each ewe during or before the mating period with 0.25 gram potassium iodide plus 0.5 gm copper or with 15 mg. zinc sulphate.

Baumgart (1967) evaluated the use of 1 % oily solution of Chlormadione acetate (Gestafortin) was given at insemination or eight days later to insemination. The author used intramuscular injection in 34 cattle requiring a second insemination; 9 animals (16.6%) returned to estrus compared with a normal return rate of 30-33% of 59 cattle treated similarly at first insemination, only 11 (18.6%) returned to

oestrus compared with a normal return rate of 30-35 %.

Khan and Luktuke (1967) reported the effect of antibiotic intra-uterine therapy in repeat breeder cows.

The authors used the following medicines:-

Penicillin	-	400,000 I.U.
Streptomycin	-	1 gm.
Distilled water		40 ml.

They treated 16 Hariana cows with the above medicine and 8 cows were kept as control. 69 % of the treated and 25 % of the untreated cows conceived. Most of the treated animals conceived with a single treatment only but for some cows 2 or 3 treatments were required for conception. Luktuke et al. (1959) also reported the use of ABIU (antibiotic intra-uterine) treatment.

Hawk et al. (1968) studied on the antifertility effect of intra-uterine devices in the cow. They inserted plastic spirals through the cervix into one or both uterine horns of cows. Cows were infertile after natural mating when a spiral was present in both the uterine horns, pregnancies resulted in cows with a spiral in only one uterine horn when ovulation occurred on the opposite side.

Jaskowski and Bernacki (1968) observed on the fertility in 38 Black Pied Lowland heifers with regular ovarian cycles were divided at random into 2 groups, one of which was kept on a water free diet (hay ad lib and 1 kg. concentrate) for 2-6 days, starting on the day of oestrus

and ending when animals lost about 15 % of its initial body weight and the other (controls) on the same diet plus 20 litres of water a day. Experimental heifers that lost more than 5.7 % of their initial weight during the first 24 hours of water restriction showed a lower conception rate.

Teresenkov (1968) reported the effect of ascorbic acid on uterine contractile activity and conception rate of the cows. The injection of a single dose of 3-4 mg ascorbic acid/kg. body weight at oestrus increased uterine motility and conception rate.

Bikkulov (1969) treated the repeat breeder cows with 17-alpha-oxyprogesterone capronate and PMS. According to the author groups of 10, 48, 44, 17 and 11 cows with a high return rate of service were given an over-all dose, by injection of 900, 1600, 1500, 8000 and 400 mg. 17-alpha-oxyprogesterone capronate respectively in addition the last group was given a dose of 6 I.U. PMS/kg.body weight. The conception rate after treatment was 70.0, 78.6, 93.6, 56.3 and 84.6 % respectively vs 38.2, 42.2, 48.3, 33.3 and 43.1 % in 3, 3, 16, 3 and 9 control groups.

Liptrap (1970) reported the rationale of hormonal therapy for repeat breeder cows.

Backstrow (1970) used lugol treatment of repeat breeder cows by painting the cervix with iodine/potassium iodide solution resulted in 10 % increase in pregnancies as compared with untreated control cows.

Kupfers and Hirschy (1970) reported on trials to improve the non-return rate in cattle by giving Chlor-madinone acetate or Choriogonadotropin with vitamin E at the time of second insemination. They divided into two groups of 274 and 246 cows which had returned to oestrus following first insemination were given 50 mg. CAP and 600 I.U. HCG + 90 mg.vit.E at the time of reinsemination, respectively. The 150th - 180th day non-return rates were 47.4 and 61.8 % respectively vs. 69.8 in 268 untreated cows that had failed to conceive to first insemination.

MATERIALS AND METHODS

MATERIALS AND METHODS

MATERIALS AND METHODS

Breeding records of Tharparkar herd of the Government Cattle Farm, Patna was analysed. A cow was considered as a repeat breeder, if it failed to settle after three services. Besides this the cases which were brought in Bihar Veterinary College Hospital were also undertaken for the study.

Skilled labourers:

Three labourers were used for the purposes. They were used for how to use the teaser bull for heat detection and to record the time of occurrence of oestrus.

Detection of oestrus during the grazing and night hours:

The attendants who were allotted with the herd during the grazing and night hours were instructed to report about the occurrence of oestrus in these animals.

Personal observation:

Besides all the measures taken to detect heat in these animals, the author also used to go in the herd for the purpose and watching of oestrus five to six times daily between 5 A.M. to 10 P.M.

Plate no.1. Showing the herd of
Repeat breeding animals in
Government Cattle Farm, Patna.



Plate no.2. Teaser detects a cow
in oestrus.

Collection of cervical mucus:

All the animals which were repeaters, brought in a yard. After taking all the hygienic, antiseptic and aseptic precautions, vaginal speculum with little liquid paraffin smeared on the outer part was inserted in the vagina after separating the two vulval lips with fingers and the cervix was located. Hand torch was also used sometimes whenever it was needed. A glass inseminating pipette was fixed to a 20 c.c. glass syringe with the help of rubber tubing for the collection of cervical mucus. The collection pipette was introduced in the os uteri carefully without touching any part of the speculum and the cervical mucus was sucked in by the syringe (Plate no.

). Soon after collection a drop of cervical mucus was taken on a neat, clean and thin slide and thin smears were prepared. The rest of the cervical mucus was kept in a glass test tube to study the chemical properties. The smear was left for drying at room temperature for 20-40 minutes for the formation of fern pattern. The slide was examined under low power (10x10) of microscope for the presence of fern like crystallization pattern of cervical mucus. The cervical mucus was collected between 0 to 12 hours after the onset of the oestrus and smears prepared from each sample were kept separately.

The main fern-like figure was represented by a central stem with venations and subvenations occurring



Plate no.3. Collection of cervical
mucus.

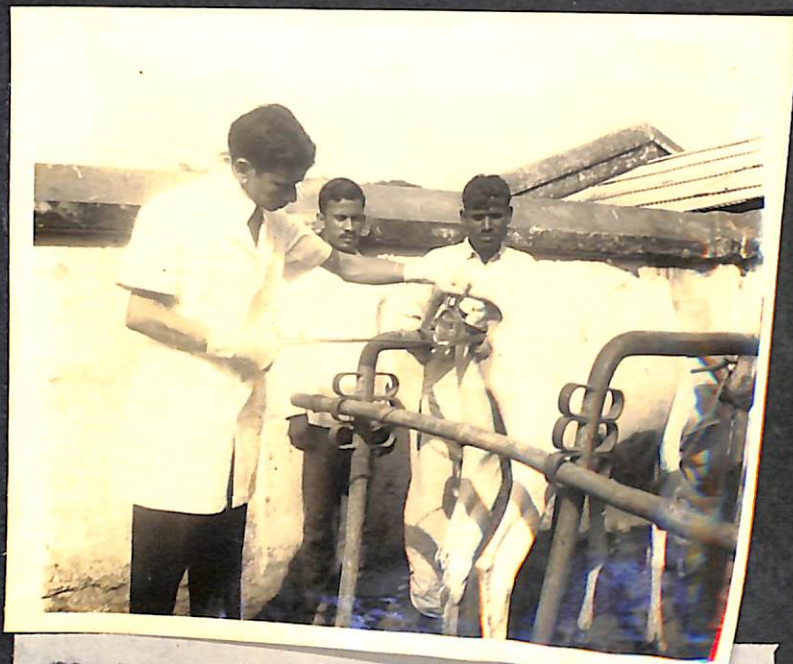


Plate no.4. Determination of pH by
B.D.H. narrow range
litmus papers.

perpendicular to each other (Figure 1).

Classification of fern pattern as typical, atypical and no pattern was made according to the pattern of crystallization arborization are as following:-

Typical Pattern:

This type of pattern gave the appearance of true fern leaf which had a central stem, long, wavy or curved with clear venations and tiny subvenations of same type and uniform size throughout the smear area nearly 90 % under study. Slides showing such patterns were included in this group (Plate nos. 5 & 6 photomicrographs show the typical pattern of cervical mucus).

Atypical Pattern:

It differs from the typical pattern in the following respects that in this type of fern pattern venations and subvenations were of different size and shape. Some of them were scattered irregularly with broad, crusiform, linear and stellate patterns. This type of pattern covered a much less area of the slide under microscope as compared to typical pattern. (photomicrographs plate no. 7, 8 & 9 show atypical pattern).

No Pattern:

In this type of pattern a few irregular stems,

venations and subvenations were found scattered here and there separately in the smear. In some smears there were no trace of patterns except than a few inclusions of cells etc. (Photomicrographs of plate no. 10 show no pattern).

In some slides black spots were seen which were due to dust particles at the time of drying in the room and also due to the effect of weather. In rainy season the slides took longer time in drying than that of summer, so in some cases artificial drying was done by keeping the slides on the top of the hot air oven.

25 animals in Government Cattle Farm, Patna were taken under the study for crystallisation pattern of cervical mucus during oestrus. Besides, 40 animals (including heifers and cows) were also examined for crystallisation pattern before and after treatment.

The results of crystallisation pattern were analysed for different group of animals before and after the treatment.

pH determination:

Collection of cervical mucus in sterilised test tubes has been mentioned in page 56 of this thesis.

The test of pH of cervical mucus was done on the same day after collection with the help of B.D.H. litmus papers having several numbers, e.g. B.D.H. pH paper no. 5570 for 5.5 to 7.0, 7085 for 7.0 to 8.5 and 8510 for 8.5 to 10.0

respectively. In some cases pH was determined also with the help of B.D.H. capillator using Brom-Thymol Blue (Range 6.0 to 7.6) and phenol red (range 6.8 to 8.4) as indicators. Each sample was tested two times with each indicator in a porcelain plate. For the process, equal volume of the indicator and cervical washings were taken in porcelain plate which was rinsed with normal distilled water before use, and were mixed thoroughly and the colour was matched with different shades supplied in the capillary set.

The pH indicator papers of different numbers having the difference of 0.3 only from each other matching colour was introduced in the cervical canal of repeat breeder cows and heifers before and after treatment. In some cases the pH of normal cows and heifers were also determined.

Treatment:

Government Cattle Farm, Patna:- All the cows and heifers in the farm which were repeaters, were separated from the herd. The number of inseminations varied from three to ten in different animals. They were clinically normal, yet failed to conceive. Out of 25 animals, in only two some abnormal discharge was present. All the cows and heifers belonging to this problem were examined per rectum and per vaginam. In almost all the cases the ovaries and

uterine horns were found normal. They were brought for the first time when they came in heat to study the effect of different drugs which were under trial. The age varied between 3 years to 5 years and 5 months in heifers and the insemination interval varied from 20 to 80 days in cows.

Selection of Drugs:

- (1) Tonophosphan solution,
- (2) Siolan 12 plus Placentrex, and
- (3) Vitablend AD₃ plus Prepalin forte.

(1) Tonophosphan (20%) solution:- Each ml.

contains - Sodium salt of 4-dimethylamino - 2 methylphenylphosphinic acid - 0.2 g.

Phenylethanol N.F. (as preservative) 0.6 % W/V

Water for injection I.P. - q.s.

It is a proprietary preparation of Hoechst.

It is available in 30 ml. bottle.

(2) Siolan 12 plus Placentrex:-

Composition of Siolan 12 injection (Sterile milk protein with vitamin B₁₂):-

Sterile milk protein

Total nitrogen 0.48 % W/V

Cyano cobalamin I.P. 100 mcg/ml.

Tricresol (Preservative) 0.3 % W/V, and

Adequate overage of vitamin added.

Siolan 12 is available in a box containing 6, 12, 25, 50 and 100 ampoules of 2 ml. and 5 ml.

Composition of Placentrex is composed of sterile aqueous extract of human placenta. It is available in a box containing 3, 6, 12, 25, 50 and 100 ampoules of 1 ml. and 2 ml.

Siolan 12 and Placentrex both are the proprietary preparation of Albert David Ltd.

(3) Vitablend AD₃ and Prepalin forte:-

Composition of vitablend AD₃:- Each gm. contains-

Vitamin A - 50,000 I.U., and
Vitamin D₃ - 5,000 I.U.

It is available in 20 gms. and 100 gms. packet.

Composition of Prepalin forte:- Each 1 ml.

ampoule contains :-

Vitamin A - 300,000 I.U., and
Chlorbutol (Preservative) 1 % in oily vehicle
(appropriate overage added).

It is available in 1 ml. ampoule.

Vitablend AD₃ and Prepalin forte both are the proprietary preparation of 'Glaxo'.

