DISTRIBUTION STUDY ON CERTAIN ANTIMICROBIAL AGENTS AND BIOCHEMICAL PROFILE IN REPEAT BREEDING COWS WITH SPECIAL REFERENCE TO ITS TREATMENT

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

PUSA (SAMASTIPUR) BIHAR

(FACULTY OF POST-GRADUATE STUDIES)

In the partial fulfilment of the requirement

FOR THE DEGREE OF

Master of Veterinary Science

IN

ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS

By

Sanjay Kumar

Reg. No. - M/VOG/61/2000-2001

Department of Animal Reproduction
Gynaecology and Obstetrics

BIHAR VETERINARY COLLEGE

PATNA - 800 014

2003

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P A T N A - 800 014

2003

In Reverent

Dedication

to

My Mother

DEPARTMENT OF ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS
Bihar Veterinary College, Patna-800014
Rajendra Agricultural University, Pusa, Bihar

Dr. M. H. Akhtar
M.V.Sc., Ph.D.
Associate Professor & Head
Deptt. of Animal Reproduction,
Gynaecology and Obstetrics
Bihar Veterinary College, Patna-800014

CERTIFICATE - I

This is to certify that the thesis entitled "**DISTRIBUTION STUDY ON CERTAIN ANTIMICROBIAL AGENTS AND BIOCHEMICAL PROFILE IN REPEAT BREEDING COWS WITH SPECIAL REFERENCE TO ITS TREATMENT**" submitted in partial fulfillment of the requirement for the degree of "**Master of Veterinary Science (Animal Reproduction, Gynaecology and Obstetrics)**" of the faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar, is the record of bonafide research carried out by **DR. SANJAY KUMAR** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that such help or information received during the course of this investigation and preparation of the thesis have been duly acknowledged.

10.12.2003

(M. H. Akhtar)
Major Advisor

Endorsed :

10.12

(Chairman / Head of the Department)

DEPARTMENT OF ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS

Bihar Veterinary College, Patna-800014
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CERTIFICATE - II

We, the undersigned, members of the Advisory Committee of **DR. SANJAY KUMAR**, a candidate for the degree of Master of Veterinary Science with Major in **Animal Reproduction, Gynaecology and Obstetrics**, have gone through the manuscript of the thesis and agree that the thesis entitled **"DISTRIBUTION STUDY ON CERTAIN ANTIMICROBIAL AGENTS AND BIOCHEMICAL PROFILE IN REPEAT BREEDING COWS WITH SPECIAL REFERENCE TO ITS TREATMENT"** may be submitted by **DR. SANJAY KUMAR** in partial fulfillment of the requirements for the degree.

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CERTIFICATE - III

This is to certify that the thesis entitled "**DISTRIBUTION STUDY ON CERTAIN ANTIMICROBIAL AGENTS AND BIOCHEMICAL PROFILE IN REPEAT BREEDING COWS WITH SPECIAL REFERENCE TO ITS TREATMENT**" submitted by **DR. SANJAY KUMAR**, in partial fulfillment of the requirement for the degree of **Master of Veterinary Science (Animal Reproduction, Gynaecology and Obstetrics)** of the faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar was examined and approved on 21-02-2004

21-02-04

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(iv) **Dr. C. Jayachandran** Jayachandran 21/2/04

(v) **Dr. J. N. Singh**
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Place : Patna

Date : 10/12/09

Sanjay Kumar

(Sanjay Kumar)

Chapter - 1

Introduction

INTRODUCTION

Repeat breading in cattle is an important constraint for profitable running of dairy industry. It affects the calving interval as well as breeding efficiency. A calving interval at 12 months is desirable for the highest milk yield and largest number of calves (Roberts, 1971). Mackay (1981) calculated $ 800 million loss to the US cattle industry as a result of reduced breeding efficiency of 10 to 15% of which repeat breeding in cattle is the major cause.

The repeat breeding cow is one that has normal or nearly normal estrous cycles and estrous periods and has been bred two or more times to a fertile bull, yet failed to conceive. The clinical examination of the animals may fail to reveal any definite lesion or condition to explain the failure of conception (Roberts, 1971). The main cause of repeat breeding is failure of fertilization and early embryonic death (Casida *et al.* 1961). Among the causes of repeat breeding suggested by Zemjanis (1963) and Roberts (1971), the infectious and nutritional causes are the most important. In modern herds, incorporating large numbers of cattle and employing artificial insemination have many problems in heat detection, timing of service and in the care and handling of semen in which the herdsman and inseminators can influence the incidence of infertility and repeat breeding. Because of the multiplicity of causes for repeat breeding

that varies from herd to herd, cow to cow and estrus to estrus, diagnosis and therapy by the veterinarians require great diagnostic skills and knowledge (Roberts, 1971).

Nutritional deficiency or excesses frequently referred as the cause of infertility and repeat breeding (Parker and Bolwey, 1976; Francos *et al.*, 1977). Nutritional deficiency impairs follicular maturation and ovulation with disturbed metabolism of carbohydrates, protein and various minerals. Blood glucose level is known to affect the pituitary gland function (Arthur, 1975). Similarly, cholesterol plays an active role as precursor of steroid hormones besides working as activators or co-factors of enzyme system. The element like calcium has been found to sensitize the female genitalia for the action of hormone (Moddie, 1965). Deficiency of one or more blood biochemical constituents directly/indirectly affect or impair the reproductive harmony. Normal levels of various biochemical constituents are indispensable for normal function of various systems of body including reproductive system.

In order to overcome the problem of repeat breeding in cattle, it is important to identity the type of infection, deficiency and its proper treatment. Culture and sensitivity tests of uterine fluid can reveal the type of infection and biochemical tests of plasma or serum can reveal the type of deficiency. For proper treatment, it is necessary to know the suitable antibiotic, its route of administration and frequency of drug administration. Distribution study may help in selection of suitable antimicrobials. By mineral supplementation,

deficiencies can be corrected which in turn help in conception of animals. In case of repeat breeding due to hormonal deficiency, administration of GnRH or its analogue at the time of A.I. has been found to increase the pregnancy rate in cows (Nakao et al 1983; Ranjan *et al.,* 1991; Lee *et al.,* 1983; Stevenson *et al.,* 1984, 1988, 1990; Pathak, 1986).

The goal of antimicrobial therapy is to produce therapeutically effective concentration of the drug at the site of infection in order to get the desired result.

Jayachandran *et al.* (1987, 1988 and 1995), Sinha *et al.* (1994) and Sood *et al.* (1999) reported distribution of antimicrobial agents in uterine tissue of healthy animals. But, very little work has been done with regard to diseased uterus. The reports on disposition of enrofloxacin and benzathine penicillin in genital tissue subsequent to parenteral route of administration are scanty.

Enrofloxacin, a recent fluoroquinolone carboxylic acid derivative is developed exclusively for veterinary use (Altreuther, 1987; Chu and Fernandes, 1989). It has a wide range of antimicrobial spectrum and has been used in several clinical trials in cows and buffaloes with metritis and other bacterial infections of the uterus (Rong-Roquiang *et al.,* 1997; Anjaneyulu *et al.,* 1999). However, the disposition study to determine the dose, route and duration of the minimum inhibitory concentration (MIC) in the uterine tissues are scanty.

Benzathine penicillin is absorbed very slowly from intramuscular depots and produces the longest duration of detectable antibiotic of all the available repository penicillins. It acts mostly on gram-positive bacteria and these bacteria are exclusively present in infected uterus. There are many literature available about distribution of benzathine penicillin in human being but not in animals, particularly in ruminants.

By taking into account of the aforesaid facts, the present investigation was carried out in repeat breeding cows with the following objectives.

1. To study the prevalence of repeat breeding cows.
2. To estimate the level of some biochemical profile in regular breeding as well as repeat breeding cows.
3. Suitable treatment of repeat breeding cows after blood biochemical examination.
4. Sub-clinical uterine infections will be treated after culture and sensitivity test with appropriate medicine.
5. To study the distribution of antimicrobial agents viz. Enrofloxacin and Benzathine Penicillin in blood and uterine fluid in normal cows and repeat breeding cows.
6. To evaluate the efficacy of treatment on the basis of conception rate.

Chapter - 2

Review of Literature

REVIEW OF LITERATURE

I. PREVALENCE OF REPEAT BREEDING

The prevalence of repeat breeding in cows is variable under various managemental conditions. It varies year-wise, month-wise, season-wise, parity-wise, and breed-wise. The records of occurrence of repeat breeding syndrome in cows were obtained from different countries.

INDIA: -

Sinha (1971) recorded the incidence of repeat breeding in Tharparkar and non-descript cows of Govt. cattle farm, Patna and Bihar Veterinary College, Hospital, Patna and found it to be 8.29 and 10.00 %, respectively.

Namboothripad and Raja (1972) studied various reproductive disorders causing infertility in Red Sindhi Cows and found the incidence of repeat breeding varied between 5.5 to 33.3 %, in different years.

Sinha (1978) studied different reproductive problems in crossbred cattle and reported the incidence of repeat breeder to be 8.41 % of Ranchi Veterinary College Clinics and 10.00 % at Namkom Military Dairy farm, Ranchi. Further, he observed that seasons had no effect on incidence of repeat breeding.

Rao and Kotaya (1980) reported 20.64 % incidence of repeat breeding in cows and found seasons had non-significant effect on the incidence.

Singh *et al.* (1981) studied the cases of infertility of outdoor clinic of Gynaecology Department, Bihar Veterinary College, Patna from 1967 to 1973 and reported that incidence of repeat breeding was 22.69 % in cows.

Sharma *et al.* (1983) stated that the incidence of repeat breeding in crossbred cows varied between 10.00 to 25.00 %.

Rahumathulla *et al.* (1986) observed the percentage of repeat breeding in cattle to be 73.70 % in Tamil Nadu.

Chetty and Rao (1987) reported that out of 1463 cattle, 132 cattle were repeat breeders in Andhra Pradesh.

Hafez (1987) established that the incidence of repeat breeding was maximum in 2^{nd} calving than 3^{rd} and 4^{th} calving, it increased upto 5^{th} calving and decreased afterwards.

Pargaonkar and Bakshi (1987) examined 354 Red Khandhari and 192 crossbred cows (with 50 % Jersey inheritance) and found the incidence of repeat breeding were 8.00 and 3.00 %, respectively.

Shukla and Pandit (1989) studied a total of 3469 reproductive cycles of cows and heifers during the period 1971 to 1984 at All India Co-ordinated Research Project on cattle at the Livestock

Farm, Adhartal, Jabalpur and found that the incidence of repeat breeding in cows was 16.23 %.

Sharma *et al.* (1991) recorded the breeding history of 475 cases of infertility in crossbred cattle H.F. × local and Jersey × local in Chotanagpur region of Bihar and reported that incidence of repeat breeding in crossbred cattle was highest (25 %) during February and lowest (10 %) during June and August.

Ahmad *et al.* (1992) examined 465 cows and 60 heifers in Assam during January 1987 to February 1991 and found that among them 120 cows and 12 heifers were repeat breeders.

Sreeramulu (1995) narrated that repeat breeding was found more in heifers (23.33 %) than the cows (16.01 %).

Narladkar *et al.* (1994) tested proficiency of 94 Deoni cows and 235 crossbred cows (Holstein Friesian × Deoni 50 percent) over a period of one year at Prabhani and observed that the incidence of the repeat breeding was 4.26 % in Deoni cows as well as in crossbred cows.

Dhable *et al.* (1996) studied the breeding records of 354 breedable cattle at military Dairy farm, Bareilly and found that out of these 63 cattle were repeat breeders. The overall incidence of repeat breeding was 17.79 %. The incidence was 15.87, 20.63, 15.87, 17.47 and 5.87 percent in 1st, 2nd, 3rd, 4th and 5th calvers, respectively.

ABROAD: -

Zemjanis (1963) reported the overall incidence of repeat breeding in cows to be 15%.

Hewett (1968) surveyed the incidence of repeat breeding cows in Sweden with reference to herd size, seasons, age and milk yield and showed the overall incidence to be 10% (9.1% to 11.1%). The highest seasonal incidence of repeat breeding was in the fall and winter months, 10.2 to 13.7 and 7.1 to 9.4 percent, respectively. The incidence of repeat breeding was lowest in heifers that had calves for the first time (5.2 %) and it rose in older cattle.

Dessouky and Jama (1973) examined 1010 cows during May 1967 to December 1970 in Iraq and found the incidence of repeat breeder was 10.8 percent.

Ronie (1973) tabulated the data of various reproductive disorders in dairy cows in 700 herds in Finland and found that 9.50 % was repeat breeder. He also observed that season had a significant effect on the incidence of reproductive disorders with the highest percentage occurring in February to March and lowest during June to September.

Francos (1974) examined 4,811 Holstein Friesian cows in Israel and reported the incidence of repeat breeding to be 5.00 %.

Rahman *et al.* (1975) inspected 1,108 indigenous cows in Bangladesh having reproductive disorders and reported that 21.66 % was repeat breeder.

Ronie and Saloniemi (1978) studied the incidence of infertility in 283 herds of dairy cows (2304 cases) over a period of

eight years (1968 to 1975) in France. They reported that total incidence of infertility (0.11 cases per cow per year) varied significantly from year to year and the incidence of repeat breeding was 8.2 %.

Shah and Usmani (1984) recorded 51.3 percent incidence of repeat breeding in crossbred cows in Pakistan.

Bartlett *et al.* (1986) studied Lactational incidence rate (LIR) of repeat breeding syndrome (RBS) among the 22 herds in UK and reported that the incidence of repeat breeding was 24.1 % (746/3309) ranged between 10 to 25 %.

Ben-Haj and Abdulmola (1988) stated that the incidence of repeat breeding in cattle accounted over 67 % in Libiya.