Grouping of Animals:-

The animals were divided into four groups in which three groups in treatment side and one group was kept as control. The grouping was done as at random in all

the suffering animals as mentioned below:-

<u>Group</u>	<u>Animal nos.</u>	<u>Drug trial</u>
I	42/63, 97/63, 75/62, 133/60, 24/5, 56/67.	Tonophosphan
II	105/6, 33/8, 59/66, 73/66, 190/66, 146/67.	Siolan-12 and Placentrex.
III	30/6, 35/7, 125/64, 37/66, 82/66, 21/68.	Vitablend AD ₃ and Prepalin forte
IV	97/T, 11/9, 24/62, 204/66, 28/67.	Control.

Group I :

All the six animals grouped under this group, were brought to the trevis, and were examined per rectum as well as per vaginum. There was no any abnormal discharge notwithstanding they were injected with 5 ml. Tonophosphan solution (injectable) and were not inseminated that day. The dose was repeated on alternate day for 6 subsequent injections and then the animals were left and watched for heat. They came in heat on different days. When they came in heat, were examined per rectum and per vaginum for ovulation and discharge respectively. They were inseminated artificially two times (morning and evening, or evening and the next morning) after ovulation took place. They were examined for conception after two months.

Group II :

The animals kept under this group were examined per rectum and per vaginum. They were administered on first day with Siolan-12 1 ampoule of 2 ml. mixed with 1 ampoule of 1 ml. of Placentrex intramuscularly. The injection was injected on alternate day. The amount was increased from the second time, i.e. Siolan 3 ml. mixed with 1 ml. Placentrex. In the third injection the amount again was increased i.e. Siolan-12 five ml. mixed with 2 ml. Placentrex and was kept constant on next alternate day till they came in heat. When they came in heat, they were examined perfectly per rectum and per vaginum for ovulation and discharge respectively and allowed for double insemination. They were examined after two months for pregnancy. The animal which repeated, was again treated as above.

Group III :

All the animals belonging to this group were separated and examined. Each animal was injected with a single dose of Prepalin forte 1 ampoule intramuscularly on the first day followed by 5 gms. Vitablend AD₃ orally once a day with gur for 20 days. When they came in heat, were examined and one ampoule of 1 ml. Prepalin forte was injected before one hour of insemination.

From second day of insemination the dose of Vitablend AD₃ was reduced i.e. from 5 gms. to 1 gm. daily for 15 days. They were examined for pregnancy after 2 months.

The animals which repeated were treated again as above.

Group IV :

The animals belonging to this group were not given any type of drug but were kept as control. Only they were watched for heat and when came in heat were examined per rectum and per vaginum and then double insemination was done. They were also examined for pregnancy after two months.

Trial in outdoor clinics of Gynaecology Department at Bihar Veterinary College, Patna :

The cows or heifers which were repeaters brought to Bihar Veterinary College Hospital were also given a trial of different drugs to see the effect of drug in field conditions. These animals varied in their breed and managerial conditions. Most of the cases were of non-descript type belonging to Patna locality. In the present investigation mostly those type of repeaters are included which had no any abnormality but repeated many times. In a few cases a little abnormal discharge was present, were also taken for the study.

A total number of 40 animals (including cows and heifers) were treated with the several drugs and comparative efficacy of those drugs were analysed. In most of the said animals the cervix, uterine horns and ovaries were normal with clear discharge. All these 40 animals were divided into 4 groups, each of 10. Grouping was done according to

drug trial as follows:-

- | | | |
|-----------|---|-------------------------------------|
| Group I | - | Steclin soluble granules |
| Group II | - | Terramycin liquid |
| Group III | - | Penicillin G sodium and Ambystatin. |
| Group IV | - | Tonophosphan. |

Group I :

Composition of Steclin granules is composed of tetracyclin hydrochloride. It is available in 30 gms. packet. It is a proprietary preparation of Sarabhai Chemicals (Squibb). It is a higher antibiotic drug. This group includes those animals in which abnormal discharge was found.

Preparation and Mode of Administration:- 15 gms. of it was dissolved in 30 ml. distilled water in a 50 c.c. record syringe. An uterine catheter having a rubber tube connection was fitted in the nozzle of syringe. One hand was introduced into the rectum, the cervix was located and with the other hand the catheter was introduced in the os of cervix through vagina and solution was pushed in the uterine horn. This drug was given twice in a week until the discharge was clear.

After a few days when they came in heat again, were examined per rectum and per vaginum. The discharge was found clear, ovulation occurred and the animals were allowed for double insemination. The animals were examined after two months for pregnancy.

Group II : Terramycin liquid.

Composition:- Each ml. contains oxytetracycline hydrochloride I.P. 50 mg.

It is a proprietary preparation of Pfizer Private Ltd. It is a broad range antibiotic and it is effective against both gram positive and gram negative organisms. It is available in 30 ml. and 100 ml. packed in bottles.

Mode of Administration:-

15 ml. of it was given as intra-uterine injection in the uterine horns with the help of syringe and catheter biweekly upto two weeks. In some animals it was continued for three weeks and when these animals came in heat, they were allowed for double insemination and pregnancies were checked after two months. The animals which repeated, were again given the same treatment for two weeks as above.

Group III : Penicillin G sodium I.P.(Squibb) - 400000 units.

The vial contains Penicillin G sodium I.P. Buffered crystalline 400000 units.

Ambystin - one vial of Ambystin-S contains Streptomycin sulphate I.P. equivalent to one gm. pure Streptomycin base.

Both are the proprietary preparation of Sarabhai Chemicals (Squibb).

Mode of Administration:- The animals of this group when came in heat were examined per rectum and per vaginum. When

it was found normal and ovulated condition, the animal was allowed for double insemination and after one hour of first insemination 400000 units of Penicillin G sodium and 1 gm. of Ambystin dissolved in 20 ml. normal saline solution was injected in the uterine horns. The animals were examined for pregnancy after two months.

Group IV : Tonophosphan (20 %) solution.

The composition and mode of administration of this drug applied to this group of animals are the same as at page 60.

Group V: Lugol's Iodine solution (1%).

Preparation of 1 % Lugol's iodine solution is as follows:-

Iodine - 10 gms.

Potassium iodide - 24 gms.

Distilled water - 1000 ml.

The ingredients were thoroughly mixed, dissolved, filtered and kept in a glass stoppered bottle.

Mode of administration:-

The animals of this group were treated with 1 % of Lugol's iodine solution as cervical paint. The os uteri was swabbed with the help of a swab holder biweekly. This treatment was done for three weeks and after that when the animals came in heat, they were allowed for double insemination, and the pregnancy was examined after two months. Those animals which repeated, they were given the

same treatment as above. In this group seven animals were included.

Group VI: Minmix (Mineral mixture).

It is a proprietary preparation of Pfizer Ltd.

It is available in 200 gm., 400 gm. and 1 kg. packet.

Composition:-

Calcium	-	22 %
Phosphorus	-	9 %
Sodium chloride		22 %
Iron	-	0.6 %
Iodine	-	0.1 %
Copper	-	0.1 %
Manganese	-	0.12 %
Cobalt	-	0.02 %
Fluorine not more than	-	0.03 %

Mode of administration:-

One oz. minmix with treacle or gur were mixed and fed orally once a day daily for one oestrus cycle and when the animals came in heat they were inseminated and examined for pregnancies after two months. Those who repeated, were fed minmix again for one oestrus cycle.

RESULTS

R E S U L T S

INCIDENCE OF REPEATERS

Government Cattle Farm, Patna:-

The breeding data were analysed in the Government Cattle Farm, Patna, for those cows and heifers which were inseminated more than three times and did not conceive. The number of the cows were more than that of the heifers. Altogether there were 25 animals suffering from the above disease out of 300 animals. So the percentage of repeat breeders was only 8.20 %. The insemination number varied between 3-10. The number of the animals are already given in the chapter 'Materials and Methods'. From the breeding record it was concluded according to age that the highest incidence was between the age of 5 to 6 years i.e. 28% and the lowest was between 7-8 and 10-11 years i.e. 4 % (Table I & fig.1), though they were of good health. The interval between insemination varied between 20-80 days but mostly between 20-40 days.

Bihar Veterinary College Hospital:-

For the correct information of repeaters in the Patna locality, the data were observed from the case record of Gynaecology section, Bihar Veterinary College, Patna. The incidence of a few years have been shown through the tables II, III, IV and V as well as through the bar diagrams (fig.2,3,& 4) showing monthly variation in each year.

Fig.: 1.

GRAPH SHOWING THE INCIDENCE OF REPEAT BREEDERS IN DIFFERENT AGE GROUP IN JHARPARKAR CATTLE.

Scale: 5 Small Square = 1 Percent

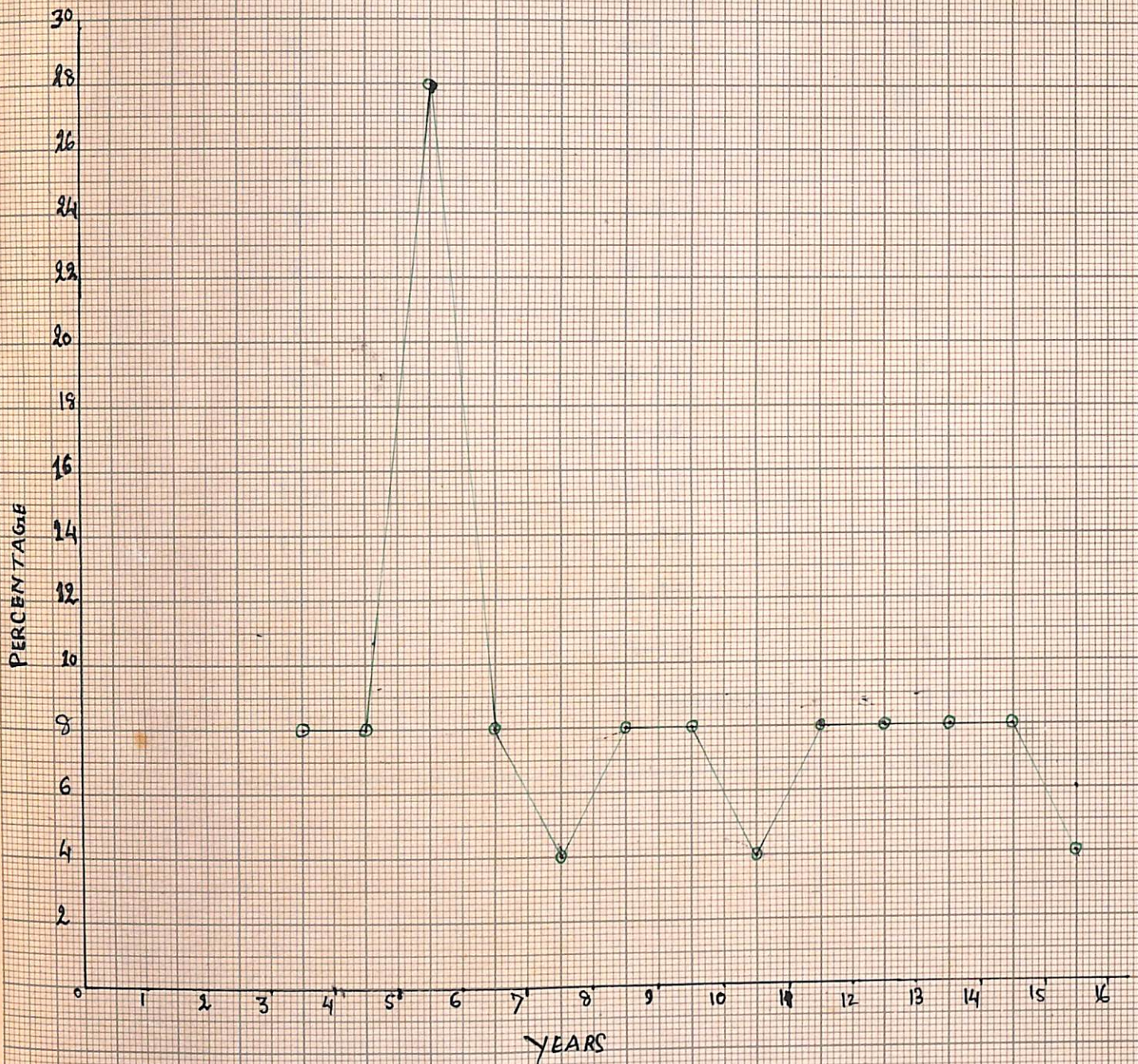


TABLE - II

Monthwise incidence of repeaters in the year 1968.

Months	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	December
Total no. of cases	112	75	105	88	96	132	180	150	180	188	180	115
No. of repeaters	21	11	28	25	24	13	21	19	18	13	11	8
Percentage of repeaters.	18.7	14.6	26.6	28.40	25.0	24.24	11.6	12.6	10.0	6.98	6.11	6.95

It is observed from the table above that out of 212 cases of the repeaters, the highest incidence was in the month of April (28.40%), March (26.6%), May (25.0%), and the lowest was in the month of November (6.11%).

Fig: 2.

Histogram Showing Monthly Variation In Occurance Of Repeat Breeders
In The year 1969 In Local Breeds.

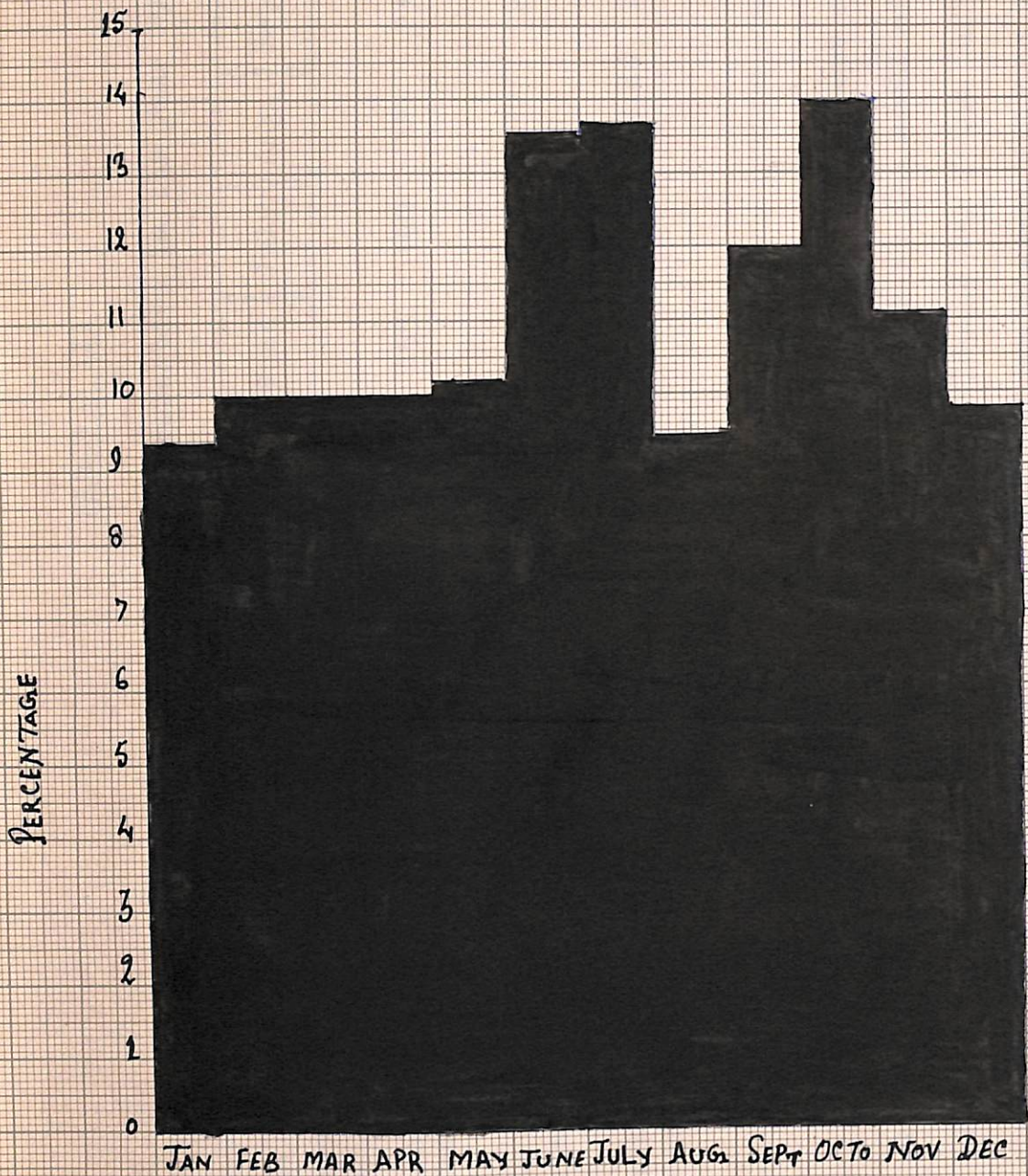


TABLE - III

Monthwise incidence of repeaters in the year 1969.

Months	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Total no. of cases	150	120	100	121	92	155	190	127	233	180	152	115
No. of repeaters	15	12	10	12	9	20	26	12	28	25	17	11
Percentage of repeaters	10	10	10	10.1	9.78	12.90	13.68	9.44	12.01	14	11.1	9.56

It is revealed from the table above that out of 197 repeaters observed in one year, the highest incidence was in the month of October (14%), July (13.68%) and June (12.90%). The lowest incidence was found during the month of January (9.37%). It is also explained through the bar diagram (fig.no.2).

Fig: 3.

Histogram Showing Monthly Variation in the Occurance of Repeat Breeders in the year 1970. In Local Breeds.

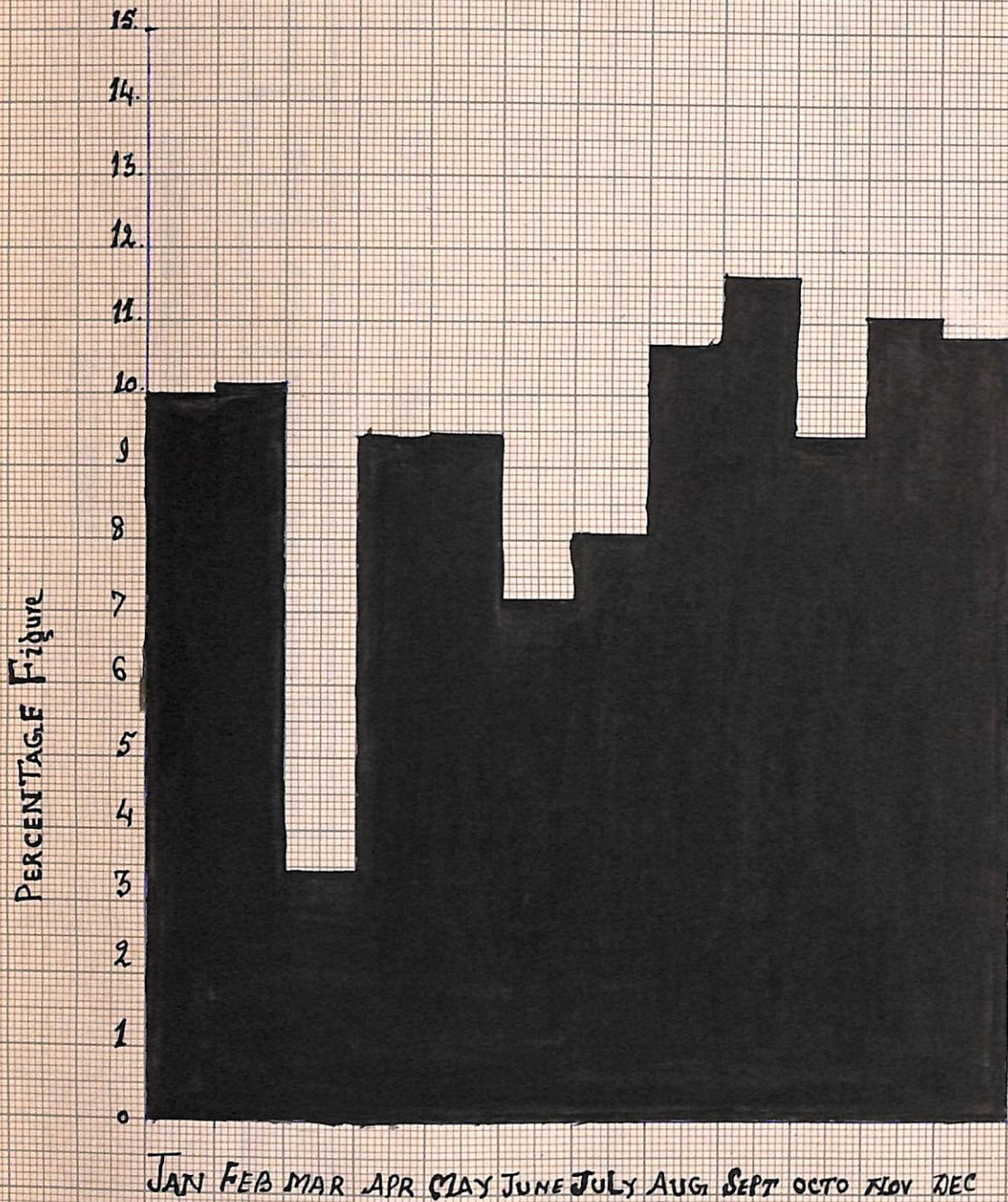


TABLE - IV

Monthwise incidence of repeaters in the year 1970.

Month	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Total no. of cases	200	138	115	85	95	180	197	140	180	169	135	120
No. of repeaters	20	14	4	8	9	13	16	15	21	16	15	13
Percentage of repeaters	10	10.14	3.47	9.41	9.47	7.22	8.12	10.71	11.66	9.47	11.11	10.83

Table shows that out of 164 cases of repeaters the highest incidence occurred in the month of September (11.66%), November (11.11%) and December (10.83%). The lowest was in the month of March (3.47%). It is also represented through the bar diagram (fig.no.3).

Fig. 4

Histogram Showing Monthly Variation In Occurance of Repeat Breeders
In the year 1971 In Local Breeds.

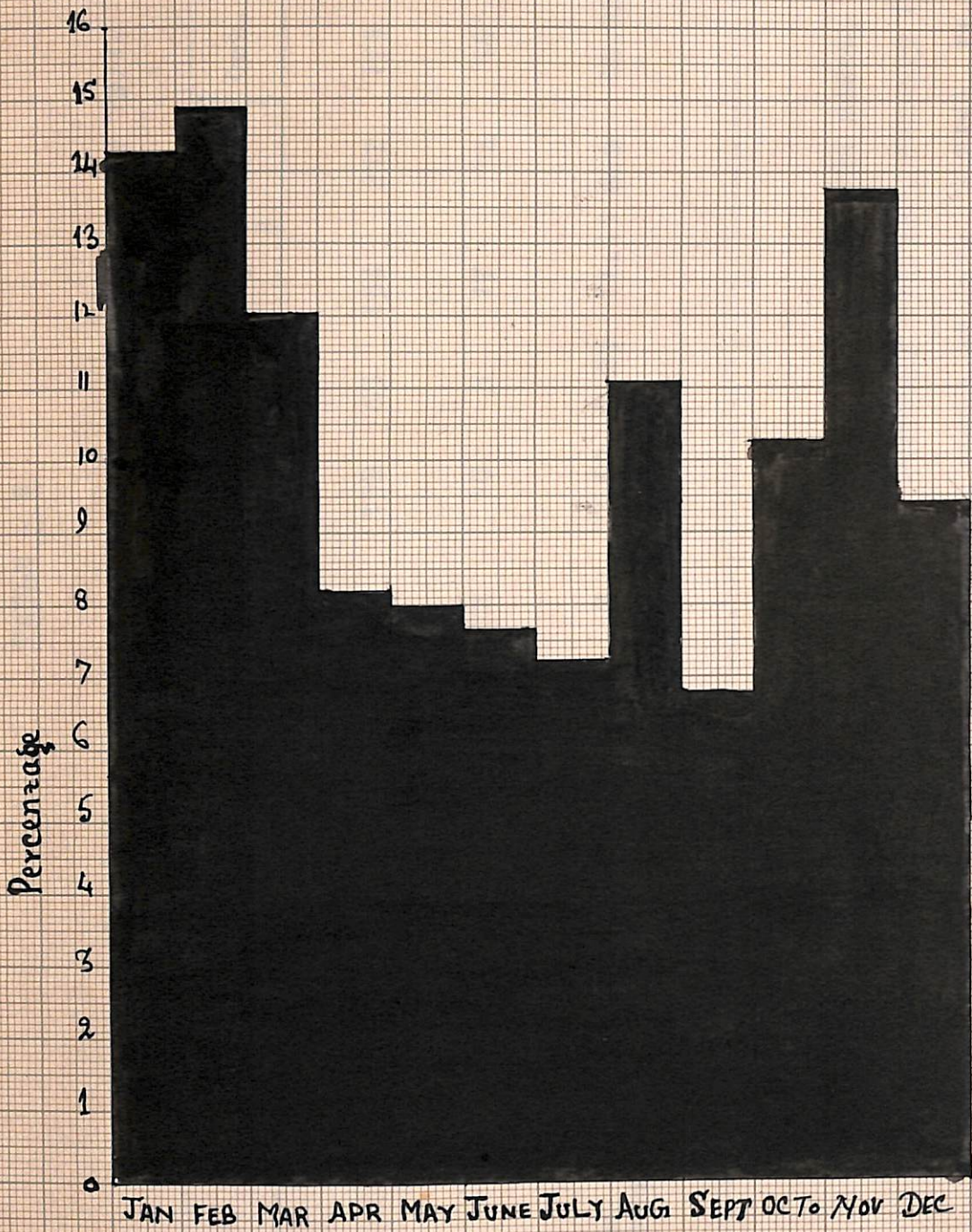


TABLE - V

Monthwise incidence of repeaters in the year 1971.