II. BLOOD BIOCHEMICAL STUDIES

Many attempts have been made to correlate the shift in normal pattern of specific blood biochemical constituents of blood, which in turn provide valuable information in the diagnosis of various diseases.

(a) Blood Glucose: -

The definite relationship between energy levels and reproductive efficiency in cattle were studied (Wiltbank *et al.*, 1962 and 1964; Dunn, 1969). Roberts (1971) related the importance of glucose with fertility. Arthur (1975) exposed to negative energy balance expressed that pituitary function might be influenced by

blood glucose level. Conception rates decreases due to energy deficiency (hypoglycaemia). The blood glucose level has been acclaimed to be reasonably accurate test to measure the energy status of animals (Madan, 1979). However, Girou and Brochest (1970) and Carstairs *et al.* (1980) failed to find any effect in energy status reproduction in dairy cows.

Rao *et al.* (1981) reported higher blood glucose (mg%) levels in repeater was 43.24 ± 2.87 and in normal dry cycling cows (42 ± 1.35) is more than that of early pregnant Ongole cows (33.54 ± 1.40).

Enkhia *et al.* (1982) reported non-significant differences in blood glucose levels between repeater and normal Rathi cows.

Parmar *et al.* (1986) found that blood sugar (mg%) levels of repeat breeding cows were significantly higher (97.78 ± 9.36 and 71.89 ± 8.33) when compared with control cows (68.84 ± 10.11 and 52.45 ± 5.16) during estrus and luteal phases, respectively.

Awasthi and Kharche (1987) found comparatively higher blood glucose level in cycling cows (54.21 ± 2.55 mg%) as compared to fertile repeat breeder (51.5 ± 90 mg%) and infertile repeat breeder (53.96 ± 3.35 mg%) with non-significant difference among three groups.

Nair *et al.* (1987) reported a significantly lower blood glucose levels in repeaters (50.11mg%) as compared to normal (66.7 mg%) crossbred cows.

Dutta *et al.* (1991) observed significantly lower blood glucose levels in repeat breeder (52.90 ± 0.51 mg%) than normal (70.83 ± 0.82 mg%) exotic cows.

Kumar and Sharma (1991) estimated the levels of haemoglobin and certain serum biochemical constituents in rural cows during fertile and non-fertile estrus and observed significantly lower serum glucose value in non-fertile (51.11 ± 2.08) than fertile (59.00 ± 3.39 mg%) cows.

Islam *et al.* (1994) reported a significantly higher serum glucose levels in repeat breeders than normal crossbred cows on day 0 and 13 of estrus cycle.

Ramakrishna (1996) reported significantly low blood glucose in repeater crossbred cows (45.72 ± 3.81 mg%) as compared to normal cycling cows (62.20 ± 5.7 mg%).

Singh *et al.* (1996) studied the physiological and biochemical attributes of three breeds (Jersey, Holstein and Gir) crossbred cows during estrus and in other stages of the estrus cycle. For cows in estrus and in other stages of estrus cycle, blood glucose levels averaged 89.90 and 106.47 mg%, respectively.

Singh and Pant (1998) reported that blood glucose levels (mg%) were 78.2 ± 2.25, 68.2 ± 2.06 and 75.0 ± 1.78 in rural, farm normal and repeat breeders cows, respectively.

Sood *et al.* (1999) studied on some biochemical attributes of cervical mucus and blood in adult, healthy, cycling cows and reported levels of glucose in cervical mucus and blood serum of cows were 1.02 ± 0.32 mg/dl and 52.23 ± 2.38 mg/dl, respectively, during estrus.

(b) Serum Total Protein: -

The role of protein in reproductive efficiency in farm animals is conflicting. However, Wiltbank *et al.* (1964) and Girou and Brochest (1970) documented the importance of protein in farm animals with respect to conceptions rate, post partum occurrence of estrus and number of insemination per conception or non-return rate. In contrast, Morrow (1977) observed that protein deficiency was not much related to reproduction.

Agarwal *et al.* (1982) reported lower serum total protein in repeater, (7.56 ± 2.08, 6.19 ± 0.32 and 4.93 ± 0.34 g/dl) when compared to non-repeater (9.24 ± 0.71, 7.80 ± 0.51 and 5.24 ± 0.57 g/dl) of crossbred cows on day 1, 13 and 16 of estrus cycle, respectively.

Enkhia *et al.* (1982) in a study found non-significant differences in serum total protein levels between normal (7.77 ± 0.36 g/dl) and repeater (7.23 ± 0.3 g/dl) Rathi cows.

Dutta *et al.* (1991) leveled the concentration of total serum protein (g/dl) was towards lower side (9.92 ± 2.06) estrus group of cows.

Gandotra *et al.* (1993) measured the values of serum total protein (9.31 ± 0.97 mg/100ml) in repeater cattle.

Islam *et al.* (1994) observed significantly higher serum total protein concentration in normal crossbred cows on day 0 compared to day 13 of estrus cycle. However, in buffaloes Salem *et al.* (1994) reported non-significant differences in blood protein concentration between normal and repeat breeders.

Burle *et al.* (1995) reported significantly higher serum total protein value in normal cycling than repeat crossbred cows and they also reported a significant difference between first (Day 0) (10.06 ± 0.17) and (8.57 ± 0.17 gm/dl) and second (Day 7) (8.92 ± 0.18) and (7.97 ± 0.13 gm/dl) blood samples in normal and repeater crossbred cows, respectively.

Srivastava (1995) obtained a non-significant difference in plasma total protein level in fertile (7.48 ± 0.09 gm/dl) and non-fertile (6.68 ± 0.12 gm/dl) cows.

Ramakrishna (1996) recorded significantly lowered serum protein levels in repeater (5.98 ± 0.098 g/dl) as compared to normal cycling (6.05 ± 0.18 g/dl) cows.

Singh and Pant (1998) studied blood biochemical profiles of rural, farm normal and repeat breeder cows and observed 7.43 ± 0.15, 7.96 ± 0.15 and 7.60 ± 0.10 levels of total protein (g%), respectively.

(c) **Calcium: -**

The calcium sensitizing the tubular genitalia to the actions of hormones is well established (Moddie and Robertson, 1962). Several workers have observed that an increased level of blood calcium may improve fertility whereas its deficiency leads to retarded reproduction in cows (Hignett, 1959; Alderman, 1963; Luktuke, 1974).

Rao *et al.* (1981) observed no difference in serum calcium levels in different stages of reproduction of normal cycling (10.38 ± 0.35 mg/100ml), pregnant animals below 3 months (10.32 ± 0.47 mg/100 ml) in Ongole cows.

Agarwal *et al.* (1982) analysed level of calcium (mg/100 ml) in repeat breeding cows to be 2.53 ± 0.17, 3.53 ± 0.18 and 4.94 ± 0.28 and in normal cows to be 3.20 ± 0.12, 3.60 ± 0.64 and 4.70 ± 0.99 on day 1, 13 and 16 of estrus cycle, respectively.

Kulkarni *et al.* (1983) tabulated overall mean value of serum calcium (mg%) 8.74 and 9.19 in Gir and crossbred lactating cows, respectively. The difference in the mean value of calcium in both the breeds was not significant.

Umashanker *et al.* (1983) reported that serum calcium concentration was high (10.59 ± 2.59 mg/100ml) in normal cyclic than repeater (8.44 ± 2.12 mg/100ml) buffaloes.

Kumar *et al.* (1986) found serum calcium level was significantly lower in repeat breeder cows (7.05 ± 0.30 mg/100ml) and repeat breeder heifers (6.55 ± 0.40 mg/100ml) than normal cyclic cows (8.98 ± 0.22 mg/100ml) and normal cyclic heifers (9.20 ± 0.34 mg/100ml).

Srivastava and Kharche (1986) reported lower (10.50-15.00 mg%) mean calcium concentration during estrus than during abnormal (repeater = 9.00 – 16.00 mg%) cycling.

Awashti and Kharche (1987) measured higher serum calcium level (8.93 ± 0.63 mg%) in fertile repeat breeders and normal cycling crossbred (7.86 ± 0.42 mg%) cows.

Rupde *et al.* (1993) stated that serum calcium concentration was 6.60 ± 2.56 and 7.32 ± 0.33 in pre treatment and post treatment repeat breeder cows, respectively. The mean value for regular breeding cow was 7.84 ± 0.659.

Ramakrishna (1996) estimated serum calcium level (9.79 ± 0.52 mg%) in repeat breeder cows with uterine infection; 9.85 ± 0.21mg% in repeat breeder cows without uterine infection and 9.95 ± 0.25 mg% in control cows.

Singh and Pant (1998) studied the blood biochemical profile of normal and repeat breeder cows in Himachal Pradesh and found that the values of calcium (mg %) were 8.42 ± 0.32, 7.42 ± 0.31 and 8.24 ± 0.22 in rural & farm normal and repeat breeder cows, respectively.

Das *et al.* (2002) tabulated the serum calcium levels in repeat breeder (10.045 ± 0.327 mg%) and in normal cyclic cows (10.50 ± 0.044 mg%). The difference in mean value did not differ significantly.

Jayanthi *et al.* (2003) studied the significantly lower calcium (10.03 ± 0.06 mg/dl) in repeat breeding as compared to normal control (10.95 ± 0.03 mg/dl) cows.

(d) Phosphorus: -

Morrow (1969) reviewed the relationship between phosphorus deficiency and infertility in dairy cattle and reported that with proper phosphorus supplementation, the number of services per conception declined from 3.7 to 1.3 in dairy heifers. Further, Morrow (1977) indicated that phosphorus deficiency disturbs hypophyseal and gonadal functions and carbohydrate metabolism causing infertility.

Rao *et al.* (1981) studied mean serum inorganic phosphorus value in normal cows (6.11 ± 0.39 mg/100 ml.) and repeaters (6.82 ± 1.13 mg/100 ml.) were slightly higher, though not significant, when compared to pregnant below 3 months (5.19 ± 0.36 mg/100ml) and pregnant above 3 months (5.30 ± 0.29 mg/100 ml).

Enkhia *et al.* (1983) in a study on electrolytes of cervico-vaginal mucus and blood during estrus in normal and repeat breeding Rathi cows observed no significant difference between repeat breeder and control cows in serum concentration of calcium, but inorganic

phosphorus was significantly higher (5.76 ± 0.28 Vs 3.16 ± 0.11 mg/100ml.) in controls than in repeat breeders.

Umashankar *et al.* (1983) found higher (7.92 ± 1.18 mg/100ml.) serum phosphorus concentration in cyclic as compared to repeater (7.57 ± 1.12 mg/100 ml) buffaloes.

Kumar *et al.* (1986) reported level of serum phosphorus was 4.98 ± 0.06 mg/100 ml in normal cyclic and 5.16 ± 0.21 mg/100 ml in repeater cows.

Srivastava and Kharche (1986) observed serum phosphorus concentration 6.60 mg % in repeater and 5.95 mg% in normal cyclic buffaloes.

Awasthi and Kharche (1987) found infertile repeaters had significantly lower (3.73 ± 0.29 mg %) inorganic phosphorus level than normal cyclic (5.06 ± 0.19 mg %) and fertile repeater (4.99 ± 0.25 mg %) cows.

Khan and Iyer (1993) stated that regular breeding cows showed a higher (6.07 ± 0.15 mg %) level of serum inorganic phosphorus than repeat breeding (4.84 ± 0.1 mg%) cows.

Rupde *et al.* (1993) in his experiment on biochemical profile in repeat breeders found mean concentrations of 3.375 ± 0.22 and 4.03 ± 0.21 mg% in pre treatment and post treatment repeaters, respectively, and for regular breeders cows, it was 4.466 ± 0.138.

Ramkrishna (1996) estimated mean serum inorganic phosphorus levels of 4.33 ± 0.14 mg% in repeat breeder cows with uterine infection, 4.51 ± 0.18 mg% in repeat breeder cows without uterine infection and 5.96 ± 0.18 mg% in control cows.

Singh and Pant (1998) reported level of phosphorus (mg%) 5.56 ± 0.24, 6.27 ± 0.21 and 4.89 ± 0.14 in rural, farm normal and repeat breeder cows, respectively.

Das *et al.* (2002) found significantly higher (5.513 ± 0.265 mg%) serum phosphorus concentration in cyclic when compared to repeat breeder (4.729 ± 0.150 mg%) crossbred cows.

Jayanthi *et al.* (2003) found significantly lower phosphorus level in repeat breeding cows as compared to normal breeding cows. The level was 4.12 ± 0.03 mg/dl and 5.27 ± 0.04 mg/dl in repeat breeder and normal (control) cows, respectively.

(e) Serum Alkaline Phosphatase level: -

The levels of enzymes are indicative of physiological activity and pathological conditions of tissues. Deranged enzymatic activities affect the normal reproductive behaviour of the animal, causing serious morphological and physiological alterations (Roberts, 1971).

Singh *et al.* (1972) have reported serum alkaline phosphatase activity in Rathi and Sahiwal breeds of cattle ranging from 4.3 to 5.2 and 2.7 to 4.3 Bodansky units, respectively.

Pandiya *et al.* (1977) bringout the normal values of alkaline phosphatase activity in crossbred dairy cattle and found to have a wide range (1.82 to 7.08 Bodansky units).

Parmar and Mehta (1989) observed lower (8.07 ± 0.75, 8.20 ± 0.96, 9.52 ± 1.21 and 10.41 ± 1.22) level of serum alkaline phosphatase (KAU%) in repeater cows as compared to control cows (22.55 ± 0.34, 22.80 ± 0.62, 23.58 ± 0.49 and 21.58 ± 0.49 KAU%) at day 0, 4, 11 and 16th of estrus cycle.

Mehta *et al.* (1989) reported that the mean value of serum alkaline phosphatase was increasing from estrus to 1st trimester of pregnancy. The mean values during estrus were 2.34 ± 0.27 BU% and 1st trimester of gestation 2.97 ± 0.26 BU%.