Month	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Total no. of cases	155	187	141	154	150	138	83	154	212	175	160	170
No. of repeaters	22	28	17	12	12	10	6	17	14	18	22	16
Percentage of repeaters	14.28	14.9	12.05	8.2	8	7.68	7.23	11.11	6.6	10.28	13.75	9.41

From the table-V it is observed that out of 194 cases, the incidence was found highest in the month of February (14.9%), January (14.28%), November (13.75%), and the lowest was in the month of July (7.23%). It is also explained through the bar diagram (fig.4).

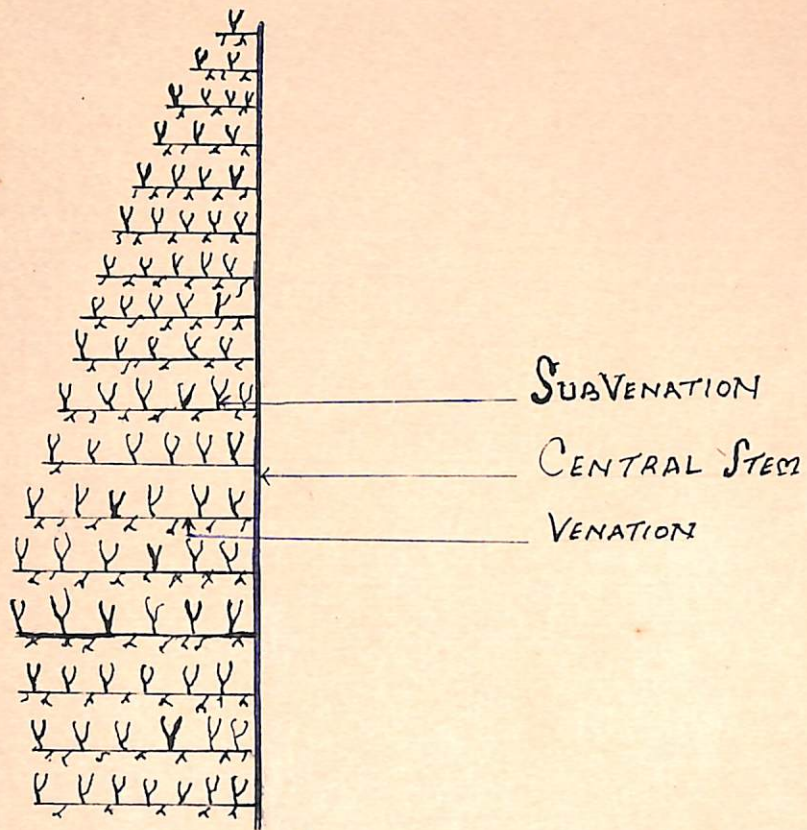


FIG. 5. SHOWING THE DIAGRAM OF FERN STRUCTURE

CRYSTALLISATION PATTERN

The cervical mucus was collected from the repeaters as well as from the normal breeders during oestrus between 0-12 hours. It was found clear, transparent and abundant in most of the cases. In majority of the cases the mucus was found soiling the external genitalia and in some cases hanging like string through vulva. When the rectal examination was done, the discharge started flowing from the vagina and accumulation of mucus was seen near the fornix in those cases in which the discharge was not seen externally. The speculum was used in the vagina and the discharge was taken out with the help of pipette and syringe as described in chapter 'Materials and Methods'. When a little discharge was to be kept on the slide, it seemed that all the discharge will remain on slide, and formed thread like structures when the pipette was lifted from the slide due to its copious nature.

The thin smears of cervical mucus prepared was examined through naked eye as well as under low power of the microscope for the presence of fern patterns (typical, atypical and no pattern) and observations were made between 0-12 hours of oestrus before and after the treatment.

Before Treatment:-

'0' hour stage:- During this stage the pattern was long, clear, close to each other and well developed

Plate no.5. Typical crystallisation
pattern before treatment
during 0 hr.of oestrus.

Plate no.6. Typical crystallisation
pattern after treatment
during 6 hr.of oestrus.

throughout the whole smear. It presented three central stems long and some short with small venations and sub-venations but not perpendicular to each other.

Out of 20 smears, 15 (75%) exhibited typical pattern, 4 (20%) atypical and 1 (5%) no pattern. Plate no. 5 shows the model of typical pattern.

Between 6-12 hours:- During this stage the crystallisation pattern was found more clear, fern like, having no cellular infiltration in most of the cases. Central stems were longer than '0' hour stage, venations and subvenations were clear and in organised fashion. These were seen also through naked eye. The smears possessed thick, long central stems with clear venations and sub-venations during '12' hours of oestrus. Nearly 90% of the area of the smears were covered with the patterns. Out of 75 smears, 58 (77.3%) showed typical pattern, 12 (16.0%) atypical and 5 (6.6%) no pattern. Plate no.6 shows typical pattern, 7 & 8 show atypical and plate no.10 shows no-pattern of this stage.

After Treatment:-

'0' hour stage:- The cervical mucus was again collected from the animals of repeat breeders in Government Cattle Farm, Patna. After treatment in some cases more than four central stems were seen. It was found a little change in the pattern. Out of 20 smears, 16 (80%) exhibited typical pattern, 3 (15.0%) atypical and 1 (5%) no pattern.



Plate no.7. Atypical crystallisation
pattern before treatment
during 8 hr.of oestrus.

Plate no.8. Atypical crystallisation
pattern after treatment
during 8 hr.of oestrus.

Between 6-12 hours:- The crystallisation pattern was also examined of the same animals which were examined before treatment during this stage. This time the structure of the patterns was approximately similar to that of before treatment, but the number of the animals showing typical pattern increased. In this case out of 75 smears, 62 (82.6%), 10 (13.3%) and 3 (4.1%) were observed typical, atypical and no pattern, respectively.

In 30 normal heifers and cows, patterns were also examined during the oestrus between 0-12 hours. There was slight variation in structure of the patterns to that of the repeaters, but typical patterns were found a little more in normal cases. Out of 30 smears, 26 (86.6%) showed typical and the rest showed atypical and no pattern.

TABLE - VI

Observations on Crystallisation pattern during oestrus in repeat and normal breeders (cows & heifers) before and after treatment.

Sl. No.	Period	No. of obser- -vation	Typical pattern		Atypical pattern		No pattern	
			No.	%	No.	%	No.	%
1	'0' hr.	20	15	75	4	20	1	5
2	6-12 hr.	75	58	77.3	12	16	5	6.6
3	'0' hr.	20	16	80	3	15	1	5
4	6-12 hr.	75	62	82.6	10	13.6	3	4.1
5	0-12 hr.	30	26	86.6	2	6.7	2	6.7

N.B.- Sl.nos. 1 and 2 for before treatment; sl.nos.3 and 4 for after treatment of repeaters, and sl.no.5 for the normal breeder.

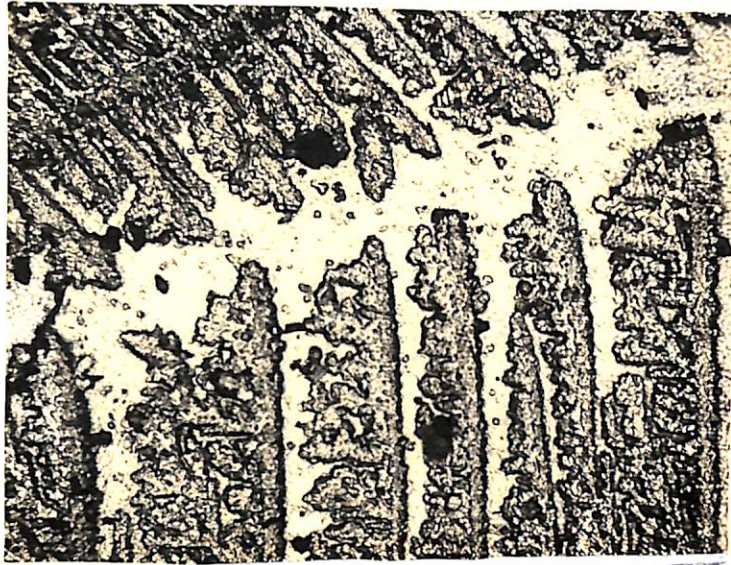


Plate no.9. Atypical crystallisation
pattern during 12 hr.of
oestrus.

Plate no.10. No pattern during 18 hr.
of cestrus showing
inclusions of cells.

From table VI it is revealed that the typical pattern was 80 % before treatment and 85% after treatment in cases of repeaters, whereas in normal cases typical pattern was 86.6% during '0' hr. of oestrus. It slightly declined during 6-12 hrs. of oestrus i.e. 77.3% before treatment and 82.6% after treatment. Atypical pattern presented its appearance during '0' hr. 20 % before treatment and 15% after treatment. During 6-12 hrs. of oestrus atypical was found 16% and 13.6% before and after treatment respectively. Atypical pattern in normal cases during 6-12 hrs. observed was 6.7 %. No pattern was seen during '0' hr. to be 5% before and after treatment as well. During 6-12 hrs. no pattern in normal cases was observed 6.7 %. In the repeaters during 6-12 hrs. of oestrus no pattern was found 6.6% and 4.1 % before and after treatment respectively.

Crystallisation pattern in relation with ovulation:-

Crystallisation pattern of cervical mucus was observed in 160 oestruses to know the relationship with the ovulation rate. The results are shown in table - VII.

From the table it is observed that out of 160 oestruses crystallisation pattern, in 108 (67.5%) was typical, 40 (25.0%) atypical and 12 (7.5%) was no pattern. Out of 108 oestruses showing typical pattern 88 (81.49%) were ovulatory and 20 (18.51%) were anovulatory. Out of 40 oestruses showing atypical pattern 26 (65%) were ovulatory and 14 (35%) anovulatory. Out of 12 oestruses showing no pattern 4 (33.33%) were ovulatory and 8 (66.66%) anovulatory.

TABLE - VII

Crystallisation pattern in relation with ovulation.

Type of fern pattern	No. of observation	Ovulatory		Anovulatory	
		No.	%	No.	%
Typical	108	88	81.49	20	18.51
Atypical	40	26	65.0	14	35.0
No pattern	12	4	33.33	8	66.66
Total	160	118		42	

Crystallisation pattern in relation to conception:-

From the animals, crystallisation pattern was observed at the time of insemination and conception rates were concluded with relation to crystallisation pattern which are represented in table - VIII.

TABLE - VIII

Crystallisation pattern in relation to conception.

Type of pattern	No. of observation	% of pattern	No. conceived	% of conception
Typical	80	69.56	32	40
Atypical	27	23.47	7	25.92
No pattern	8	6.95	8	12.5

From the above table it is revealed that out of 115 oestruses which were inseminated, crystallisation pattern was typical in 80 (69.56%), atypical in 27 (23.47%) and no pattern in 8 (6.95%). Out of 80 oestruses which showed typical pattern conception occurred in 32, showing the conception rate 40 %.

In the atypical pattern out of 27 oestruses, only 7 conceived having a conception rate of 25.92% and out of 8 oestruses showing no pattern, conception occurred in only one (12.5%).

HYDROGEN-ION CONCENTRATION: (pH)

The cervical mucus was tested for the pH values from 79 repeaters (cows and heifers), 25 normal cows and 10 pregnant animals. All the samples were collected between 0-12 hours of oestrus stage.

The mean pH value, standard error, standard deviation and coefficient of variation were calculated as per methods described in Snedecor's (1957) method.

From the Snedecor's formulae the mean pH value of cervical mucus was observed 8.24 with the standard error of 0.052, standard deviation of 0.264 and coefficient of variation 3.20 in the repeat breeder cows and heifers in Tharpar-kar breed in Government Cattle Farm, Patna.

The mean pH values of the cervical mucus of those cases which were brought to the Gynaecology section of the Bihar Veterinary College, Patna, was found to be 7.96 with the standard error of 0.212, standard deviation of 0.26 and coefficient variation of 3.26.

The pH was also determined with the help of BDH pH papers having pH of 5.5 to 10.0, showing differences of 0.3 only. Besides, it was determined with the help of Phenol Red indicator. The pH was found alkaline in all the cases showing 7.5 as minimum and 8.6 as maximum.

The pH was determined in 25 normal cases of Tharparkar herd. From the result, the mean values observed between 4-8 hours of oestrus was 8.12 having standard error 0.0473, standard deviation 0.184 and coefficient variation 2.26.

The pH was also obtained in 10 animals of one month pregnancy and the result was calculated as per methods described by Snedecor (1957). The mean pH value was 8.25 having a standard error of 0.0816, standard deviation 0.816 and coefficient variation of 9.89.

From the above results, it was concluded that there was a slight difference between pH taken before and after treatment.

The statistical analysis of pH of repeaters, normal and pregnant animals (cows and heifers) during oestrus are given in table IX below.

TABLE - IX

The statistical analysis of pH of repeaters, normal and pregnant animals.

Type of animals	No. of observation	Max. pH	Min. pH	Mean	S.E.	S.D.	C.V.
Repeaters in G.C.F.	25	8.5	7.6	8.24	0.052	0.264	3.20
Normal in G.C.F.	25	8.4	7.8	8.12	0.0473	0.184	2.26
Pregnant in G.C.F.	10	8.6	7.5	8.25	0.0816	0.816	9.9
Repeaters in B.V.C.H.	30	8.3	7.4	8.18	0.124	0.426	5.20
Normal in B.V.C.H.	15	8.1	7.2	7.81	0.0783	0.303	3.88

From table - IX it is revealed that of repeaters taken at Bihar Veterinary College Gynaecology department, in 18 the pH varied between 8.3 and in 12 the pH varied between 7.4 to 8.0. The mean value of pH was obtained 8.18 showing a standard error 0.124, standard deviation 0.426 and coefficient 5.20.

TREATMENT:

Government Cattle Farm, Patna:-

Group I :- Tonophosphan (20%) solution as a drug of first group in treatment side. In this group of treatment there were six animals including 5 cows and 1 heifer. The number of infertile services in heifers after last calving in cows varied between 3-8. Out of two conceived to first post-treatment service for a efficiency of 40 %. One cow belonging to this group after 2½ months of service and on postmortem it was found that she died due to tuberculosis and was not pregnant. Another cow belonging to the same group which was treated third time post-treatment died also after one month service. This cow died due to old age and could not settle. This group settled after third service without any treatment. Out of rest 4 animals, 3 conceived showing a rate of 75%. The data of this result are given below:-

From table - IX it is revealed that out of 30 cases of repeaters taken at Bihar Veterinary College Hospital in Gynaecology department, in 18 the pH varied between 8.0 to 8.3 and in 12 the pH varied between 7.4 to 8.0. The mean value of pH was obtained 8.18 showing a standard error of 0.124, standard deviation 0.426 and coefficient variation 5.20.

TREATMENT:

Government Cattle Farm, Patna:-

Group I :- Tonophosphan (20%) solution was taken as a drug of first group in treatment side. In this group of treatment there were six animals including 5 cows and 1 heifer. The number of infertile services in heifers and after last calving in cows varied between 3-8. Out of six, two conceived to first post-treatment service for a breeding efficiency of 40 %. One cow belonging to this group died after 2½ months of service and on postmortem it was found that she died due to tuberculosis and was not pregnant. Another cow belonging to the same group which was inseminated third time post-treatment died also after one month of service. This cow died due to old age and cold. One cow of this group settled after third service without further treatment. Out of rest 4 animals, 3 conceived showing conception rate of 75%. The data of this result are given in table - X.

TABLE - X

Showing the results of Tonophosphan solution in repeat breeders (cows and heifers)
 (columns 4 & 5 are not concerned with heifers)

Sl. no.	Cow no.	Date of birth	No. of calving	Date of last calving	No. of infertile services after last calving.	Date of last insemination before treatment.	Date of last insemination after treatment	Results	Remarks
1.	43/63	24.8.63	2	28.6.70	6	24.8.71	11.11.71	Pregnant	
2.	97/63	15.6.63	3	23.11.70	3	3.5.71	24.8.71	-	Died on 7.11.71 due to T.B.
3.	75/62	22.5.62	4	19.12.70	3	10.5.71	10.8.71	Pregnant	
4.	133/60	24.9.60	6	23.12.70	3	16.4.71	6.11.71	Pregnant	
5.	24/5	24.2.55	9	31.3.70	6	10.1.71	20.8.71	-	Died on 20.10.71 due to Old age & cold.
6.	56/67	22.4.67	-	-	8	24.3.71	22.8.71	Not preg.	

Group II :- In this group 6 animals were kept for the experiment. Out of six, three were cows and three heifers. The number of calving varied between 1-8 in cows and the number of infertile inseminations varied between 3-9 in cows and heifers both.

Out of six animals, 2 conceived in first post treatment service for a breeding efficiency of 40 %. The rest animals repeated at a regular oestrus cycle and were again inseminated after the same treatment. One cow conceived after second service. One heifer again repeated and she was inseminated without further third treatment. One heifer died due to T.B. after first post treatment service. However out of 5 animals, three were conceived showing the conception rate of 60%. The data are summarised in table - XI.

Group III:- There were six animals in this group including 5 cows and one heifer. The number of calving varied between 1-8 in cows and the number of inseminations varied between 3 to 6 in both. Out of 6 animals, 3 conceived to first post treatment service for a breeding efficiency of 50%. The rest three animals were given the same treatment when they came in heat for the second time, were again inseminated among which one conceived. The rest two were again inseminated but did not conceive. The total conception rate in this group obtained was 66.6 %. The data are given in table - XII.

TABLE - XI

Showing the results of Siolan-12 and Placentrex in repeat breeders (cows and heifers) (columns 4 & 5 are not concerned with heifers).

Sl. No.	Cow no.	Date of birth	No. of calving	Date of last calving	No. of infertile services after last calving	Date of last insemination before treatment	Date of last insemination after treatment	Results	Remarks
1.	105/6	9.12.56	7	6.2.71	3	13.6.71	19.7.71	Pregnant	
2.	33/8	16.3.58	8	26.2.71	3	24.5.71	6.7.71	Pregnant	
3.	59/66	9.4.66	1	26.5.69	8	16.1.71	23.9.71	Not Preg.	
4.	73/66	24.5.66	-	-	9	24.5.71	1.12.71	Not preg.	
5.	190/66	28.11.66	-	-	4	12.11.70	23.8.71	Died on 17.11.71.	
6.	146/67	16.11.66	-	-	8	13.1.71	16.7.71	Pregnant	

TABLE - XII

Showing the results of Vitablend AD₃ and Prepalin forte in repeat breeders
(cows and heifers) (columns 4 & 5 are not concerned with heifers)

Sl. no.	Cow no.	Date of birth	No. of calving	Date of last calving	No. of infertile services after last calving	Date of last insemination before treatment	Date of last insemination after treatment	Results	Remarks
1.	30/6	10.2.56	8	21.11.70	3	1.5.71	21.9.71	Pregnant	
2.	35/7	22.2.57	7	10.11.70	3	22.7.71	14.9.71	Pregnant	Preg. after 2nd treatment.
3.	125/64	21.8.64	3	18.2.71	4	15.6.71	10.11.71	Not preg.	
4.	37/66	5.3.66	1	19.6.70	5	16.6.71	24.11.71	Pregnant	
5.	82/66	7.5.66	1	23.12.69	6	20.5.71	4.9.71	Not preg.	
6.	21/68	1.2.68	-	-	5	17.3.71	18.7.71	Pregnant	



Group IV :- The repeaters which were not given any type of treatment, were kept under this group. The number of calving varied between 4-8 in cows and the number of infertile inseminations varied between 3-10 in cows and the number of infertile inseminations were 5 in heifers.

Out of 5 control animals, none conceived in first service and they were watched for next heat. When they came in heat they were artificially inseminated. One heifer conceived after second service and rest 4 were again inseminated for the third time but conception did not occur. The conception rate was obtained 20 % in this group. The data are given in table - XIII.

Comparative efficacy of the drugs:-

The result for the effect of the different drugs applied for the experimental purposes in repeaters at the Government Cattle Farm, Patna has already been given drugwise in tables X, XI, XII & XIII. Table - XIV shows the comparative efficacy of the drugs. From the table it is observed that efficacy of applied drugs are 75 %, 60 % and 66.6 % in Tonophosphan, Siolan-12 and Vitablend + Prepalin forte respectively. The efficacy of the control group is 20 %.

TABLE- XIII

Showing the results of repeaters (cows and halfers) under Control group.
(columns 4 and 5 are not concerned with heifers)

Sl. no.	Cow no.	Date of birth	No. of calving	Date of last calving	No. of infertile services after last calving.	Date of insemination.	Results	Remarks.
1.	97/T	unknown	5	17.10.69	10	9.10.71	Not pregnant	
2.	11/9	17.1.59	8	2.3.71	3	27.11.71	Not pregnant	
3.	24/62	12.2.62	4	27.12.69	4	24.10.71	Not pregnant	
4.	204/66	15.12.66	-	-	5	31.10.71	Not pregnant	
5.	28/67	4.3.67	-	-	5	17.10.71	Pregnant	

Fig:-6

Histogram Showing The Comparative Efficacy Of different drugs against Repeat Breeders In Tharparkar Breed (Cattle).

DRUGS:-

- A) Tonophosphan Solution.
- B) Sialan₁₂ and Placentrex.
- C) Vitablen AD3 + Prepalin forte
- D) Control.

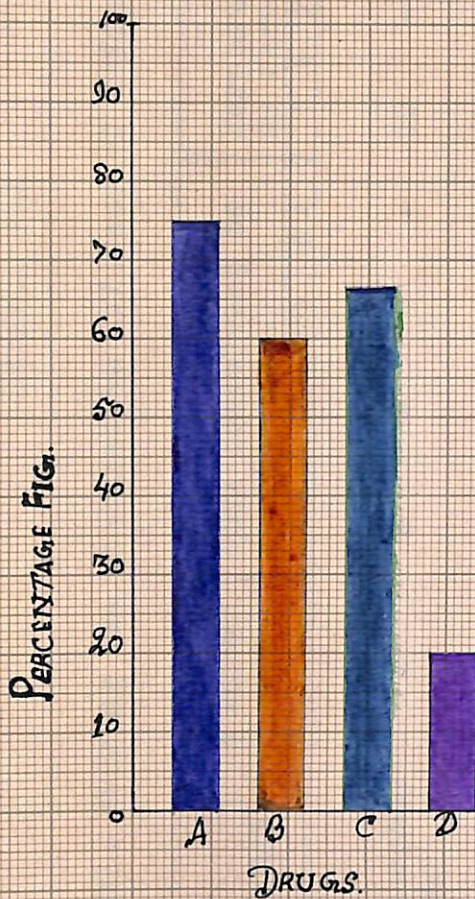


TABLE - XIV

Table showing the comparative efficacy of different drugs.

Sl. no.	Groups no.	Drugs	Animal nos.				Cows conceiving on service nos.				Percentage conceived on service nos.				Total percentage of conception
			1	2	3	4	1	2	3	4	1	2	3	4	
1.	I	Tonophosphan.	6	2	-	1	-	50	-	25	-	-	-	75	
2.	II	Siolan & Placentrex	6	2	1	-	-	40	20	-	-	-	60		
3.	III	Vitablend & Prepalin forte	6	3	-	1	-	50	-	16.6	-	-	66.6		
4.	IV	Control	5	-	1	-	-	-	20	-	-	-	20		

Bihar Veterinary College Hospital:

Group I : - Ten animals were included in this group. They were all nondescript. The number of calving varied between 2-4. The number of infertile inseminations varied between 3-12. Out of 10 animals, 4 were heifers.

It will be observed from table - XV that out of 10 animals, 3 conceived to first post treatment service for the breeding efficiency of 30 %. The rest of the animals when came in heat for the second time, were allowed for double insemination without further treatment. Out of six animals, one conceived in 2nd time showing a breeding efficiency of 10% and the rest did not conceive in second time. Out of the rest 5, 2 again conceived after 3rd insemination and rest 3 did not conceive. The total percentage of conception rate obtained in this group was 60 %. The data are summarised in table - XV.

Group II :- There were ten animals under this group. The number of calving varied between 2-4. The number of infertile insemination varied between 3-6. Out of 10 animals two were heifers and 8 cows. All animals belonged to non-descript breed.

Out of ten animals, 4 conceived to first post treatment service for the breeding efficiency of 40%. The rest of the animals were further treated and when they came in heat were inseminated, but no one conceived. After a few days when they came in heat were inseminated third time, one conceived. Conception rate was 50%. The data are shown in table - XVI.