Mahmood *et al.* (1991) observed marginally higher level of AKP during estrus (48.89 ± 6.91 unit/litre) than in mid pregnancy (51.16 ± 5.89 unit/litre) in 3/4 Friesian crosses.

Gandotra *et al.* (1993) reported values of serum alkaline phosphatase were non-significant 52.6 ± 0.27 (normal phenol produced/min/ml. of serum) in normal cows and 60.3 ± 10.1 (n mol phenol produced/min/ml of serum) in repeater cows.

III. ISOLATION, IDENTIFICATION AND SENSITIVITY OF MICROFLORA IN REPEAT BREEDINGS COWS

It is generally accepted that a variety of specific and non-specific microorganisms are present in the cervico-vaginal mucus of

cows, which are responsible for repeat breeding. Various reports on isolation of microbes and their sensitivity patterns are described below:

Gunter *et al.* (1955) isolated *Streptococci* spp., *Corynebacterium* spp., *Micrococci* spp., and *Diptheroid* spp. from cervical mucus of repeat breeder animals.

Roberts (1971) reported that infection in the genital tract resulted in early embryonic death and repeat breeding. He concluded that *V. foetus* and probably *Brucella abortus* and *Mycoplasma* might cause early embryonic death and repeat breeding. Further, he stated that other organisms associated with repeat breeding were *C. pyogenes. Ps. aeruginosa, Streptococci, Staphylococci* and *E. coli.*

Hartigan *et al.* (1972) examined 80 repeat breeding cows and isolated bacteria (*Streptococcus*, *Staphylococci* and Microaerophilic *Streptococci* only) from 27 positive samples (34%).

Leclerc *et al.* (1972) reported that most frequently *E. coli* strains were isolated from the cervical mucus of repeat breeding cows than other bacterial strains.

Krishnamurty *et al.* (1974) examined 106 cervical mucus samples from repeat breeding cows and recovered bacteria from 86 animals (81.87%) while remaining 20 animals (18.13%) were apparently sterile. The various species of bacteria encountered were *Streptococcus pyogenes, Staphylococcus, Micrococcus flavous, M.*

aureus. *Bacillus* spp. *C. pyogenes, E. coli, Aerobacter aerogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Paracolobacterium*.

Verma and Tyagi (1974) cultured 30 uterine samples from repeat breeding cows. 27 samples were bacteriologically positive and isolates included *Micrococcus*, *Staphylococcus aureus*, *Proteus* spp., *Pseudomonas*, *Corynebacterium*, *Diptheroids* and *Anthracoids*. *E. coli* was the predominant organism isolated.

Namboothiripad and Raja (1976) isolated *Proteus* (7), *Pseudomonas* (7), *Escherichia* (4), *Aerobacter* (1), *Staphylococci* (1) and mixed infection from 6 samples of uterine discharge of 26 cows that failed to conceive.

Panangala *et al.* (1978) studied microflora in cervico-vaginal mucus of 72 normal fertile and 70 repeat breeding cows. Bacteria isolated from all the cases and *Streptococcus* was the most predominating. Mixed infections with upto 6 isolates were common and mycoplasma was isolated from 21 per cent.

Mutiga (1978) examined 100 repeat breeder dairy cows and found that 60 % were clinically normal but bacteria were isolated from majority of uteri (90%) in which 30 % of the isolates were mixed. The bacteria isolated were *Pseudomonas aeruginosa*, *Pasturella*, *E. coli*, haemolytic *Streptococci*, *Staphylococci* and *C. pyogenes*.

Sharma *et al.* (1978) studied microflora of cervical discharge of 36 repeat breeder cows at I.V.R.I. dairy farm and found *E. coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Proteus* and *Klebsiella* species in the samples and found that most of the organisms were sensitive to Neomycin, Gentamicin, Nitrofurazones and Chloramphenicol.

Deka *et al.* (1979) found that microflora in the cervicovaginal mucus of repeat breeding cows were *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Streptococcus dysagalactiae*, *Streptococcus pyogenes*, *C. pyogenes* and *Klebsiella aerogenes*.

Awad and El-Hareri (1980) obtained 148 bacterial isolates from mucus samples of 20 cows and 50 buffaloes which were repeat breeder. *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacterium pyogenes*, *Klebsiella* spp. and *Proteus* were main organims isolated. However, the bacteria isolated were more sensitive to Chloramphenicol, Polymyxin-B, Gentamicin and Ampicillin but less sensitive to Colistin sulphate.

Naik (1982) isolatd *Staph. aureus*, *Streptococcus pyogenes*, *E. coli*, *Pseudomonas*, *Corynebacterium* and *Bacillus* species from the genital discharge of the repeat breeding cows and buffaloes.

Kharde *et al.* (1983) studied 112 isolates from 40 repeat breeder cows. The isolated organisms were *Corynebacterium pyogenes*, *C. bovis*, *C. renale*, *Pseudomonas aeruginosa*, *Proteus vulgaris*,

Streptococcus feacalis and *Candida albicans*. Further, they carried out the antibiogram of different isolates obtained from the genital samples of 10 normal and 40 repeat breeder cows in Bombay. The drugs used were Penicillin (10 IU), Ampicillin (10 μg), Sulphadimidin (300 μg), Nitrofurantoin (300 μg), Streptomycin (10 μg), Tetracycline (30 μg), Oxytetracycline (30 μg), Gentamicin (10 μg), Kanamycin (30 μg), Neomycin (30 μg) and Chloramphenicol (30 μg). They found that 63 percent of the isolates from repeat breeders and 47 per cent of the isolates from normal breeders were resistant to one or more of the antimicrobial agents. The maximum inhibitory activity was demonstrated by Gentamicin and Kanamycin closely followed by Neomycin, Streptomycin, Chloramphenicol, Sulfadimidin and Nitrofurantoin in case of repeat breeders while in fertile cows it was demonstrated by the Gentamicin. Kanamycin and Neomycin closely followed by Chloramphenicol, Streptomycin, Nitrofurantoin, Sulfadimidine, Oxytetracycline, Ampicillin and Tetracycline, respectively.

Singh *et al.* (1983) carried out bacteriological investigation on the biopsy material of uteri of 36 repeat breeding and 15 normal breeding cows and found 95 percent repeat breeding cows were positive for bacterial infection while 40 percent of normal cows also possessed uterine infections of lesser intensity. The microorganisms isolated were *Corynebacterium* spp., *Streptococci* spp., *Staphylococci* spp. Gram positive variable cocci, Gram negative bacilli, Anthracoids and Gram positive bacilli.

Bora (1984) studied 172 cervical mucus samples of repeat breeding cows and recovered bacteria from 142 (82.56%) animals, while remaining 30 (17.44%) were sterile. The isolated bacteria were *E. coli* (60), *C. pyogenes* (31), *Ps. aeruginosa* (27), *Staph. pyogenes* (15), *Staph. epidermidis* (14), *Kl. aerogenes* (4), *Streptococcus pyogenes* (1), *Proteus microbilis* (1) and *Bacillus* species (36).

Dholakia *et al.* (1987) found changing pattern of sensitivity of microbial flora of various antibiotics during the six-year periods (1980-81 to 1985-86). The effectiveness of antibiotics has gradually decreased; in the case of Penicillin, it decreased from 26.66 to 14.28 percent, in case of Streptomycin from 50.8 percent to 36.17 percent, in case of Chloramphenicol from 74.5 percent to 46.80 percent and in Tetracycline it decreased from 46.29 percent to 34.78 percent while to Gentamicin, adequate sensitivity around 70 percent was maintained.

Malik *et al.* (1987) studied 395 mucus samples from infertile cattle between January 1984 and December 1985. Bacteria were grown from 370 samples; 226 showed a single bacterial infection while 144 showed a mixed infection. *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacterium pyogenes*, *Bacillus subtilis* and *E. coli* were cultured from 22.98, 31.08, 3.24, 1.35 and 2.43 percent, respectively.

Ben–Haj and Abdulmola (1988) carried out antibiotics sensitivity test of bacterial isolates from 60 repeat breeder cows in

Libya and reported that the isolated bacteria were sensitive to Chloramphenicol, Cephaloridine, Gentamicin, Colistin Sulphate and Neomycin order of sensitivity while these were resistant to Erythromycin, Penicillin, Streptomycin, Tetracycline and Lincomycin.

Sharma *et al.* (1988) performed antibiogram of isolated microorganisms from 43 repeat breeder cross bred cattle (35 cows and 8 heifers) at Ranchi using separate Pasteur bio-discs for gram negative and gram positive organisms. The results indicate that non of the drugs tried was effective against all the isolates; however, most of the organisms were sensitive to Co-trimoxazole, Chloramphenicol and Kanamycin. The sensitivity pattern also revealed that none of the gram positive organism was sensitive to Penicillin and Ampicillin and that many of the strains of bacteria were resistant to higher antibiotics.

Shukla (1989) carried out bacteriological investigation in the biopsy material from the uteri of normal and repeat breeder cows. The uteri of normal cows harboured *Esch. coli*, *Staphylococcus* and *Streptococcus* spp. In 30 percent of the cows and in 91.12% repeat breeder cows had either single or mixed form of bacterial infection in the uterus. However, in repeat breeder cows, the uteri were free from bacterial infection.

Singh *et al.* (1989) obtained a total of 179 isolates of bacteria from uterine swabs of 86 repeat breeding cows of which 72

(83.7%) showed single bacterial infections. The bacteria isolated were *Staph. aureus* (61), Haemolytic *Streptococcus* (43), *Corynebacterium* spp. (29), *E. coli* (18), *Micrococci* (19) and *Pseudomonas* spp. (68). They conducted antibiogram of isolated bacterial strains. The antibiotics used were Kanamycin, Cloxacillin, Tetracycline, Chloramphenicol, Nitrofurantoin, Penicillin, Streptomycin, Ampicillin, Erythromycin and Bactrim. The result of antibiogram revealed that, *Staph aureus* exhibited highest sensitivity towards Kanamycin closely followed by Erythromycin, Cloxacillin and Tetracycline. *Streptococci* spp. showed highest sensitivity against cloxacillin. The maximum sensitivity of *Esch. coli* and *Corynebacterium* isolates were noted against Cloxacillin and Bactrim, respectively.

Sharda *et al.* (1991) reported that the cervico-vaginal samples of 50 repeat breeding animals, 32 (64%) samples were bacterial positive while the remaining 18 (36%) were bacteriologically sterile which are identified as *Staph. aureus* (26.0%). The antibiotic sensitivity test found that all the isolates were sensitive to more than one antibiotics but Gentamicin was found most effective in which percentage sensitivity of isolates were 73.68 followed by the penicillin (68.12%), Chloramphenicol (65.79%), Oxytetracycline (60.53%), Triple Sulpha (57.89%), Streptomycin (55.26%), Nitrofurantoin (52.63%) in the decreasing order, while Ampicillin (59.79%) was found to be the least effective. More than 40% of the isolates showed multiple resistance of 4 or more drugs.

Singla *et al.* (1991) studied 52 cases of repeat breeding in cows and found that in most of the cases, 86.54% cows yielded either single or mixed type of bacterial growth while 13.46% cows were completely sterile and did not yield any bacterial growth. The organisms isolated were *E. coli* (22.65%), *Pseudomonas aeruginosa* (15.10%), *Staphylococcus aureus* (13.20%), *Proteus vulgaris* (11.32%), *Streptococci* (11.32%), *Staph. epidermidis* (11.32%), *Klebesiella* spp. (9.43%) and *Micrococcus* spp. (5.66%). Further, they carried out *in-vitro* drug sensitivity test against 9 different chemotherapeutic agents and found the percentage of sensitivity of the isolates towards these Chemotherapeutic agents in decreasing order were Gentamicin (77.33%), Chloramphenicol (66.03%), Co-trimoxazole (45.28%), Tetracycline (22.78%), Furazolidone (20.75%), Ampicillin (18.86%), Streptomycin (11.32%), Erythromycin (5.66%) and Penicillin (3.77%).

Venkateshwaran and Rajeswar (1991) carried out bacteriological investigation in 252 infertile cattle and isolated E. *coli* as well as genera *Staphylococcus*, *Pseudomonas*, *Streptobacillus*, *Klebsiella*, *Bacillus*, *Proteus* and *Micrococcus* from 26 samples in which random identification was done.

The *in-vitro* sensitivity test using Nitrofurantoin (300 μg), Oxytetracycline (30 μg), Penicillin G (10 IU), Ampicillin (10 μg), Co-trimoxazole (25 μg), Streptomycin (10 μg) and Chloramphenicol (30 μg) and found the percentage of sensitivity of the isolates towards these antibiotics were 13.49%, 23.41%, 23.41%, 29.76%, 30.95%, 33.73% and 41.27%, respectively, in ascending order.

Verma *et al.* (1991) collected cervical mucus samples from infected cattle and they found 100% sensitive to Enrofloxacin, 80% to ampicillin, 84.6% to chloramphenicol and 86.6% to erythromycin.

Ramaswamy *et al.* (1992) reported the antibiogram of the bacterial isolates with regards to susceptibility. They noted that out of all the drugs tested, Gentamicin and Kanamycin were found tobe most effective (74.20 % each) followed by Neomycin (70.97 %), Nitrofurantoin (64.52 %), Cephalexin (61.30 %), Amikacin (58.07 %) and Streptomycin (54.04 %). 70 percent of the isolates were resistant to Ampicillin, Colistin, Penicillin, Polymycin-B, Tetracycline Sulphamethxole, Vancomycin, Amoxycillin and Chloramphenicol.

Gupta and Deopurkar (1993) obtained isolates from 5 cows showing gynecological problems. These isolates were identified as *Staphylococcus aeureus*, *E. coli*, *Pseudomonas aeruginosa* and *Salmonella* spp. The drug sensitivity pattern revealed that out of 13 isolates, maximum number of isolates were found sensitive to Chloramphenicol (61.53 %) followed by Streptomycin (53.84 %), Neomycin (46.15 %), Cephaloridine (30.76 %) and Erythromycin 15.38 %).

Singla *et al.* (1993) reported that out of 36 uterine discharge samples, 34 (94.44%) yielded pure growth of bacteria belonging to single genus. The organisms isolated were *Staph. aureus* (26.31%), *Esch. coli* (18.43%), *K. pneumoniae* (13.16%), β-haemolytic

Streptococci (10.53%), *C. pyogenes* (10.53%), *Ps. aeruginosa* (7.89%), *Pr. vulgaris* (5.26%), *B. cereus* (5.26%) and *Staph. epidermidis* (2.63%). The most of the isolates recovered from repeat breeder cows were sensitive to Gentamicin followed by Chloramphenicol, Septran, Streptomycin and Tetracycline. The percentages of sensitivity of isolates towards these antibiotics were 78.94, 68.42, 42.08, 18.41 and 15.78, respectively. They also found that the sensitivity was minimum with Erythromycin (5.26 %) and Furazolidone (2.63 %) and no organism was sensitive to Penicillin and Ampicillin.

Krishnan *et al.* (1994) performed antibiogram of different isolates and found that all the isolates were sensitive to various antibiotics. The sensitivity percentages of the Gentamicin, Chloramphenicol, Furazolidone, Nitrofurantoin, Chlortetracycline, Ampicillin, and Penicillin were 92, 84, 72, 62, 52, 48 and 44, respectively. They used a total of 128 cervico-vaginal samples from repeat breeder and normal breeder cows for isolation of bacteria and isolated *Bacillus*, *E. coli*, *Staph. aureus*, *C. pyogenes*, *Pr. vulgaris* and *Streptococci*.

Sarmah (1994) carried out the bacterial isolation from the 36 repeat breeder cattle and out of which 28 samples (77%) were yielded bacterial growth on culture. The isolated bacteria were *E. coli* (10), Haemolytic *Streptococcus* (8), *Staph. aureus* (8), *Pseudomonas aeruginosa* (3), *Proteus vulgaris* (3) and *Bacillus* spp (3).

Verma *et al.* (1994) obtained 56 isolates from 46 samples of uterine discharge from repeat breeder crossbred cows, of which *Esch. coli* was found to be the commonest micro organism followed by *Pseudomonas* spp., *Staph aureus*. The other microorganisms isolated were *Staph. epidermidis*, *Micrococcus*, *Corynebacterium* spp., *Streptococcus* spp., *Proteus* spp. and unidentified Gram negative *Bacilli*. They found that majority of the isolates were sensitive to Gentamicin, Chloramphenicol, Furazolidone, Nitrofurantoin and Streptomycin, while some of them were sensitive to Nalidixic acid, Cephalexin and Ampicillin and Penicillin-G. Oxytetracycline and Cotrimoxazole were found to be least effective.

Dabas *et al.* (1995) observed 58 (76.3%) cervical samples, were positive to bacterial infection. Out of 76% cervical samples taken from repeat breeder cows, the isolated bacteria were Gram positive *cocci* (60.4%) mixed infection (27.6%) and Gram negative rods (12%).

They carried out *in-vitro* antibiotics sensitivity/ resistance test against 13 antimicrobials and found that the majority of cervical samples were sensitive to Erythromycin (89.6%), Cloxacillin (93.1%), Ampicillin (86.2%), Chloramphenicol (89.6%), Nalidixic acid (94.8%), Nitrofurantoin (87.9%) and Co-trimoxazole (82.7%). They also found that most of the samples showed resistance against Penicillin, Streptomycin, Oxytetracycline and Tetracycline.

Singh and Sekhon (1995) reported that the isolated culture of different organisms showed maximum sensitivity towards

Gentamicin followed by Chloramphenicol, Apicillin and Penicillin where as all the culture were found resistant to Oxytetracycline.

Takel *et al.* (1995) performed antibiogram of the isolates against different antibiotics and found that all the isolates were sensitive to more than one antibiotic. The majority of isolates were sensitive to Chloramphenicol and Oxytetracycline followed by Framycetin, Streptomycin, Gentamicin and Neomycin.

The isolated organisms were *E. coli*, *Strept. pyogenes*, *Staph. epidermidis*, *Staph. aureus*, *C. pyogenes*, *Anthracoids* and un typed Gram positive *rods* from cervico-vaginal mucus samples of 18 repeat breeder cows. *C. pyogenes* (2.80%), *C. renale* (0.93%), *Pr. mirabillis* (0.93%), *Pr. vulgaris* (0.93%) and *L. dentrificans* (0.93%).

Das *et al.* (1996) carried out bacteriological investigation in 82 repeat breeding cows and found that 34 cows yielded single type of micro-organism while remaining 48 yielded mixed type of infection. The isolated microorganisms were *Ps. aeruginosa* (17.34%) a predominant one, followed by *Staph. aureus* (14.28%), *E. coli* (13.26%), *Corynebacterium pyogenes* (10%) and *Acholeplasma* spp. (10%). Further, they showed Ciprofloxacin, Norfloxacin, Gentamicin and Kanamycin were the effective drugs for the treatment. When bacteria is the sole cause of repeat breeding, the percentages of sensitivity of the samples towards these antibiotics were 97.56, 85.36, 73.1 and 51.21, respectively.

Dhabale *et al.* (1996) carried out bacterial isolation from cervical mucus samples of 30 repeat breeder cattle. The different bacterial isolates obtained were *E. coli*, *C. pyogenes*, *C. ranale*, *Strept. pyogenes*, *Staph. aureus* and *Pseudomonas aeruginosa*. They found that the sensitivity and resistance percentage of various isolates recovered from repeat breeder cattle towards different antibiotics were Gentamicin (94.44% and 5.55%), Chloramphenicol (88.88% and 11.11%), Streptomycin and Nitrofurantion both had same sensitivity percentage (61.11% and 38.88%) and Tetracycline (33.33% and 66.66%). Ampicillin was found to be 100% resistant.

Ramkrishna (1996) studied the susceptibility pattern of different isolates against various antibiotics and found that the organisms were highly sensitive to Gentamicin (89.1%), Chloramphenicol (73.9%) and Nalidixic acid (73.9%) followed by Kanamycin (69.5%), Nitrofurazone (65%), Tetracycline (58.6%), Trimethoxazole (58.6%), Streptomycin (34.7%), Sulfamethoxazole (14.5%) and Pencillin (13%).

Singh *et al.* (1996) studied 122 cervicovaginal mucus samples from repeat breeding bovines and isolated 107 strains of bacteria. The isolated microorganisms were *B. cereus* (11.21%), *B. magaterium* (4.67%), *B. mycoides* (3.74%), *B. subtilis* (0.93%), *Bacillus* species untyped (32.71), *Esch. coli* (24.3%), *K. genitalium* (1.87%), *Klebsiella* untyped (5.61%), *S. aureus* (8.74%) and *S. albus* (1.87%).

Gupta *et al.* (1997) studied cervico-vaginal mucus of 108 repeat breeding cows and found 58 (53.7%) having genital tract infection. The organisms isolated were *Staphylococci*, *Streptococci*, *E. coli*, *Salmonella* spp., *Klebesiella* spp., *Enterobacter* spp., *Proteus* spp. Gram positive bacilli and unidentified gram negative rods. The antibiotic sensitivity was maximum for Ciprofloxacin, Chloramphenicol, Amikacin, Gentamicin, Kananycin and Norfloxacin. Most of the cultures were found resistant to Oxytetracycline and Penicillin.

Kumar (1997) tested antimicrobial agents to different isolates in his study on cattle suffering from repeat breeding problems. Ciprofloxacin was found to be the most sensitive (91.86%) followed by Gentamicin (84.88%), Cephalexin (63.95%) and neomycin (58.14%). Septran and Ampicillin had limited effect of 41.86 and 32.55% respectively, where as tetracycline was sensitive only in 27.90% of the isolates.

Baishy *et al.* (1998) studied the antibiogram of bacteria isolated from uterine discharges of repeat breeding cattle. Out of 36 uterine samples collected from repeat breeder cattle, 31 (86.11%) were culturally positive for one or other type of bacteria. Six (16.67%) samples yielded mixed type of bacteria. Most common isolates were *Esch. coli* (29.73%) followed by *Staphylococcus* spp. (24.32%), *Streptococcus* spp. (18.92%), *Bacillius* spp. (18.22%) and *Corynebacterium* spp. (8-10%). The sensitivity pattern of

antimicrobial agents revealed that 97.27% organism were sensitive to Ciprofloxacin, 83.78% to Gentamicin, 78.37% to Kanamycin, 72.29% to Erythromycin, 43.24% to Cloxacillin, 40.54% to Ampicillin and 18.91% each to Nitrofuraxone and Oxytetracycline. Ciproflocxacin was found to be the most effective drug *in-vitro*.

Arora *et al.* (2000) undertook bacteriological studies on the genital infections in repeat breeder bovines. Out of 225 samples processed, 190 (84.44%) yielded a total of 305 different isolates while the remaining 35 (15.56%) were negative for any bacterial growth. *Esch. coli* was the predominent organism comprising 25.25% of the total isolates, followed by *Staphylococus aureus* (17.05%), *Proteus* spp. (13.44%), *Streptococci* (12.79%), *Bacillus* spp. (8.52%), *Klebsiella pneumoniae* (6.89%), *Pseudomonas aeruginosa* (6.56%) and *Cornybacterium pyogenes* (1.64%). In mixed bacterial cultures, the most common combinations were *Esch. coli* and *Staphylococus aureus*. The antimicrobial sensitivity of these isolates revealed that Gentamicin (94.43%) is the most effective drug closely followed by Pefloxacin (93.11%). A fairly high degree of sensitivity was also observed against Nitrofurantoin (87.87%), Chloramphenicol (74.43%) and Neomycin (68.52%) However, a large number of isolates were resistant to Oxytetracycline, Co-trimoxazole, Erythromycin, Streptomycin, Ampicillin and Penicillin.

Moharana *et al.* (2000) collected total of 168 uterine mucus samples from different area of Orissa during (1995-96). They

found Ciprofloxacin (75%), Gentamicin (69.7%), Chloramphenicol (68.5%), Streptomycin (41.6%) and Nitrofurantoin (48.2%) to be highly sensitive while Penicilin G, Ampicllin and Cloxacillin showed higher percentages of resistance. The isolated organisms identified were *Streptococcus* spp. and *Staphylococcus* spp. The other organisms associated were *Esch. coli*, *Proteus* spp., *Pseudomonas* spp., *Klebsiella* spp., *Corynebacterium* spp. and *Bacillus* spp.

Seh *et al.* (2000) tested antimicrobial agents of different isolates in their study on cows suffering from repeat breeding problems. The sensitivity pattern of the isolates revealed that 98.48% organisms were sensitive to Gentamicin, 92.42% Ciprofloxacin, and Chloramphenicol and Nitrofurantoin, respectively. Erythromycin was the only antibiotic that showed least effective (3.03%).

Chandrakar *et al.* (2002) noted overall frequencies of different bacterial isolates identified from uterine fluid of repeat breeding crossbred cows that were *Staph. aureus* (29.16%), *Esch. coli* (20.83%), *C. pyogenes* (16.66%), *Ps. aeruginosa* (16.66%), *Streptococcus* (12.5%) and *Proteus* (4.16%) the antibiotic sensitivity pattern of these bacterial isolates revealed that Chloramphenicol (91.6%) was most effective drug followed by Gentamicin (70.8%). However, lower sensitivity was evident against Oxytetyacycline (37.5%), Cephalexin (33.3%) and Streptomycin (33.3%) whereas Ampicillin and Penicillin did not show any sensitivity (0.0%).

IV. DISTRIBUTION OF ANTIMICROBIAL AGENTS IN UTERINE TISSUE

Distribution studies are necessary for determining the therapeutic dose and concentration, route as well as frequency of administration of a drug. The drug must reach the site of infection in concentration high enough to destroy or inhibit the infecting organisms. The largest organ involved in genital tract infection is the uterus where the drug should reach in sufficient quantity to eliminate the infection. Two main methods are employed for the treatment of genital tract infections with antimicrobials.

1. Systemic (i.v/i.m)
2. Local (intrauterine)

After systemic administration, an antimicrobial absorbed in the blood and there after transported to the genital tissues where its concentration may be guided by several factors like plasma protein and tissue binding of the drugs, blood flow to the uterus, lipid solubility and diffusion characteristics of the drug etc.

If an antimicrobial agent reaches in therapeutic concentration in the uterine tissues and the uterine lumen, when given via the systemic route, this route of administration may be preferred to intrauterine infusion since it is the most convenient route and the drug concentrations can be easily predicted as well as can be accurately be maintained. Sometimes, uterine infections are

also associated with systemic infections which can be cured when a drug was given by systemic route. Therefore, a number of studies have been conducted to explore its feasibility in the treatment of repeat breeder cows associated with uterine infections.

Masera *et al.* (1980a) found that on i.m. administration of Oxytetracycline resulted in more rapid and complete absorption of the drug into the blood than that following intrauterine (i.u.) infusion. Detectable concentrations of the drug were present in all tissues of the reproductive tract 24 h after i.m. injection.

Masera *et al.* (1980b), also studied the peak blood concentration of sodium penicillin G after i.m. injection and they found a high peak blood concentration occurred more quickly and therapeutic concentration lasted longer than those achieved after i.v. infusion. Endometrial concentrations were present for 8 h but were considerably less than those achieved following i.u. administration.