TABLE - XV

Showing the results of Steclin granules in Repeaters (cows & heifers).

Sl. no.	Case no.	No. of calving	Date of last calving	No. of infer- tile services after last calving.	Date of last insemination before treatment.	Date of last insemination after treatment.	Results	Remarks
1.	m1	2	26.7.70	5	28/6/71	5/8/71	Pregnant	P. after 3rd service, P.T.
2.	m2	-	-	5	21/7/71	7/8/71	Not pregnant	
3.	m3	3	14.9.70	12	29/6/71	9/8/71	Pregnant	P. after 3rd service, P.T.
4.	m4	2	27.11.70	3	19/6/71	10/8/71	Not pregnant	
5.	m5	-	-	4	2/7/71	11/8/71	Pregnant	P. after 1st service, P.T.
6.	m6	4	2.1.71	4	15/7/71	12/8/71	Pregnant	P. after 1st service, P.T.
7.	m7	2	25.9.70	4	1/9/71	27/9/71	Pregnant	P. after 1st service, P.T.
8.	m8	4	25.2.71	4	29/7/71	30/8/71	Pregnant	P. after 2nd P.T. service.
9.	m9	2	26/7/70	5	28/6/71	5/8/71	Not pregnant	
10.	m10	-	-	5	21/7/71	7/8/71	Not pregnant	

P.T. = Post treatment.

TABLE-XVI

Showing the results of Terramycin liquid in Repeaters (cows & heifers).

Sl. no.	Case no.	No. of calving	Date of last calving	No. of infertile services after last calving.	Date of last insemination before treatment.	Date of last insemination after treatment.	Results	Remarks
1.	n ₁	2	10/2/71	5	30/6/71	10/8/71	N.P.	
2.	n ₂	3	2/1/71	4	7/7/71	16/8/71	P.	P. after 1st P.T.S.
3.	n ₃	3	19/2/71	6	16/7/71	19/8/71	N.P.	
4.	n ₄	2	16/1/71	5	18/7/71	19/8/71	N.P.	
5.	n ₅	2	22/10/70	5	20/7/71	26/8/71	P.	P. after 1st P.T.S.
6.	n ₆	-	-	5	26/7/71	2/9/71	P.	P. after 1st P.T.S.
7.	n ₇	3	3/3/71	4	22/8/71	25/9/71	N.P.	
8.	n ₈	3	12/1/71	3	22/8/71	4/10/71	P.	P. after 3rd P.T.S.
9.	n ₉	-	-	6	24/8/71	4/10/71	P.	P. after 3rd P.T.S.
10.	n ₁₀	4	15/9/70	5	20/8/71	20/10/71	N.P.	

P = Pregnant; N.P. = not pregnant; P.T.S. = Post treatment service.

Group III :- In this group ten animals were taken for the experimental purpose. Out of ten, 8 were cows and two heifers. The number of calving varied between 2-4. The number of infertile services varied between 3-7.

Out of ten animals, 5 including one heifer conceived to the first post treatment service for the breeding efficiency of 50 %. The rest unconceived animals were again given the same treatment in which two conceived. The rest three were inseminated for the third time but not conceived. The total percentage of conception in this group was 70 %. The data of the result are shown in table-XVII.

Group IV :- Ten animals were also kept in this group including 8 cows and 2 heifers, for the treatment as experimental purposes. The number of calving and infertile services varied between 1-4 and 3-6 respectively. All were of non-descript.

Out of 10 animals, 2 cows and one heifer conceived to first post treatment service for the breeding efficiency of 30 %. The rest animals which did not conceive to first service, were again inseminated for second time and two of them conceived, without further treatment. The rest 5 did not conceive even after 3rd and 4th services. The total conception rate in this group was 50 %. The details are given in table - XVIII.

TABLE - XVII

Showing the results of Penicillin-G Sodium and Ambystrin-S in repeaters
(cows and heifers)

Sl. no.	Case no.	No. of calving	Date of last calving	No. of infertile services after last calving	Date of last insemination before treatment.	Date of last insemination after treatment.	Results	Remarks
1.	P ₁	-	-	4	2/6/71	26/7/71	P.	P. after 1st P.T.S.
2.	P ₂	3	15/10/70	7	22/6/71	26/7/71	P.	-do-
3.	P ₃	3	19/1/71	4	8/7/71	29/7/71	P.	-do-
4.	P ₄	3	14/1/71	4	12/8/71	12/9/71	P.	P. after 2nd P.T.S.
5.	P ₅	3	6/1/71	3	15/7/71	9/8/71	N.P.	
6.	P ₆	2	21/8/70	6	19/7/71	10/8/71	N.P.	
7.	P ₇	2	20/10/70	5	20/7/71	11/8/71	P.	P. after 1st P.T.S.
8.	P ₈	4	26/11/70	4	23/7/71	12/8/71	P.	P. after 2nd P.T.S.
9.	P ₉	-	-	5	25/7/71	16/8/71	N.P.	
10.	P ₁₀	2	26/12/70	4	20/7/71	20/8/71	P.	P. after 1st P.T.S.

P. = Pregnant; N.P. = Not pregnant; P.T.S. = Post treatment service.

TABLE - XVIII

Showing the result of Tonophosphan in repeaters (cows and heifers).

Sl. No.	Case no.	No. of calving	Date of last calving	No. of infertile services after last calving.	Date of last insemination before treatment.	Date of last insemination after treatment.	Results	Remarks
1.	q1	-	-	5	2/7/71	12/8/71	P.	P. after 1st P.T.S.
2.	q2	4	26/10/70	4	29/6/71	16/8/71	P.	P. after 2nd service
3.	q3	3	5/1/71	4	15/7/71	24/8/71	N.P.	
4.	q4	1	12/3/71	5	27/7/71	8/9/71	P.	P. after 1st P.T.S.
5.	q5	3	15/4/71	3	9/8/71	20/9/71	P.	-do-
6.	q6	3	15/3/71	4	25/8/71	12/10/71	N.P.	
7.	q7	2	5/4/71	3	30/8/71	10/10/71	P.	P. after 2nd service
8.	q8	-	-	6	10/8/71	22/9/71	N.P.	
9.	q9	2	30/6/71	3	15/9/71	25/10/71	N.P.	
10.	q10	1	8/2/71	6	1/10/71	13/11/71	N.P.	

P. = Pregnant; N.P. = Not pregnant; P.T.S. = Post treatment service.

Group V : - Seven animals were kept under this group and all were non-descript. The number of calving and the number of infertile inseminations varied between 1-4 and 3-6 respectively. Out of 7 animals, 6 were cows and 1 heifer.

Out of 7 animals, 1 conceived to first post treatment service for the breeding efficiency of 14.28%. The rest were again inseminated after treatment when they came in heat. Out of 6, 1 conceived in second time having a breeding efficiency of 14.28%. The rest 5 were again treated and inseminated but no one conceived. The total percentage of conception in this group was 28.57 %. The data are given in table - XIX.

Group VI :- Seven animals including 5 cows and two heifers were also kept in this group, for the drug trial. The number of calving and the number of infertile inseminations varied between 1-4 and 3-6 respectively. All the animals belonged to non-descript.

From the table XX it will be observed that out of 7 animals, 2 conceived after 1st post treatment service for the breeding efficiency of 28.57 %. The rest 5 were again treated and inseminated, but not conceived. Among these 5, one conceived after third treatment showing a breeding efficiency of 14.28 %. The total percentage of conception rate was found 42.85% in this group. The data are summarised in table- XX.

TABLE - XIX

Showing the results of Lugol's solution paint in repeaters (cows & heifers).

Sl. no.	Case no.	No. of calving	Date of last calving	No. of infer- tile services after last calving.	Date of last insemination before treatment.	Date of last insemination after treatment.	Results	Remarks
1.	R ₁	1	12/9/70	5	2/8/71	1/9/71	P.	P. after 1st P.T.S.
2.	R ₂	2	25/10/70	3	7/7/71	10/8/71	N.P.	
3.	R ₃	-	-	6	2/7/71	17/8/71	P.	P. after 2nd P.T.S.
4.	R ₄	3	29/1/71	4	28/7/71	30/8/71	N.P.	
5.	R ₅	3	5/1/71	3	30/6/71	3/8/71	N.P.	
6.	R ₆	2	15/2/71	4	10/8/71	14/9/71	N.P.	
7.	R ₇	4	8/3/71	4	22/9/71	18/10/71	N.P.	

P. = Pregnant

N.P. = Not pregnant

P.T.S. = Post treatment service.

TABLE - XX

Showing the results of Minnix in repeaters (cows & heifers)

Sl. no.	Case no.	No. of calving	Date of last calving	No. of infertile services after last calving.	Date of last insemination before treatment.	Date of last insemination after treatment.	Results	Remarks
1.	s ₁	2	19/12/70	4	26/6/71	4/8/71	N.P.	
2.	s ₂	3	25/2/71	3	10/7/71	19/8/71	N.P.	
3.	s ₃	-	-	4	18/7/71	30/8/71	P.	P. after 1st P.T.S.
4.	s ₄	4	8/2/71	5	12/8/71	16/9/71	P.	-do-
5.	s ₅	-	-	5	6/8/71	17/9/71	N.P.	
6.	s ₆	1	30/1/71	6	8/8/71	22/9/71	P.	P. after 3rd P.T.S.
7.	s ₇	2	10/4/71	3	20/8/71	25/9/71	N.P.	

P. = Pregnant

N.P. = Not pregnant

P.T.S. = Post treatment service.

Fig. 7

Histogram Showing The Comparative Efficacy of different drugs against Repeat Breeders in Local Breeds of Cattle.

DRUGS:-

- a) Penicillin Sodium & Ambystrin
- b) Steclin Granules.
- c) Terramycin liquid.
- d) Tonophosphan Solution.
- e) Minmix.
- f) Lugol's Solution.

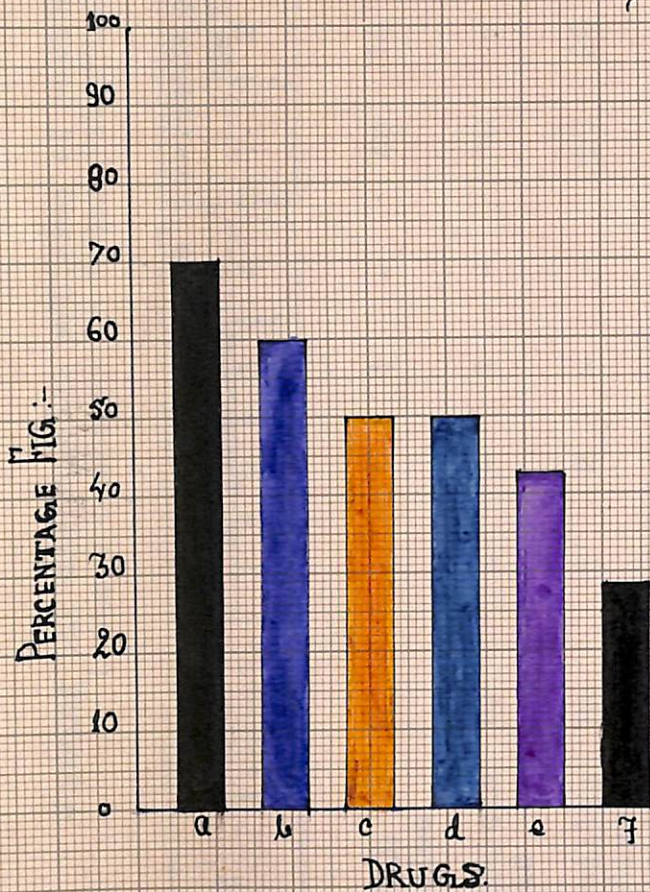


TABLE - XXI

Showing the comparative efficacy of various drugs tried in repeaters (cows & heifers) in Bihar Veterinary College Hospital.

Sl. no.	Group	Drug	No. of animals	Cows conceiving on services nos.		Percentage conceived in services nos.				Total percentage of conception.			
				1	2	3	4	1	2		3	4	
1.	I	Steclin Granules	10	4	1	1	-	-	40	10	10	-	60
2.	II	Terramycin liquid	10	4	-	1	-	-	40	-	10	-	50
3.	III	Penicillin-G sodium & Ambystarin-S	10	5	2	-	-	-	50	20	-	-	70
4.	IV	Tonophosph-an.	10	3	2	-	-	-	30	20	-	-	50
5.	V	Lugol's solution.	7	1	1	-	-	-	14.28	14.28	-	-	28.57
6.	VI	Minmix	7	2	-	1	-	-	28.57	-	14.28	-	42.85

From table - XXI, it is observed that the comparative efficacy of drugs such as Steclin granules, Terramycin liquid, Penicillin-G sodium plus Ambystrin-S, Tonophosphan, Lugol's solution and Minmix are 60, 50, 70, 50, 28.57 and 42.85 percent respectively. Penicillin-G sodium plus Ambystrin-S gave the best result among all the drugs tried in Bihar Veterinary College Hospital, Patna.