Caudle *et al.* (1983) observed the endometrial levels of Amikacin in mare for three consecutive days. Four mares were infused with 2.0 g once a day and four others were infused with 3.0 g a day. Biopsy result indicates that Amikacin is readily absorbed into the equine endometrium. The 3.0 g dose has no therapeutic advantage over the 2.0 g dose.

Jayachandran *et al.* (1987) studied distribution of streptomycin in uterine fluid of she buffaloes after i.m. injection of Streptomycin (10 mg/kg). They observed that the drug was detectable upto 12 h in uterine fluid but therapeutic level was not achieved.

Jayachandren *et al.* (1988) also noted non attainment of therapeutic level when sulphadimethcoxine (100 mg/kg) when it was administered orally in buffaloes. The authors suggested that this drug could not be used by oral route for the treatment of uterine infections. In contrast, Singh *et al.* (1988) showed the maintenance of therapeutic concentration for a period of 12 h in uterine fluid when sulphadimidine was given by i.v. route @ 200 mg/kg.

Jayachandran *et al.* (1995) showed maintenance of therapeutic concentration of oxytetracycline for a period of 0.5 – 24 h when administered at the dose rate of 5 mg/kg, i.v. and the drug can be effectively used by systemic route for the treatment of endometritis in buffaloes.

Sood *et al.* (1999) studied the disposition kinetics and uterine tissue levels of Neomycin in six reproductive healthy and normal cycling female buffaloes after i.v. administration during estrus period at the dose of 5 mg/kg body weight. At 10, 30 and 720 min, the levels of Neomycin in uterine tissues were 42.43 ±2.84, 26.72 ± 1.28 and 2.13 ± 0.43 μg/kg, respectively. They suggested that dosage regimen of Neomycin in bufflalo should be 5 mg/kg followed by 3 mg/kg at 12 h interval.

(a) Enrofloxacin: -

Enrofloxacin, one of the newly developed fluoroquinolones, was synthesised in 1983 by Bayer Research Laboratory in Germany. It is used as a drug of choice for animal

treatment only. Enrofloxacin is metabolised into cipfloxacin in liver, which also exerts potential antimicrobial activity.

Enrofloxacin is a broad spectrum antimicrobial with bactericidal action. It is effective against gram negative and gram positive bacteria as well as Mycoplasma. In addition, some anaerobic pathogens are also susceptible. It is also effective against micro organisms that are resistance to β-lactum antibiotics, tetracyclines and aminoglycosides. The MIC values of enrofloxacin for different species of microorganisms ranged between 0.01 to 2.0 μg/ml in veterinary practice although enrofloxacin is being extensively used in cows for treatment of genital infections. Literature on its disposition studies in the reproductive tract is particularly scanty. However, few studies on enrofloxacin in animals are described below.

Luna *et al.* (1991) administered enrofloxacin by uterine infusion (3 $mg.kg^{-1}$) and noted rapid absorption through uterine mucosa and rapidly excreted in the milk within 12 hr.

Amorena *et al.* (1992) administered enrofloxacin s.c. and i.v. to 6 buffalo at the dose rate of 2.5 $mg.kg^{-1}$ after i.v. administration. They observed the peak plasma concentration to be 1.756 ± 0.346 μg/ml while after s.c. it was to be 0.210 ± 0.037 μg/ml after 70 minutes. The elimination half lives were similar for both the routes. They have recommended that enrofloxacin should be administered at the dose rate of 2.5 $mg.kg^{-1}$ body wt. repeated at every 8h interval.

Kuhn (1993) noted in pig that the peak plasma concentration of 0.68 μg/ml at 225 minutes after single i.v. injection given at the dose rate of 2.5 mg/kg. He also reported that since the amount in urine exceeded 4 mg/L during 12 h after injection, the drug might be suitable for treating urinary tract infections.

Tras *et al.* (1993) noted the enrofloxacin concentration in milk samples of dairy cow after i.m. injection of enrofloxacin (2.5 mg/kg) to be 0.035 ± 0.005, 0.025 ± 0.009 and 0.005 ± 0.003 μg/ml at 24, 48 and 72 hr, respectively. They also noted that enrofloxacin could not be detected at 96 and 120 hrs in milk.

Walser *et al.* (1993) conducted kinetic study of enrofloxacin after i.v., i.m. and s.c administration of 2.5 mg/kg body weight in cows. They noted that enrofloxacin penetrated into milk in higher concentrations and persisted longer period than that of blood.

Kaartinen *et al.* (1995) estimated elimination half life of 1.7, 5.9 and 5.6 h after i.v., i.m. and s.c. administration of enrofloxacin (5 mg/kg). Mean absorption time were 6.2 and 6.9 h after i.m. and s.c. administration. The bioavailability after i.m. administration was 82% and 137% after s.c. administration. They noted volume distribution over 1 L/kg for enrofloxacin after i.v. injection. The peak concentration of enrofloxacin in milk was reached between 0.7 and 1.2 h. After i.m. and s.c. administration, the concentration time curves for enrofloxacin in milk were shallow and there were no obvious peak.

Gigure *et al.* (1996) conducted pharmacokinetic studies of enroflxacin in adult horse and estimated the concentrations of the drug in serum, body fluid and endometrial tissues after repeated intragastrically administered dosage. They noted mean absorption half life of 0.68 and 0.3 h and elimination half life of 5.94 and 6.09 for the post i.v. dosage of 2.5 and 5 mg/kg body weight, respectively. Endometrial tissue concentration exceeded plasma concentration by as much as 3 fold. For the 5 mg/kg dosage, mean endometrial concentrations (10.19 mg/g at 74 hrs and 6.56 μg/g at 84 hrs) exceeded the MIC for most gram positive and gram negative aerobes including *Pseudomonas* spp., *S. zooepidemicus*, *Klebsiella* spp. and *E. coli* which are the agents most frequently isolated from mares with endometritis.

Mengozzi *et al.* (1996) noted a rapid distribution phase and a slower elimination phase with a half life ($t_{1/2}$ β) of 3.78 ± 0.44 h after i.v. dose of 2.5 mg/kg. When the same dose was administered i.m., the drug was rapidly absorbed, reaching mean peak plasma conc. in 1.2 ± 0.11 h; after that time, it appeared to decrease with a half life of 3.65 ± 0.31 h, the bioavailability (F) of enrofloxacin by i.m. route was calculated to be 85.28 ± 3.40% and volume distribution (Vd_{SS}) was noted to be 3.02 ± 0.22 and 3.03 ± 0.31 L/kg for i.v. and i.m. routes, respectively.

Gatne *et al.* (1997) administered enrofloxacin by i.m. route at the rate of 2.5 mg/kg body weight. They found a variation in the persistence of enrofloxacin in serum, between 6 to 8h. They have

also suggested that the dose of enrofloxacin should be repeated every 12 h and in acute cases every 8 h.

Kumari Sudha (1998) conducted kinetic study of enrofloxacin after single i.v. and s.c. administration of enrofloxacin in healthy lactating goat at the dose rate of 5 mg/kg body wt. they noted mean absorption half life ($t_{1/2}$ ka) and distribution half life ($t_{1/2}$ α) of 0.60 ± 0.01 and 0.20 ± 0.03 hr in goat. Elimination half lives ($t_{1/2}$ β) were also observed as 2.82 ± 0.33 and 1.42 ± 0.15 h for i.v. and s.c. administration, respectively. Vd_{area} of 2.34 ± 0.54 and 5.26 ±1.23 L/kg were reported for i.v. and s.c. administration, respectively.

Kumar (2002) administered enrofloxacin i.v. at the dose rate of 5 mg/kg in healthy cows and cows suffering from endometritis. He observed the drug reached its peak plasma concentration 4.67 ± 0.23 μg/ml and 3.47 ± 0.21 μg/ml at 0.5 h in healthy and endometric cows respectively. The drug reached its peak concentration in uterine fluid was 2.20 ± 0.08 μg/ml at 4h and 9.00 ± 0.73 μg/ml at 3h in healthy and endometric cows respectively. In plasma the mean therapeutic concentration was maintained upto 12 h in both healthy cows and cows suffering from endometritis. The drug maintained its therapeutic concentration (0.12 ≥ μg/ml) in uterine fluid from 2 to 12 h in healthy cows where as 1 to 24 h in cows suffering from endometritis.

(b) Benzathine Penicillin: -

Hagdrup *et al.* (1986) treated twelve human suffered from syphilis weekly with injection of 1.44 gm (2.4×10^6 I.U) of Benzathine Penicillin G for upto 3 weeks. Almost daily, serum penicillin concentrations were measured by a sensitive microbiological agar cup method. An individual and inter individual variation was found. Concentrations below the recommended 0.018 μg/ml were found 7 days after the first or second injection in 5 samples. So, shorter intervals between injections are recommended.

Kaplan *et al.* (1989) collected a total of 193 samples that were studied after administration (i.m.) Benzathine Penicillin G in young human and the dose was 1,200,000 units and serum penicillin levels were determined at 1, 3, 10, 21 and 28 days. The mean serum penicillin levels remained greater than or equal to 0.02 μg/ml for 21 days, but by 28 days only 44% of the serum samples had detectable levels of penicillin and only 36% had levels greater than or equal to 0.02 μg/ml.

Nathan *et al.* (1993) studied 25 healthy gravidas at 38-39 weeks gestation scheduled for selective repeat cesarean delivery under spinal anaesthesia receiving Benezathine Penicillin G, 2.4 million IU intramuscularly preoperatively. Ten women delivered 1 day after injection, five delivered 2-3 days after and ten delivered 7 days after. They collected maternal serum and cerebrospinal fluid, amniotic fluid (AF) and cord serum at delivery. Penicillin levels measured using a

validated agar disc diffusion methods (sensitivity 0.006 μg/ml) with *Micrococcus lutea* as the test organism. There was no significant difference in mean penicillin levels at days, day 2-3, or day 7 for maternal serum, maternal cerebrospinal fluid, cord serum, or AF. The mean (± standard error) penicillin concentration (range 0.005 – 0.59 μg/ml) in maternal serum declined from 0.14 ± 0.04 μg/ml 1 day after injection to 0.08 ± 0.06 mg/ml 7 days after injection. The proportion of patients with a penicillin concentration at or above 0.018 μg/ml in the maternal serum declined significantly from day 1 to day 7. Overall, nine of 25 women (36%) had serum penicillin levels that were less than 0.018 μg/ml.

Papich *et al.* (1994) studied plasma concentration of penicillin G was in beef steers after administration of either a combination of Bnezathine Penicilline and Procain Penicillin G in a 1:1 mixture at a dosage of 9, 000 I.U/kg of body weight i.m. (n=5), 24,000 I.U/kg i.m. (n=5) or 8,800 I.U/kg s.c. (n=5) or Benzathine Penicillin alone at a dosage of 12,000 I.U/kg i.m. (n=7). Plasma concentration of penicillin G was measured by use of high performance liquid chromatography assay that had a limit of determination of 0.005 μg/ml. At a dosage for this combination of 9,000 I.U/kg i.m. and 8,800 I.U/kg s.c. mean ± S.E.M., peak plasma concentration was recorded 0.58 ± 0.15 and 0.44 ± 0.02 μg/ml respectively. Although the plasma penicillin concentration was quantifiable for 7 days in steers that received 9,000 IU/kg i.m. for 4

days that received 8,800 IU/kg s.c., the concentration was < 0.1 μg/ml in both groups after the first 12 hours.

After administration of the combination at dosage of 24,000 IU/kg i.m., there was initial peak plasma concentration at approximately 2 hours, thereafter plasma concentration decreased slowly with half life of 58 hrs. Although plasma penicillin G concentration was quantifiable for 12 days at this dosage, concentration was < 0.1 μg/ml for the first 48 h.

Belov *et al.* (2000) studied comparative randomized pharmacokinetic of Benzathine Penicillin in three dosage forms. Benzathine Penicillin was used as Extencilline (2.4 million unit or 1.2 million unit, "Rhone-Poulene Rorer", France) and as Billin-5 (1.5 million unit, "Synthesis" Russia). 33 patients were included in investigation (23 women and 10 men aged 16-60 years). Benzyl penicillin concentration was estimated by microbiology test in blood samples taken 1, 3, 24 h and 7, 14 and 21 days after intramuscular drug injection. After 2.4 million unit of Extencilline administration, the inhibition level for beta-hemolytic *Streptococcus* group A (25 ng/ml) was maintained in 83.3% of patients for 3-week period. After 1.2 million unit Extencillin injection (10 patients) or 1.5 million unit Bicillin-5 injection (12 patients) the above mentioned concentration was achieved on the 21st day in 30 and 0 percent of patients, respectively.

Ranheim *et al.* (2002) injected 33,000 IU/kg or 100,000 IU/kg Benzathine Penicillin + Procaine Penicillin G i.m. or s.c. or 100,000 IU/kg Procaine Penicillin G i.m. or s.c. in piglets. Intramuscular injection of Benzathine Penicillin + Procaine Penicillin resulted in higher maximum concentrations in plasma than did s.c. injection. The mean residence time (MRT) of Penicillin G was longer when the drug was injected s.c. rather than i.m. They also recorded secondary peaks between plasma concentration versus time profile of the s.c. injections of Benzathine + Procaine Penicillin which is possibly reflecting a certain degree of inflammation at the injection site.

IV. THERAPEUTIC MEASURES

(a) Conventional therapy: -

For efficient reproduction, the balanced supply of nutrients is essential. Several investigators indicated the direct relationship between nutrients and the number of services per conception (Mc Donald *et al.*, 1961). Hypoglycaemia is responsible for infertility in lactating cattle and affects pituitary functions (Roberts 1971). Lower serum protein level is associated with inactive ovaries and affects the process of implantation (Roberts, 1971). Calcium plays an important role in sensitizing the tubular genitalia for the action of hormone therapy increasing the fertility rate in animals (Moddie, 1965). Serum inorganic phosphorus levels falls following insufficient dietary intake (Blood *et al.*, 1980). Minerals and enzymes play an

important role in animals by increasing the efficiency of livestock production and reproduction.

McDonald *et al.* (1961) used an organic iodine compound fed orally for 8 to 12 days before the next service in 1036 repeat breeding cows and 58 percent conceived on the first service, while in the control cows conception rate was below 50 percent.

Mehta *et al.* (1986) treated repeat breeding crossbred cattle with "Triple Sulphate Mixture" ($CuSO_4$ – 150 mg; $CoSO_4$ – 2g and $FeSO_4$ – 1000 mg) was given daily for ten days beginning with the day of estrus and found 66.66% conception rate.

Ranjan *et al.* (1991) treated 7 repeat breeding cows with 5 g iodized salt daily for 1 month that resulted in a sharp increase in thyroxine level and 50% of repeat breeders conceived in 1st insemination.

Rupde *et al.* (1993) treated repeat breeders by corrective supplemental therapy with mineral-vitamin mixture (Alvite-M) at the rate of 20 g per animal per day once in the feed of each animal for a period of 15 days that helped to raise the lower serum biochemical levels.

Kumar (2000) treated repeat breeding crossbred cows with Supplevite M 30 g orally once daily. The conception recorded to be 50% in repeat breeder cows and 20% in control animals.

(b) Gonadotropin releasing Hormone (GnRH) therapy: -

Gonadotropin releasing hormone (GnRH) modulates the secretion of Leuteinizing Hormone (LH) and Follicle Stimulating

Hormone (FSH) by the secretary cells of the adenohypophysis in cattle (Kaltenbach *et al.*, 1974). The mechanism of apparent GnRH induced improvement in fertility has not yet been established. It is conjectured that GnRH might induce an earlier luteininzing hormone (LH) surge and ovulation, resulting in better synchronization of ovulation and insemination for improved conception rates. Other possible cause for improved fertilization is GnRH, which induces an additional surge of LH to enhance active luteinization of granulosa and theca cells, to ensure adequate production of progesterone in the developing corpus luteum in order to maintain pregnancy following successful fertilization. Repeat breeding cattle treated with GnRH at the time of insemination have higher pregnancy rates than to controls (Lee *et al.* 1983, Stevenson *et al.* 1984, Pathak *et al.* 1986, Sonwane *et al.* 2001).

Schelms and Mostafawi (1978) injected 109 Holstein Friesian pure-bred cows with 0.125 mg of synthetic GnRH analogue at the time of first service to study the effect of GnRH on the fertility of inseminated cows. 58.7% cows were diagnosed as pregnant after one insemination whereas 49.5% animals were pregnant in the untreated control group. Total pregnancy rate of 81.65% in the treated group was higher than 8.33% noted in the controls while the service per conception also improved from 1.49 to 1.39. It was concluded that GnRH administration at the time of estrus could improve herd fertility though its effect on follicular rupture and regulatory influence on ovarian function.

Elemer (1983) treated 145 cows with Receptal (Hoechst) at the time of insemination and reported that the number of inseminations per conception was 2.10 vs 2.79 in 198 untreated controls and the pregnancy rate 47.6 vs 35.9 per cent.

Lee (1983) reported that repeat breeder cows (n = 346) given saline solution (n = 161) or GnRH (n=185) at the time of 3rd breeding, the conception rate for repeaters given GnRH (72.97%) were 25% higher than those controls (47.83%).

Pathak *et al.* (1986) studied the conception rate for repeat breeder cows given 100 mg GnRH at the time of 4th insemination, which was higher as compared to controls (47% vs 37.7%).

Stevenson *et al.* (1988) treated repeat breeder dairy cows with GnRH at the dose rate or 100 mg i.m. given within 30 seconds after insemination. They found higher (54%) conception rate in repeat breeders than controls (39%).

Majumdar (1989) administered 2.5 c.c of GnRH (Receptal) at the time of A.I. in 38 repeater bovines. Of 38, 23 (60%) repeat breeding bovines were confirmed pregnant.

Stevenson *et al.* (1990) assigned repeat breeding cows randomly to four treatment groups when detected in estrus as single A.I. plus no injection, single A.I. plus 100 mg GnRH at A.I.; double A.I. plus no injection and double A.I. plus 100 mg GnRH at A.I., and reported over all pregnancy rates of the four treatments were 112/353

(32.1%); 165/406 (41.6%), 119/364 (33.5%) and 135/359 (37.5%), respectively.

Bon Durant *et al.* (1991) studied effect of GnRH on fertility in repeat breeder California dairy cows, by administrating GnRH 100 mg (n=495) or placebo (n=468) at the time of third insemination and found 43.2% and 39.3% conception rates, respectively.

Rao (1991) conducted clinical trials on repeat breeder cows given intramuscularly injection of 5 ml GnRH (Receptal) at A.I., resulted in a conception rate of 54.2 per cent.

Roy *et al.* (1995) administered 5 ml receptal i.m. at the time of onset of heat and the conception rate was 33.3% in control and 73.6% in repeat breeding treatment group.

Senthilkumar and Rajasekar (1998) reported that in GnRH treated animals (100 mg at A.I.), percentage of conception rate was 53.35%.

Sonwane *et al.* (2001) found that the 65% conception rate in repeat breeder treated crossbreed cows as compared to 40% control animals after 2.5 ml Receptal i.m. at the time of insemination.

Rangnekar *et al.* (2002) injected 2.5 ml Fertagyl (GnRH and analogue) intramuscularly at the time of insemination. The conception rate in treatment group was 70% and control group was 40%. The conception rate in treatment group was boosted by 30% than control group.

Shelar *et al.* (2002) studied and assessed pre insemination use of GnRH in 20 non-infectious repeat breeding Gir cows and its crossbreds (G×HF, G×J). Buserline 10 μg was injected i.m. just before insemination in treatment group of animals. They found that conception rate in the treatment group was 20% higher than the control.

(c) Antimicrobial therapy: -

Pre-requisites of any rational therapy include a correct diagnosis, selection of proper and judicious use of drug and thereby achieving the therapeutic objectives. The beneficial effect of specific antimicrobial and chemotherapeutic agents selected on the basis of *in-vitro* sensitivity tests in repeat breeding cows had been reported by various workers (Kharde *et al.,* 1983; Das *et al.,* 1996 ; Gupta *et al.,* 1997).

Uterine infections commonly lead to repeat breeding (infertility) in cattle by causing denudation of the uterine mucosa and altering the pH of the uterine content and thereby adversely affecting the survival of spermatozoa in the female genital tracts or by causing inflammation of the uterus and thus affecting implantation of the ova.

The efficacy of antibiotics and anti-microbial treatment of uterine infection in repeat breeding cows by parentral route depends upon several factors including the sensitivity of the offending organism to the antimicrobial selected, dosage and duration of the treatment, route of administration, the time at which the treatment

was initiated during the course of the disease, presence of concurrent maladies, nutritional status, and stress resulting from environmental or managemental factors. Several studies were conducted for isolation of the uterine and cervico-vaginal microflora in diseased conditions as repeat breeder and antimicrobial therapy by intrauterine route. But very few works have been done for the treatment of repeat breeding by parentral route.

Shoaib (1975) treated 15 repeat breeding animals with Hostacycline powder (15 g of Hostacycline powder dissolved in 30 ml of sterile distilled water) and found 80 percent conception rate in the treatment groups as compared to 20 percent in the control.

Sharma *et al.* (1978) treated 36 repeat breeder cows with specific antibiotics and chemotherapeutic agents selected on the basis of *in-vitro* sensitivity test at I.V.R.I. Izatnagar. The drugs used were Neomycin 0.35 g, Terrmycin 5 g, Nitrofurazone 1 g. Chloramphenicol 0.35 g and Streptomycin 1 g. These drugs were dissolved in 10 to 15 ml of normal saline and infused intrauterine 6 to 24 hours post insemination. This approach of treatment of repeat breeders cows resulted 86.6 percent conception rate in treated group while it was 16.6 percent in control group.

Purbey and Umashankar (1985) carried out treatment of 64 repeat breeder cows of Military dairy farm, Bareilly with intrauterine antibiotics 8 to 12 h post A.I. The drugs used for treatment were Penicillin. Hostacycline. Tetracycline, Furea bolus and Mastalone U. They found that 41 were conceived out of 61 cows treated (64.04 percent) with these treatments.

Singh *et al.* (1986) treated the repeat breeder crossbred cattle in 2 groups with 2 different dosages of Ampicillin at R.V.C., Ranchi. The conception rate observed in group 1 (250 mg of Ampicillin) was 59.09 percent as compared to 44 percent in control whereas, it was 72.73 percent in group 2 (500 mg of Ampicillin intrauterine) as against 44 percent in control group.

Awasthi and Karche (1987) treated 50 repeat breeder crossbred cows at J.N.K.V.V., Jabalpur. The best results were obtained in group 1 and group 2, which were treated with Gentamicin sulphate and Septalone U. The overall conception rate of 60 percent was noted in each group. Poor result was obtained in group treated with Streptopenicillin with the conception rate of 40 percent only.

Saini *et al.* (1989) treated 254 repeat breeder of crossbred cows during the period of August 1983 to July. 1986 at Hissar with Streptopenicillin, Betadine and Mastalone U. The drugs were given 24 horus after insemination through Intra-Uterine route. The higher conception rate of 51.94 percent was found in Streptopenicillin treated group followed by 47.06 percent in Betadine. No difference in conception rate was observed between Mastalone U treated group and control group. Both these groups had low conception rate viz. 42.50 percent and 40 percent, respectively.

Maurya *et al.* (1992) stated that 88 repeat breeder animals (cattle and buffaloes) were given treatment on the basis of sensitivity test. The intrauterine infusion along with parentral injection of the most effective drug/drugs, either single or in

combination of two for 6 days at the time of estrus resulted in 67 (84.8%) conception within next two estrus cycles. No animal in the control group recovered during this period.

Singla *et al.* (1993) treated the animals on the basis of bacteriological examination and *in-vitro* drug sensitivity test. The intra-uterine infusion of Gentamicin 200 mg and Chloramphenicol 1.0 g for 3 consecutive days at the time of estrus resulted in 88.88 percent and 38.9 percent conception rate, respectively. The overall conception rate was found to be 68.89 percent.

Awasthi and Nema (1995) treated 60 repeat breeder crossbred cows belonging to private organised farms at Durg and Bhilia. The drugs used were Gentamicin, Ampicillin-Cloxacillin combination and Cephalexin. The best results were obtained with Cephalexin (90 percent) at the dose of 1.5 g and 750 mg intrauterine and 24 h post A.I. followed by Ampicillin-Cloxacillin combination where conception rate was 70 percent and 50 percent at the dose of 1 g and 500 mg, respectively. The lowest conception rate of 50 percent was obtained with 500 mg and 200 mg of Gentamicin sulphate.

Dabas *et al.* (1995) treated 76 repeat breeder crossbred cows with specific antibiotics selected on the basis of *in-vitro* sensitivity test at Pantnagar and found that intrauterine infusion along with parentral administration of the most effective drug or drugs either single or in combination of two for 4 to 5 days at the time of estrus resulted in conception in 46 (79.31 percent) animals within next two estrus cycles post treatment.

Dhabale (1995) treated 15 cattle and 10 buffaloes by single intra uterine medication with one of the antibiotics. As per antibiogram results after 24 hours post A.I., he noted 7 cattle and 5 buffaloes became pregnant, making the conception rate of 46.66 and 50.00 percent, respectively.

Singh and Sekhon (1995) reported that repeat breeding cows treated with Gentamicin 800-1200 mg gave 73.70 percent conception rate at first A.I. and 13.2 percent in subsequent A.I. Chloramphenicol given at the dose of 2 g resulting in 40 percent conception rate at first and 20 percent conception in the subsequent A.I.

Chandrakar *et al.* (2002) treated repeat breeding cows based on sensitivity test. Group I (n=10) was treated with 1 g Chloramphenicol diluted in 20 ml distilled water and group II (n=10) 400 mg Gentamicin diluted in 20 ml distilled water through intrauterine route for three consecutive days. 80%, 70% and 20% conception were recorded with Chloramphenicol, Gentamicin and untreated control (n=10), respectively.

Roy *et al.* (2002) treated with Gentamicin at the rate of 400-800 mg I/U per animal after A.I. Out of 22 treated cows, 17 (77.3%) conceived at first A.I. and 2 (9.11%) in subsequent A.I.

Chapter - 3

Materials and Methods

MATERIALS AND METHODS

The present study was carried out on cows, which came for gynaecological check-up at the Department of Animal Reproduction, Gynaecology and Obstetrics of Bihar Veterinary College, Patna, organised Khatals in and around Patna and cattle farm of RAU, Pusa, Samastipur. In each case, history of the animal was recorded in relation to number of calving A.I./natural service done etc. Repeat breeding cows having normal or nearly normal estrus cycle and estrus period and has been bred two or more times to a fertile bull (yet failed to conceive) were selected. The repeat breeding cows were evaluated on the basis of history and special gynaeco-clinical examination. For this examination, cow was properly restrained in the travis and the tail of the cow was held to one side by an attendant. The vulvar and perineum region were cleaned and dried. Afterward the cows were subjected to recto-vaginal examination for colour of mucus membrane and genital discharge, cervical relaxation, uterine tone, follicular and luteal status of ovaries and other observations were also recorded.

In the present study to know the sub-clinical form of infections, isolation of etiological agents were undertaken. After isolation of infective agents, treatment was done and conception in repeat breeding cows was noted to know the efficacy. Concentrations

of Enrofloxacin and Benzathine Penicillin in serum and uterine fluid at specific time intervals were estimated to know the effectiveness by systemic route. Ninety (90) cows weighing between 250 to 430 kg were selected for the present study.