DISCUSSION

DISCUSSION

Repeat breeding in cows due to various reasons accounts for heavy economic loss, the incidence of which fluctuates depending upon breed, feed, season, age and hygienic conditions of the animal and regional climatic variations. The number of incidence noted, indicated the importance of the condition in cattle in Tharparkar and local breeds.

The findings of the present study revealed, 8.29 % incidence in Tharparkar breed.

For the better understanding of the problem the observations recorded by different workers considered are as follows:-

Olds and Seath (1941), cited by Robert, reported that 10 % of the herds or individual cow was affected per year by sterility problems which required more than two services per pregnancy, continued to be affected the following year. They also reported that the number of problem herds remained constant from year to year at 6.5 %.

Tanabe & Casida (1947) compared the incidence problem between two herds of Dairy Cattle and found a higher incidence of 18.2 % in Holstein data and a lower value of 2.0 % in Guernsey data, with an average of 10.1 % when both were included.

Casida (1950) and Ulberg et al. (1952) reported a 50 % and 40 % incidence of repeaters in dairy cows respectively, in which cows requiring more than one service were included

in the list of repeaters, whereas in the present study a minimum of three services to produce a calf was taken into account.

Khan and Luktuke (1967) reported a range of 14 to 27 % incidence of repeaters in Haryana breed of dairy cattle.

The various works cited above so also the present study lead us to infer that there is only slight variations in the percentage of repeat breeders in Tharparkar breed except a bit higher result in the Haryana breed. As far the incidence of local breeds are concerned, there was found a higher variation from month to month and all over average it was 16.23 % in 1968, 10.98 %, 9.30 % and 10.29 % in 1969, 1970 and 1971, respectively.

Out of the repeat breeding cows and heifers studied, it was observed that 62.5 % of the repeaters came in heat within 17 to 25 days which seems in parallel with the finding of Flerchinger and Erb (1953), a 57.4 % of repeaters coming into heat within 17 to 26 days and Lutke-Vestert (1946) also reported that about 50 % of the animals returning to service were reinseminated after an interval of more than 24 days.

The high incidence in the animals of 5 to 6 years of age was quite striking and significant (fig.1). About one third of the incidence was found in heifers in Tharparkar breed and 22.2 % in the local breed. Hewett (1968) had also surveyed the incidence in cows and concluded that the incidence was affected by age.

The highest incidence in local breed was found in those cows which calved three times and a little less in cows who calved two times which is in close proximation with the

work of Beljaevara (1970) who recorded the highest incidence within first to third gestation.

The effect of season on repeaters was also noted on the basis of material collected in Bihar Veterinary College Hospital since 1968 in local breeds and it was observed that the incidence varied in different seasons and also from year to year, e.g. it was highest in the month of February (14.9%), September (11.66%), October (14%) and April (28.40%) in the year 1971, 1970, 1969 and 1968 respectively (fig.2,3,4). The results of the observations carried out by Bellani (1961) on the basis of early and late returns to heat after artificial insemination stated that the incidence was highest from March to July. Hewett (1968) in his investigation, stated that the incidence was higher in autumn than in Spring.

It has been observed from the survey that the incidence in one breed is more than the other. The animals (cows and heifers) of Tharparkar breed are given a better balanced ration and better management maintained in Government Cattle Farm, Patna whereas on the other side local breeds are not given proper diet and good management. Therefore, managerial and nutritional conditions may also be the factors for repeat breeding. Age also plays a part for the cause of repeat breeding, but the effect of climate does not interfere too much in these breeds. The present conclusion is based purely on assumption.

CRYSTALLISATION PATTERN:

Crystallisation pattern of cervical mucus is of great importance as evinced by various workers, the literatures of which have been given earlier in detecting the oestrus period, various stages of oestrus cycle, pregnancy and ovarian cycle, but very few literatures were available in cases of repeat breeding animals. Redberg (1948) and Scott Blair (1957a) had reported that the formation of crystals in vaginal mucus was due to the presence of sodium chloride and mucin, while Devyust and coworker (1961) reported that the cervical discharge contains three main things i.e. sodium and potassium chlorides, muco-proteins and high amount of water. These three factors help in making crystalline appearance when smears were dried in air.

The crystallisation patterns were classified during the present study by taking the structure of a 'true fern leaf' as basis for typical pattern, and difference from it as atypical and the absence of any structure as 'no pattern' which is similar with the classification done by Roy Chaudhury (1964), Luktuke & Roy (1967) and Sinha (1969) as typical, atypical and no pattern.

In the present investigation, the crystallisation patterns were determined during the oestrus period in cases of normal and repeat breeding animals. In repeat breeding animals, attempts have been made to see any difference in crystallisation pattern with that of normal breeders. The attempts have also been made to see the difference in crystallisation pattern

in repeaters before and after treatments. The present observations in normal breeders in case of typical pattern resembled with (i) and (ii) of Scott Blair and Glover (loc.cit.) and type A & B of Abusineina (1962) and atypical and no pattern of present study also resembled with their (iii),(iv),(v) and (vi) and type C and no pattern respectively.

The crystallisation pattern observed in the present study during oestrus in normal breeders is comparable with the works done by so many workers (Garm & Skjerven,1952; Coluzzi & Battistacci,1954; Fedrigo,1955; Scott Blair & Glover,1957a; Alliston et al.,1958; Abusineina,1962; Roy Choudhury, 1964; Hukeri,1965; Luktuke & Roy,1967; Roy Choudhury & Rajdan,1968; Sharma et al.,1968; Sinha,1969, and others). The crystallisation pattern was ascertained through naked eye also (Sinha, 1969), besides under low power of microscope. In normal breeders typical patten was observed maximum during 0 to 12 hours and slight less in repeat breeders. The average percentage of typical pattern between 0 to 12 hours stage was found to be 86.6 % and the rest were atypical and no pattern which is comparable with the result of the above workers.

The findings of crystallisation patterns in repeat breeders revealed that the average typical pattern during 0 to 12 hours was 75 %, atypical 20 % and no pattern 5 % before treatments. The crystallisation pattern was also obtained in all the said animals after treatment when they came in heat for the next time. There was slightly difference in the pattern and the typical pattern was found 80 %, atypical 15 % and no pattern 5 % between 0 to 12 hours stage of oestrus.

Perhaps no literature seems to be available stating the structure of fern patterns in repeat breeding animals before and after treatments as observed during present findings. This trial was also done in local breeds of Patna and the result obtained was 77.3 % as typical, 16.0 % as atypical and 6.6 % no pattern between 6 to 12 hours stage in repeat breeders before treatment. They were treated with various drugs according to trial and when they came in heat for the next time, the crystallisation pattern was again examined. The result after treatment observed was 82.6 % as typical, 13.6 % atypical and 4 % as no pattern. Sinha (1969) established correlation between the crystallisation pattern of normal breeders and repeat breeders and the results found by him was insignificant. The results are incorporated in table VI.

Crystallisation pattern in relation with ovulation :

It will be revealed from table VII that the crystallisation phenomenon of cervical mucus with ovulatory and anovulatory conditions of oestrus, the incidence of ovulatory cases was the highest (81.49%) when the smears exhibited typical pattern and lowest (33.33%) with 'no pattern'. The ovulation rate showing atypical pattern was 65.0 percent.

The results of the present findings in ovulatory cases of oestrus seems to be similar with the result of Papinicolaou (1945), Hukeri (1965), & Sinha (1969). The value of present findings in case of atypical pattern is higher than Hukeri (58%) and a bit higher than Sinha (63.38%) which may be due to

managerial error, individual variation and breed difference. The results of crystallisation pattern obtained in anovulatory conditions reveal that 'no pattern' (66.66%) was the highest, which is a bit lesser than Sinha, and typical pattern was the lowest (18.51%) which is also a bit lesser than Sinha (19.45%). Atypical pattern obtained was 35 %. The level of oestrogen is responsible for the causes of typical pattern and a deduction on this basis can be made that the level of oestrogen is a contributory factor in causing ovulation and so the hormone plays a role between ovulation and crystallisation pattern as stated by Sinha (loc.cit.).

Crystallisation pattern in relation to conception :

Similarly, study of crystallisation pattern at the time of insemination shows that in typical pattern there was the highest conception rate (40 %) which is even more than the total conception rate (38.42%) as compared to atypical pattern (25.92%) and no pattern (12.5%). But, no literatures are available in cases of repeat breeders concerning crystallisation pattern in relation to conception rate. A lot of works on optimum time to breed and crystallisation pattern in relation to conception rate is on record only in normal cases. But, the literatures on this aspect are not available to compare the results for better understanding.

HYDROGEN ION CONCENTRATION (pH):

The pH of cervical mucus observed in the present study during oestruses either in normal or in repeat breeding cases was alkaline. The mean pH value in 40 normal cases during oestruses is comparable with the work of Woodman and Hammond (1925) Miller et al.(1941), Olds and VanDemark (1957a), Hartwig (1959), Toubrich (1959), Gupta (1962), Hukeri (1965) and Peterson(1965) on cervical mucus.

The mean pH value in normal cases during oestrus obtained here is 8.12 ± 0.0473 in Tharparkar and 7.81 ± 0.0783 in local breeds. The result of local breed is exactly similar with the result of Olds and VanDemark (loc.cit.) and Gupta(loc.cit.) who reported the average pH value of cervico-vaginal and cervical mucus respectively as 7.8, with a slight variation from the result of Chaurasia (1962). The difference of pH in both the breeds that is between Tharparkar and local breeds might be due to variations in the breed and management of the animals.

The average pH of cervical mucus in 55 repeat breeding cases during oestrus obtained in the present study is 8.24 ± 0.052 in Tharparkar and 8.18 ± 0.124 in local breeds showing a little difference due to variation in the breed, management and collection made during different hours of oestrus. Very few literatures seem to be available regarding the pH of cervical mucus of repeat breeding animals.

From the findings of Roark (1953) it is observed that the pH of cervical and vaginal mucus of repeat breeding animals

is acidic whereas the data obtained in the present study show the pH value as alkaline in all the cases of repeat breeders.

From the data of pH observed in normal and repeat breeding cases of Tharparkar it seems that there is no appreciable difference whereas among normal and repeat breeding cases of local breeds, there is a slight variation and it might be due to collections made at different hours.

The present records of the pH of cervical mucus from cases of repeaters do not seem to coincide with the findings of Shipley (1924) and Roark (1953) who reported acid reaction on the basis of litmus paper. The present study is supported by Chaudhary and Prasad (1954) who reported on pH of vaginal washings of repeat breeding cases and found no abnormalities whereas the present study stands for the cervical secretion. In the present study pH was determined with the help of BDH pH narrow range paper and also with the help of BDH cappilator using Brom-thymol Blue and Phenol Red as described in chapter 'Materials and Methods', although there was not so marked difference between the two methods.

The pH of cervical secretion during one month of pregnancy in Tharparkar in 10 animals was also determined and the pH observed was 8.25 ± 0.0816 which is exactly similar with the result of Hukeri (1965) and is comparable with the observations recorded by Shalash (1958) who concluded that the differences of pH during oestrus, dioestrus and pregnancy are statistically significant, but they are of no diagnostic value. In the present study the finding has been given on the basis of service

records and other probable factors associated with infertility have not been ruled out.

From the observations recorded herein and review of the various works on this aspect it can be concluded that the pH of cervical mucus in the stages such as oestrus, oestrus cycle and pregnancy was distinctly alkaline and differentiable among the said conditions and no significant difference was found on statistical analysis. There was no significant difference in pH before and after treatment in repeat breeding animals. The controlled experiments could not be conducted due to shortage of time and other factors. So controlled experiments are very essential to conclude a more definite and authentic results.

From the present investigation, it appears that there is no appreciable difference between the pH of cervical mucus of normal and repeat breeding animals. From the findings of some of the workers reviewed and from the present investigation as well, it can be presumed that pH of cervical secretion might not be a factor associated with repeaters. It requires more further investigations. Biochemical analysis of the cervical secretions might be helpful to substantiate the effects of this disease on fertility of the animals.

TREATMENT:

In the present study attempts have been made for the treatment of repeat breeding animals in two different breeds (Tharparkar and local) with the use of various drugs and

efficacy of all the drugs have been observed. These days, several works are being done for the development of animal wealth, and a great economic loss in animal wealth is also seen due to repeat breeding animals. Although various treatments have been tried in reducing fertility in different farm animals at several places. In the fields, veterinarians feel also great difficulties when they find the cases of repeaters. The cows or heifers should normally conceive at an average of 1.5 services but instead of that the animals do not conceive even after 3 or more inseminations though they are clinically normal.