For carrying out the present study in case of healthy (normal) and repeat breeding cows the following proforma were used.

Proforma – I

Anamnesis and case record of animals

1. Case No.
2. Date
3. Name of the owner
4. Address
5. Species
6. Breed
7. No. of calving
8. Last calving
9. Estrus cycle
10. Number of services
    (i) Natural
    (ii) Artificial
11. Time of heat
12. General Condition

Proforma – II

Special examination of animals

1. Vagina
2. Os Cervix
3. Discharge (colour, consistency, odour etc)
4. Cervix
5. Uterine horn
   (a) Left
   (b) Right
6. Oviduct
   (a) Left
   (b) Right
7. Ovaries
   (a) Left
   (b) Right
8. Diagnosis

All the experimental animals were free from ecto as well as endoparasites. Cows having irregular estrus cycle, mucopurulant discharge and cystic ovaries were excluded from the study.

The animals thus selected, were divided randomly into various groups for different experiments.

PREVALENCE

The prevalence of repeat breeding cases were obtained from gynaecological records of cows during the period of August 2002 to July 2003 as maintained in the Gynaecology Department of Bihar Veterinary College, Patna. Month, season-wise and parity-wise prevalence were analysed among selected cows.

Methods of Collection of Samples

Uterine samples (Cervical mucus): -

Samples of uterine fluid were collected for physical and bacteriological study by the methods described by Dabas and Maurya (1988) at the time of estrus. The equipments needed were an A.I. gun with its sterilize sheath and a 20 ml glass syringe. The inseminating guns were wrapped in a craft paper and sterlised in hot air oven at 160^0C for one hour and were kept in dry cabinet till use. Factory sterilized polythene bag containing sheaths was kept in a clean dry tray with a clean dry towel. The sheath was with drawn one by one through a small opening at the time of use.

The animal from which sample to be collected was restrained properly in a travis. The vulva and perineum were washed with soap water and dried with a piece of sterilized gauze. Then rectified sprit was applied on the area with a cotton swabs and allowed to dry. Taking other strict routine precautions, the vulvar lips were spread by an assistant while inseminating gun along with sheath

was passed through the vagina in rotating movement. By rectal palpation, the cervix was located and inseminating gun was manipulated until the tip of sheath was introduced into the body of the uterus. The sterlised glass syringe was fitted to the back end of the sheath so that it could become airtight. A negative pressure was applied by retracting the syringe plunger to withdraw the uterine content into the sheath. Since the uterine mucus was quite viscid, the syringe plunger was slowly and maximally retracted and held in position with slight movement in sheath for about 20 second. The sheath with aspirated fluids was withdrawn along with fitted syringe again by spreading the vulvar lips and immediately the content was poured into sterilized test tubes near the flame of a burner. The samples were marked and brought to laboratory for further microbiological studies, identification of bacteria and sensitivity test. In few animals, distribution studies of antimicrobials were also done on uterine samples collected at specific time interval after single i.m. administration of two drugs i.e., Enrofloxacin and Benzathine Penicillin. The dose rate of Enrofloxacin was 5 mg/kg and Benzathine Penicillin was 12000 IU/kg.

Collection of blood samples and its preservation: -

Before collection of blood, hairs around the jugular vein on either side of neck of the animals were shaved and the area was cleaned with ether. The site was sterlised prior to collection with rectified sprit. The blood samples from each cow were collected in a

sensible manner. Attempts were made to evade a violent effort on them. Blood samples were drawn directly from jugular vein through vene-puncture by sterilized hypodermic needle (18 gauze) and disposable syringe (20 ml). Approximately, 20 ml blood from each cow was collected. About 2 ml of blood was mixed with anticoagulant (sodium fluoride) and rest (about 18 ml) blood was kept in glass test tube in slanting position for 20 minutes at room temperature for separation of serum. The serum was separated and centrifused for 10 minutes at 3000 rpm. The clean serum was pipetted out with the help of sterilized Pasteur pipette and kept in a 10 ml clean stopper vial. The serum thus separated was kept in refrigerator till further analysis. Approximately, 3 ml blood was collected and serum was separated after drug administration at different interval for distribution study.

COLLECTION AND PROCESSING OF BIOLOGICAL FLUIDS (FOR DISTRIBUTION STUDY)

The samples of biological fluids (serum and uterine fluid) of healthy cows and repeat breeding cows were collected after i.m. administration of Enrofloxacin (5 mg/kg) and Benzathine Penicillin (12000 I.U/kg). The samples of biological fluid (serum and uterine fluid) of Enrofloxacin were collected at 1, 2, 4, 8, 12, 24, 30, 36 and 48 h while in case of Benzathine Penicillin the samples of biological fluids were collected at 1, 4, 8, 12, 24, 36, 48, 60, 72, 96 and 120 h.

Schedule of blood sampling

The blood was collected from normal breeding and repeat breeding cows on following different occasion for biochemical analysis:-

1st : Blood was colleted on 0 day (i.e., estrus day)

2nd : Blood was taken on 21st day.

3rd : Blood was collected on 42nd day.

Analysis of blood: -

The collected blood samples were processed for estimation of biochemical profiles. The blood samples having anticoagulant were processed for determination of glucose within 30 minutes of its collection. The estimation of total protein, calcium and phosphorus were done in serum within 3-4 hrs after blood collection. The enzyme Alkaline Phosphatase (AKP) was estimated within 8-18 hrs of the separation of serum samples.

Blood glucose was estimated by Folin–wu method (1920).

Determination of total serum protein was done by biuret method as per Reinhold (1953).

Serum calcium was estimated by using the Clark Collip (1925) method.

Inorganic phosphorus in serum was estimated by the method by Fiske and Subba Rao (1925).

Serum Alkaline phosphatase was estimated by Bodansky method (1932).

TREATMENTS

60 repeat breeding cows having no any visual abnormality was taken in the following groups.

(i) Group – I (T_1)

Mineral mixture group: - 20 repeat breeding cows were supplemented with mineral mixture feed supplement. Powder KALMIN-L at the dose rate of 35 g/animal/day orally in feed for a period of 60 day. Serum examinations were conducted on estrus (0 day) before treatment and 21st and 42nd day post treatment.

(ii) Group II (T_2)

Gonadotropin releasing hormone (GnRH) group: - 10 repeat breeding cows were treated with GnRH 10 µg (Receptal) i.v. at the time of insemination. Serum examinations were conducted on estrus (0 day), 21st and 42nd day post treatment.

(iii) Group III (T_3)

GnRH and mineral mixture group: - 10 repeat breeding cows were treated with mineral mixture (KALMIN-L) powder 35 g/animal/day and GnRH (10 µg) on estrus day. Serum examinations were conducted on estrus (0 day) before treatment and 21st and 42nd day.

(IV) Group IV (T_4)

Enrofloxacin group: - 10 repeat breeding cows were treated after culture and sensitivity test with Enrofloxacin and biological samples were collected at various time intervals.

(V) Group V (T_5)

Long acting Penicillin group: - 10 repeat breeding cows were treated after culture and sensitivity with Benzathine Penicillin and biological samples were collected at various time interval.

Control animal

This group consists of 20 regular (normal) breeding cows with normal and healthy genitalia, exhibiting physiological estrus evidenced by the presence of mature graffian follicle in the ovary and showed regular conception. 10 repeat breeding cows included in control group of animals, which were bred for more than 3 times in successive estrus but failed to conceive. No treatment was carried out but blood biochemical and uterine fluid were examined by usual manner.

Group – I (control C_1 group): -

It consists of 20 normal cyclic cows without treatment

Group –II (control C_2 group): -

It consists of 10 repeat breeder cows without treatment.

Media used and their preparation

1. ***Nutrient broth***: -

Nutrient broth was prepared from the readymade media manufactured by Hi-media, Mumbai (Appendix-I). The prepared broth was poured into sterilized test tubes and incubated at 37^{0}C for 24 h to test the sterility of the media. Then, the tubes were stored at 4^{0}C in a refrigerator for further use.

2. ***Nutrient Agar***: -

Nutrient agar plates and slants were prepared from the readymade media obtained from Hi-media, Mumbai (Appendix II). The prepared media was then incubated at 37^{0}C for 24 hrs to test the sterility and then stored in a refrigerator at 4^{0}C for further use.

3. ***Mac-Conkey agar***: -

Mac-Conkey agar plates were prepared from the readymade media obtained from Hi-media, Mumbai (Appendix-III). The plates were incubated at 37^{0}C for 24 hours to test the sterility and then stored in a refrigerator at 4^{0}C for further use.

4. ***Blood Agar***: -

Blood agar plates were prepared by adding 10% defibrinated sterile sheep blood to the nutrient agar media at 45^{0}C at the time of pouring (Appendix-IV). The plates were incubated at 37^{0}C for 24 hours to test sterility and then stored in a refrigerator at 4^{0}C for further use.

BACTERIOLOGICAL STUDIES

Samples collected were inoculated on blood agar plates and Mac-Conkey's agar plates by streak method and incubated at 37^{0}C for 24 hours. After incubation, plates were examined for any type of colonial growth.

Organisms having colonial growth on blood agar plates were taken in pure form on nutrient agar slant and then were incubated again at 37^0C for 24 hours for further study.

MacConkey's agar plates were examined for the preseence of lactose fermenter (LF) and Non-lactose fermenter (NLF) colonies. All the representative LF and NLF colonies were obtained in pure form on nutrient agar slants and were kept for further studies.

The mixed cultures were also purified and obtained in pure form by conventional method i.e, inoculated in nutrient broth and then streaked on nutrient agar plate. Pure representative colonies were then taken on nutrient agar slants and were incubated at 37^0C for 24hrs. Growths obtained cultures were kept at 4^0C in a refrigerator for further study. Various types of organisms were grown in different media. Colonial morphology of various types grown on different media was examined for their morphological characters and gram staining reactions.

Gram positive *cocci* found in cluster, formed smooth, glistening, opaque, convex golden and/or white colour colonies on nutrient agar. These cocci resembling to *Staphylococci* were differentiated from genus *Micrococcus* using Haugh and Leifson test. Differentiations of pathogenic cocci to that of non-pathogenic cocci were performed by coagulase test. All coagulase positive *Staphylococci* were designated as *Staphylococus aureus* irrespective of pigment productions where as coagulase negative were *Staphylococcus epidermidis.*

Gram positive *cocci* were arranged in chains resembling to *Streptococci* and were formed pinhead colories on blood agar plates. Clear zone of hemolysis was indicative of *Streptococus pyogenes*, partial haemolysis indicated *Streptococcus viridens* and non-haemolysis was *Streptococcus salivaris.*

Both Gram–negative organisms from pink colony (lactose fermenter) on Mac-Conkey's agar plates were studied for IMVC (indol, methyl red, Voges-proskauer, Citrate utilization test) and sugar fermentation tests using lactose, maltose, glucose, sucrose and mannitol to identify the organisms. Cultures were identified according to the classification of *Enterobacteriaceae* described by Edward and Ewing (1972).

Gram positive rods found in an overall plate resembling to Chinese letter appearance and clubbing at both poles (beaded appearance) were considered to be *Cornybacterium* spp.

Gram negative, non-spore forming and motile form, smooth mucoid blue green colonies with musty smell, both oxidase and catalase positive but negative for Indol, M.R. and V. P. tests were treated as *Pseudomonas aeruginosa.*

Test for identification of isolates

To identify the organisms, some specific bacterial tests were performed as described by Cruickshank *et al.* (1975), which are as follows. The isolates were identified based on morphological and biochemical properties as described under Table – I.

1. ***Oxidase activity test***: - It was done by dropping oxidase reagent (1% aqueous solution of tetra-methyl-p-phenylene diamine) on the filter paper and rubbing the isolate on the moist surface. Development of dark purple colour on the paper within few seconds confirmed positive.

2. ***Catalase activity test***: - 4-5 drops of 18 hours broth culture was taken on a slide and a drop of 3% H_2O_2 was added over it. Production of gas bubbles indicates a positive reaction.

3. ***Coagulase test***: - 0.1 ml of 24 hours broth culture was added to 3 ml of diluted rabbit plasma (1 in 10) and incubated at 37^0C for one to six hours. The tubes were observed for coagulase production at one, three and six hour intervals. Presence of coagulase indicates positive result.

4. ***Haemolysis***: - *Streptococci*, grown on 10% sheep blood agar plate incubated at 37^0C for 24 hours, were examined for haemolysis. A clear zone of haemolysis around the colonies was indicative of β - haemolysis whereas partial haemolysis immediately surrounding the colonies was called as α-haemolysis. No haemolysis was termed as α - haemolysis.

5. ***Indol production test***: - 0.5 ml Kovac's reagent was added in peptone water, inoculated and incubated at 37^0C for 48 hours and shaken gently. Development of red colour in alcohol layer indicates the presence of indol.

6. ***Methyl red (M.R.) test***: - Glucose-phosphate-peptone-water medium was inoculated and incubated at 37^0C for 48 hours.

Few drops of 0.4% methyl red reagent was added. Red colour due to acidity confirms positive whereas in negative case it remains yellow.

7. ***Voges-Pro***skauer (V.P.) ***test***: - 1-2 drops of inoculum was dropped in tube containing glucose-phosphate-peptone-water and incubated at 37^{0}C for 48 hours. Then 1 ml of 40% KOH and 3 ml of 5% Naphthol were added. A pinch of creatinine powder was added too and shaken well. A positive reaction was indicated by development of a pink colour in 2-5 minutes becoming crimson in 30 minutes.

8. ***Citrate utilization test***: - One drop of 24 hours broth culture was added to 5 ml of Koser's medium and incubated at 37^{0}C for 48 hours. Utilization of citrate was indicated by growth and consequent turbidity of the medium. A control non-inoculated tube was incubated for comparison.