In Tharparkar, minerals and trace elements were given as a trial in those animals which were clinically normal. In local breed, such cases were taken under study in which a low grade of infection was present and they were treated with the various antibiotics, e.g. steclin granules, terramycin liquid, etc. and the clinically normal animals were given minerals and trace elements as trial.

In the present study, Tonophosphan solution was observed to give a good effective response against repeaters in Tharparkar breed. The conception rate was found to be 75 % (Table X and XIV). This observation is in agreement with the reports of Hignett & Hignett (1952), and Snook (1964). Snook (loc.cit.) observed a good result in Australian dairy herds. From the present observation, it seems that there may be deficiency of phosphorus in diet in the herd. Snook also recommended the use of phosphatic supplements in the feed. Tonophosphan did not give satisfactory result in the local breed as that of Tharparkar. From the table XVIII and XXI, it is observed

that 5 out of 10 animals (50%) settled after the treatment. The number of animals for experimental purposes used are little due to lack of such animals, so it should be tried on a large number of animals also for better results.

From the literatures reviewed, no reports are available on Siolan₁₂ and Placentrex. Too little attention has been given to the physiology and pathology of the placentrex and Siolan₁₂ in cases of failure of implantation. Kingman (1948) tried the placentome of the cow in cases of failure of implantation whereas the placentrex is a preparation of human placenta and Siolan₁₂ is mixed with it for the beneficial results. Frank (1934) used ovarian extracts in cases of repeaters after removal of corpora lutea and observed a good result.

From the present study, Siolan₁₂ and Placentrex seem to have been given a trial for the first time against repeaters. The conception rate obtained in this group was 60 % (table XI & XIV).

As Vitablend AD₃ and Prepalin forte not being so much old preparation, no trials have been done of these drugs in cases of repeaters in cattle. Veterinarians are applying these drugs in the fields also and found a good result. Niemann(1964) reported that repeaters may be due to vitamins deficiency as he found in his research work in South Africa. From the data obtained in the present study, the conception rate was observed 66.6 % (table XII & XIV). Though, due to lack of such animals, experiments could not be done in a large number of animals, it seems that vitablend and prepalin forte play an important role

in treating the cases of repeaters. It might be due to the deficiency of vitamins in the feed.

The repeat breeding animals were selected at random on the basis of records for the control group. The animals of this group were examined per rectum and per vaginum but they were not given any treatment. They were examined daily by vasectomised bulls for the detection of heat and inseminated. The result obtained in this group was very poor (20%) (Table XIII & XIV). This result seems to be similar to that of McClure (1965) and Khan and Luktuke (1967) who reported 13 % and 25 % conception rate in control group respectively. Person (1962) observed 48% conception rate in control group. Hjerpe (1961) reported no significant difference between treated and control groups. The present observation is in great proximation with the result of Filkins (1964) who reported 20 % conception rate in control group.

Steclin granules which is a preparation of tetracycline hydrochloride was used as intrauterine injection biweekly at the rate of 15 gm. dissolved in 30 ml. distilled water in those cases, suffering with slight subclinical infection. Hjerpe (1961) reported the use of tetracycline hydrochloride in repeat breeding cases, but the result was not satisfactory. In the present observation, Steclin granules was found effective against repeaters (60%) (table XV & XXI).

Observations regarding terramycin liquid which contains oxytetracycline hydrochloride, was found not too much satisfactory in the present investigation, though it is broad spec-

spectrum antibiotic. Five cases got cured out of ten(50%), shown in table XVI & XXI, whereas Sacchi et al.(1958) got good result by irrigating at insemination time or 24-72 hours later. They observed 70 % conception in repeaters. In the present study, the result might be less due to concentration of the drug, because 15 ml., each ml. containing equivalent to 50 mg. bioactivity, whereas they used 10 ml. oxytetracycline preparation, each ml. containing equivalent to 100 mg. bioactivity. The result of Jeganathan and Maheswaram (1961) is also a bit higher than the present and the reason might be due to polymyxin B-sulphate combined with oxytetracycline hydrochloride. Petersson (1963) also reported the use of oxytetracycline preparation and finally he concluded that intra-uterine treatment of repeat breeder cows with germicidal or bacteriostatic drugs should be avoided if pathogenic agents do not appear to be present.

The animals showing subclinical infection were treated with Penicillin G sodium and streptomycin sulphate after one hour of insemination. Uterine infection is one of the main causes of repeat breeding. In the present study, 10 animals were treated and out of 10, 7 (70%) settled to conception in local breeds. The results obtained during study is in agreement with the reports of Chambers (1948), Easterbrooks and Plastringe (1950), Herrick (1951), Lindley (1954), Luktuke et al.(1959), Luktuke and Joshi (1961), Persson (1962), Gibbons and Kiesel (1964) and Khan & Luktuke (1967). Lindley (loc.cit.) has recommended the use of Penicillin and streptomycin on the 2nd day following insemination, whereas Luktuke & Joshi (loc.cit.),

Khan and Luktuke (1967) conducted controlled experiments using 12-24 hours after insemination. In the present study, it has been given one hour after insemination. Luktuke and Joshi (loc. cit.) found a very good result and Khan and Luktuke (loc.cit.) found 69 % conception rate in repeat breeding animals, of Haryana breed. Ulberg et al. (loc.cit.) used a mixture of dihydrostreptomycin, aureomycin and penicillin but did not get satisfactory results.

Luktuke et al. (1959) observed 75 % conception rate after a single treatment in heifers and 83.3 % in cows in first and second insemination after treatment. It will be observed from table XVII and XXI that 70 % of the animals settled as a result. From the study of various workers, it is observed that there is no harm in giving intra-uterine treatment one or two days immediately following the oestrus because the fertilised ovum reaches the uterus three to four days after service. The best result of conception rate in repeat breeding animals was obtained with the treatment of Penicillin G sodium and Ambystarin.

It is revealed from the present study that with the application of 1 % lugol's solution, the conception occurred in only two out of seven (28.57%) in local breed (table XIX & XXI). Hingerford (1946) in his experiment with the use of aqueous solution of iodine injected into the uterus followed by 30 mg. stilboestrol intramuscularly, obtained a conception rate of 70 % in repeat breeders. McKay and Thomson (1958) reported encouraging result (more than 60%) with the use of organic

iodine compound. Janzen (1959) observed a better result with the uterine infusion of very dilute solution into the uterus 2-4 days after service with the combination of the sulphone "Lotagen" 24-48 hours after insemination. Backstrom (1970) found 10 % increase in conception rate when he used lugol's treatment by painting the cervix in repeat breeder cows. However, from the present study, it is observed that lugol's solution does not response satisfactory result in repeat breeders. It is believed that iodine deficiency may be restored in the system by the application of lugol's solution.

Minmix, which is a mineral mixture, containing many trace elements, and organic compound was found to be effective as 42.85 % (table XX & XXI) in repeat breeders. Bentley *et al.* (1951) who concluded that the animals required more services per conception than those fed manganese supplements. Bentley and Philips (1951) also investigated the level of manganese in calves. Tesink (1962) observed an increase rate in conception when he fed mineral supplements containing copper and manganese.

Wilson (1964), McClure (1965) reported that repeaters are also suffered due to nutritional deficiency, and hence they treated the cases of repeat breeders with nutritional supplements. Nedyalkov (1966) reported the use of trace elements in ovine sterility, and before mating the ewes were given 0.25 gm. potassium iodide and 0.5 gm. copper or 15 mg. zinc sulphate. As far as the minmix is concerned, it contains calcium, phosphorus, sodium chloride, iron, iodine, copper, manganese, cobalt and fluorine, it is effective against repeaters.

From the observations obtained in the present study, it is evident that no single cause was responsible for the repeat breeders. The causes of repeat breeding are numerous, but it seems that the content of trace elements are lower than the average in the farm given in the feeds. As far as the basic causes of repeaters are concerned, e.g. failure of fertilisation and early death of embryo, it requires more research work in controlled experimental animals and upon a large number of animals.

In the present study, more stress have been given upon nutritional deficiency and subclinical uterine infection. It is also realised that the biological significance of this study would have been improved with a considerably greater number of animals. Hygienic measures must be adopted for prevention in all gynaecological infections.

Many attempts have been made by various workers to get at a difference in anterior pituitary gland function which might be responsible for the repeat breeding condition. The possibility that lactogenic hormone (LTH) might be deficient in repeaters as it was investigated by Kiddy *et al.*(22) and Cupps and Laben (7), but no comparable difference was found between them and control in the gland content of this hormone.

From the literatures, which were available, it appears that the efficacy of different drugs reported by the different workers seem to be based on the individual response of a particular drug and not on a comparative basis. Further, more research works should be extended for the discovery of basic causes of repeaters and their treatments.

SUMMARY

S U M M A R Y

A systematic survey at the Government Cattle Farm, Patna, as well as the cases of Bihar Veterinary College Hospital were undertaken to observe the incidence of repeat breeding in Tharparkar and local breed.

In the farm animals, overall average percentage of incidence observed, was 8.29 % whereas in local breed it was 10.98, 9.30 and 10.29 % in the year 1969, 1970 and 1971 respectively. Monthwise and yearwise incidence was observed in local breed and it varied in different months of different years.

The disease was obtained more in the animals of 5-6 years of age in Tharparkar and after second and third lactation in local breed.

The repeat breeding animals returned to oestrus mostly between 17-25 days after insemination.

A total number of 79 cases of repeat breeders were taken under the present study including 25 Tharparkar and 54 local breed.

The crystallisation pattern was observed in repeat breeders before and after treatment, and in normal breeders during different hours of oestrus. A little difference was observed in repeat breeders before and after treatment.

Out of 20 oestruses in Tharparkar, the crystallisation pattern was observed typical in 15 (75%), atypical in 4 (20%)

and no pattern in 1 (5%) before treatment and in 16 (80%) as typical, in 3 (15%) as atypical and in 1 (5%) as no pattern after treatment. Out of 75 oestruses in local breed, the crystallization pattern was obtained in 58 (77.3%) typical, in 12 (16%) atypical and in 5 (6.6%) no pattern before treatment, and after treatment it was observed typical in 62 (82.66%), atypical in 10 (13.6%) and no pattern in 3 (5.1%).

Out of 160 oestruses, crystallization pattern of cervical mucus was found typical, atypical and no pattern in 108 (67.50%), 40 (25%) and 12 (7.5%) respectively. Ovulation occurred in 81.49, 65 and 33.33% in typical, atypical and no pattern, respectively.

The conception rate was also calculated on the basis of crystallisation pattern and it was 40, 25.92 and 12.5% in typical, atypical and no pattern, respectively.

The cervical mucus samples of 55 repeat breeding, 40 normal cases and 10 pregnant animals were tested with the help of B.D.H. pH narrow range paper number 5570, 7085 and 8510 for the pH value of 5.5-7.0, 7.0-8.5 and 8.5-10.0, respectively. The pH obtained in all the repeat breeding cases during oestrus was alkaline varying between 7.4 to 8.5 and overall average pH was found 8.24 ± 0.052 in Tharparkar and 8.18 ± 0.124 in local breed.

In normal cases average pH observed was 8.12 ± 0.0473 in Tharparkar and 7.81 ± 0.0783 in local breed. The average pH of one month pregnant animals was 8.25 ± 0.0816 .



Altogether 8 drugs were tried to find out their comparative efficacy with the control group as well, against repeat breeding cases in 79 animals including Tharparkar and local breed. The drugs administered in the present investigations are :- Tonophosphan solution, Siolan₁₂ and Placentrex, Vitablend AD₃ and Prepalin forte, Steclin soluble granules, Terramycin liquid, Penicillin G sodium, and Ambystrin-S, 1 % lugol's solution of Iodine, and Minmix.

In the farm animals, out of three drugs applied, Tonophosphan solution gave the best result in repeat breeders. The next effective drugs were Vitablend AD₃ and Prepalin forte, and Siolan₁₂ plus Placentrex.

In the local breed, out of six drugs applied, Penicillin G sodium and Ambystrin-S gave the best curative response against repeat breeders. The next effective drugs in this breed were Steclin granules, Terramycin liquids, and Tonophosphan solution. Minmix also gave a good result in some cases.

The aim of the present investigations was to find out the incidence of repeat breeders in Tharparkar and local breeds and to see the difference, if any, in the crystallisation pattern and pH values of cervical mucus and their treatments, which were done as far as practicable, but it seems that further investigations should be carried out in controlled way so that complete recovery of repeat breeder be made.

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