9. ***Sugar fermentation test***: - Test of culture for the liberation of acid and gas from sugars were performed in peptone water with a particular sugar added in such a way that the concentration of sugar became 1%. pH was determined with the help of Andrade's indicator. A Durham's tube was included to detect the presence of gas. Un-inoculated control tubes for each sugar were also incubated along with inoculated tubes to trace out any type of contamination. The tubes containing sugars like lactose, maltose, glucose, surcose and mannitol were arranged. A drop of 18 hours incubated peptone water was inoculated to each tube. The inoculated tubes were incubated for 24-72 hours

and the production of acid and gas was noted on every 24 hours. The tube showing pink colours was considered positive for acid and presence of air bubbles in Durham's tube was indicative for presence of gas.

10. ***Hugh and Leifson test***: - It was done to separate the two genera of *Styphylococcus* and *Micrococcus* on the ability of former genus to grow and to produce acid anaerobically from glucose. Hugh and Leifson medium was prepared. Duplicate sterilized tubes filled with medium to depth of 2 cm were taken for each organism to be tested. The tubes were inoculated with a heavy inoculum by Stab method with the help of a straight plantinum wire. After inoculation, surface of each tube was covered with 1-2 cm layer of sterile liquid paraffin. All the tubes were incubated for 5-10 days and examined for acid production. *Staphylococcus* grew and formed acid throughout in both open and sealed tubes whereas *Micrococcus* produced acid in open tubes only and failed to grow and produce acid in the closed tubes.

11. ***Nitrate reduction test***: - The test strain was grown in broth medium containing 2% potassium nitrate for 96 hour at 37^0C. Presence of nitrite was tested by adding a few drops of test solution 'A' (containing 8 g of 0.5% sulphonic acid in dilute sulphuric acid) and test solution 'B' (containing 6 ml of dimethyl α- naphthylamine in one litre of acetic acid) to about 2 ml of culture. Production of red colour denotes the presence of nitrites.

Table - I

Properties of bacterial isolates for identification

Isolates	Basis of identification	
	Morphological characters	**Biochemical properties**
Escherichia coli	Gram-negative bacilli formed white glistening colonies on nutrient agar and pink colonies on Mac Conkey's agar.	Lactose fermenter, production of gas from glucose. Positive for indol and MR tests. Negative for VP and did not produce H_2S.
Klebsiella pneumoniae	Gram–negative bacilli with pink mucoid cultures growth similar to *Esch. coli*	Lactose fermenter, produced gas from glucose, positive for M.R. negative for Indol, V.P. and H_2S
Staphylococcus aureus	Gram-positive cocci arranged in grapes like clusters.	Produced acid from glucose aerobically and anaerobically. Positive for V.P. coagulate
Isolates	Morphological characters	Biochemical properties
Staphylococcus aureus (contid)	Produced cream or golden coloured, circular smooth colonies on blood agar.	Positive, ferments lactose, maltose and mannitol.
Streptococcus pyogens	Gram–positive *cocci* arranged in chains of varied length. Colonies were small smooth and haemolysis on blood agar.	Acidify the litmus but no coagulation. Ferments sugars with production of acid only.
Corynebacteri-um pyogens	Gram-positive rods appeared as picture likened to chinese lettering.	Produced acid from carbohydrates (Glucose, Maltose, and Sucrose), negative to catalase, oxidase, urease, indol and nitrate reduction.
Proteus vulgaris	Gram-negative rods with swarming growth on nutrient agar	Urease positive, Indol positive, V.P. negative, fermenting maltose and produced H_2S
Pseudomonas aeruginosa	Gram-negative with bluish pigment colonies gave a ground grass appearance	Oxidase positive, negative for Indol, MR, V.P., and not produced H_2S.

***In-vitro* antibiotic sensitivity test: -**

In-vitro antibio-gram of isolates were carried out by paper disc diffusion technique as per Bauer *et al.* (1966). Antibiotic discs (Hi-Media Lab. Pvt. Ltd., Mumbai, India) viz., Enrofloxacin, Penicillin, Amikacin, Oxytetracyclin, Chloramphenicol and ciprofloxacin, were used.

Table – II

Antimicrobial disc used for in-vitro sensitivity along with concentration.

Sl.No	Name of Antimicrobials	Symbol	Concentration per disc (µg/IU)
1	Enrofloxacin	En	5 (µg)
2	Penicillin	P	10 (IU)
3	Amikacin	A	10 (µg)
4	Oxytetracyclin	O	30 (µg)
5	Gentamicin	G	10 (µg)
6	Chloramphenicol	C	30 (µg)
7	Ciprofloxacin	Cp	5 (µg)

These antimicrobial disc supplied by Hi-Media Laboratories Ltd. (India) were used (symbols assigned to each disc along with their concentrations were given in Table - II. The pure

colony obtained from each sample was inoculated in a nutrient broth tube and incubated at 37^0C for 6 to 8 h in order to obtain growth in log phase. The content of the tube was poured on nutrient agar plate and thoroughly spread over the entire plate to prepare lawn. The excess fluid was discarded. The plate was allowed to dry in incubator for 10 minutes then antimicrobial discs were placed into the plate accordingly to the symbol marked on the bottom of the plate. The plate was incubated at 37^0C for 24 hours. The zone of inhibition was noted there after. In case of mixed culture, isolates were used separately for the antibiogram after obtaining into its single form. The result of the sensitivity was explained on the basis of the size of zone of inhibition.

DISTRIBUTION STUDY OF ANTIMICROBIALS IN SERUM AND UTERINE FLUID

Enrofloxacin and Benzathine Penicillin were used in the present experiment. Enrofloxacin (100 $mg.ml^{-1}$) and Benzathine Penicillin (48 lakh IU powder dissolved in 16 ml sterile distilled water - 300, 000 IU ml^{-1}) were used.

Enrofloxacin at the dose rate of 5 mg/kg and Benzathine Penicillin at the dose rate of 12000 IU/kg i.m. were used.

Animals: -

The number of animals used for distribution study of Enrofloxacin and Benzathine Penicillin are given in table - III.

Table – III

Name of drugs and number of animals used for distribution study

Name of drugs	Healthy cows	Repeat breeding cows
Enrofloxacin	5	5
Bezathine Penicillin	5	5
Total	10	10

Procedures adopted for the microbiological assay: -

The concentration of Enrofloxacin and Benzathine Penicillin in serum and uterine fluid were determined by employing the standard cylinder Plate bio-assay technique (Arret *et al.*, 1971). The details of the estimation methods are noted below.

Sterilization of glasswares, needle and porcelain assay cylinders: -

All glasswares, needle and porcelain assay cylinders were washed with detergent solution in running tap water. Rinsed with glass distilled water and then air dried, test tubes, centrifuge tubes, vials, porcelain assay cylinders placed in vials and needles put in test tubes were plugged with cotton wool. Assay plates, pipettes and syringes were wrapped by paper. All these materials were sterilized in hot air oven at 160^0C for an hour.

Preparation of assay agar plates: -

For estimation of concentrations of Enrofloxacin and Benzathine Penicillin in biological fluids, readymade antibiotic assay media of Hi-media, Mumbai was used (Appendix-V). Melted Enrofloxacin and Benzathine Penicillin antibiotic assay media (20 ml), was poured separately with aid of a sterile measuring cylinder into each of the separate sterile special assay plates kept on a horizontally plane surface to get uniform thickness of media. The plates were kept inside the incubator at 37^{0}C for 24 hr to ascertain any microbial contamination. The plates were then stored in a refrigerator until assay was carried out

Preparation of organism: -

The two test organisms viz., *Esch.coli (*ATCC 25922) for assay of Enrofloxacin and *(ATCC 9341)* for assay of Benzathine Penicillin were grown separately on the slants of culture tubes containing nutrient agar at 37^{0}C for overnight. Then it was stored under refrigeration. The organisms were transferred weekly to fresh media to maintain their normal activities.

Preparation of standards of Enrofloxacin and Benzathine Penicillin in biological samples: -

Enrofloxacin – Enrofloxacin was dissolved and diluted in sterile glass distilled water to have different strengths viz., 80 µg/ml, 40 µg/ml, 20µg/ml, 10 µg/ml, 5 µg/ml, 1 µg/ml and 0.5 µg/ml. From each

standard solution 0.1 ml was added to a sterile vial containing 0.9 ml of serum or uterile fluid (collected from healthy animals before drug administration). This yielded drug standards of 8 μg/ml, 4 μg/ml, 2 μg/ml, 1 μg/ml, 0.5 μg/ml, 0.2 μg/ml, 0.1 μg/ml and 0.05 μg/ml in the above noted biological fluids. These standards were used simultaneously with test samples in the assay plates for determination of drug concentrations in test samples.

Benzathine Penicillin – The drug was dissolved and diluted in sterile glass distilled water to have different strengths viz., 40 I.U/ ml, 20 I.U/ml, 10 I.U/ml, 5 I.U/ml, 2 I.U/ml, 1 I.U/ml, 0.5 I.U/ml, and 0.2 I.U/ml. From each standard solution 0.1 ml was added to a sterile vial containing 0.9 ml of serum/uterine fluid (collected from healthy animals before drug administration). This yielded drug standards of 4 I.U/ml, 2 I.U/ml, 1 I.U/ml, 0.5 I.U/ml, 0.2 I.U/ml, 0.1 I.U/ml, 0.05 I.U/ml, and 0.02 I.U/ml in the above noted biological fluids. These standards were used simultaneously with test samples in the assay plates for determination of the drug concentrations in test samples.

Assay procedure: -

The quantitative estimation of Enrofloxacin and Benzathine Penicillin in biological samples were done by microbiological assay method (cylinder plate diffusion method) using *Esch.coli* (ATCC 25922) and *Sercina lutea* (ATCC 9341) as test organism, respectively. The test organisms were grown in nutrient

both for 1 to 3 hours at 37^{0}C unitl the growth was seen (turbid by naked eye). Enrofloxacin and Benzathine Penicillin assay plates were flooded with the broth containing the organisms and excess broth was drained out. The plates were then dried in the incubator at 37^{0}C for a period of about an hour. Sterile porcelain assay cylinders of uniform size were placed at appropriate distance along the circumference in the inoculated assay plates. Fifty micro litres of standard solution of various strenghts of the drug as well as test samples viz., serum and uterine fluid collected after drug administration were poured in separate porcelain cylinder kept on the table for about 2 hours and then kept in the incubator at 37^{0}C for overnight to allow the growth of organism. The mean diameter of the bacterial zone of inhibition produced by the standard of the drug was measured.

The concentrations of the drugs in different test samples of a biological fluid were estimated from the standard curve plotted from the zone of inhibition versus concentrations of the drug standards in semilog scale.

TREATMENT BY ANTIMICROBIALS IN REPEAT BREEDING COWS

The chemotherapeutic agents were used for *in-vitro* sensitivity against the isolates obtained from the samples. The repeat breeding animals were treated with the drug selected on the basis of sensitivity report. Repeat breeders were treated with Enrofloxacin at

the dose rate of 5mg/kg i.m. daily for 5 days if found sensitive to this drug while in Benzathine Penicillin sensitive animals, the drug was given 12000 IU/kg i.m. at 96 hrs interval for two times. The dose and dosage interval of the above drugs for i.m. route was selected based on the distribution study of these antimicrobials.

The animals were re-examined in subsequent estrus. Discharge was examined and samples were again taken for bacteriological study. Animals found clinically diseased free after treatment were then inseminated with frozen semen. Owners of the animals were cautioned to restrict their animals for natural service.

Artificial insemination (A.I.): -

All the experimental cows were inseminated with frozen thawed semen after detection of estrus according to a.m. - p.m., p.m. - a.m. rule at proper time.

Conception rate (C.R.): -

Conception rate was calculated on the basis of pregnancy diagnosis by rectal palpation after 45-60 days of insemination.

STATISTICAL ANALYSIS

Statistically, the data were analysed according to the methods suggested by Snedcor and Cochran (1967).

Chapter - 4

Results

RESULTS

I. PREVALENCE OF REPEAT BREEDING

I.A. Month wise prevalence: -

The cases of reproductive disorders in cows brought at the out door clinics of the Department of Animal Reproduction, Gynaecology and Obstetrics, Bihar Veterinary College clinics during the period from August, 2002 to July, 2003 were recorded and the month wise prevalence of repeat breeding was calculated. The highest incidence (27.78%) was recorded during the month of January and lowest (8.85%) during September. The overall prevalence out of the total 819 infertility cases was 15.99% (Table 1 and Fig. 1). The Chi square test revealed no significant effect on months on the prevalence of repeat breeding in cows ($\chi^2_{11\ d.f} = 16.27$).

I.B. Seasonal prevalence: -

The season wise prevalence of repeat breeding cows during the study period are shown in Table 2 and Fig. 2. It was seen that the maximum percentage (20%) being recorded in Summer (May – July) and minimum (12.5%) during Autumn (August – October). The Chi square test revealed no significant effect of season in the prevalence of repeat breeding in cows.

Table - 1

Month wise prevalence of repeat breeding in cows

Months	Total no. of reproductive disorder	Number of repeat breeder cows	Month wise prevalence of repeat breeding in percentage (%)	Calculated Chi-square value at 11 d.f.
Aug., 02	105	18	17.14	16.24^{NS}
Sept., 02	113	10	8.85	
Oct., 02	78	9	11.53	
Nov., 02	90	17	18.89	
Dec., 02	93	12	12.90	
Jan., 03	36	10	27.78	
Feb., 03	48	8	16.67	
March, 03	45	5	11.11	
April, 03	31	6	19.35	
May, 03	66	17	25.76	
June, 03	54	9	16.67	
July, 03	60	10	16.67	
Total	819	181	15.99	

NS : Non-significant

Fig. 1. : Showing month wise prevalence of repeat breeding in cows

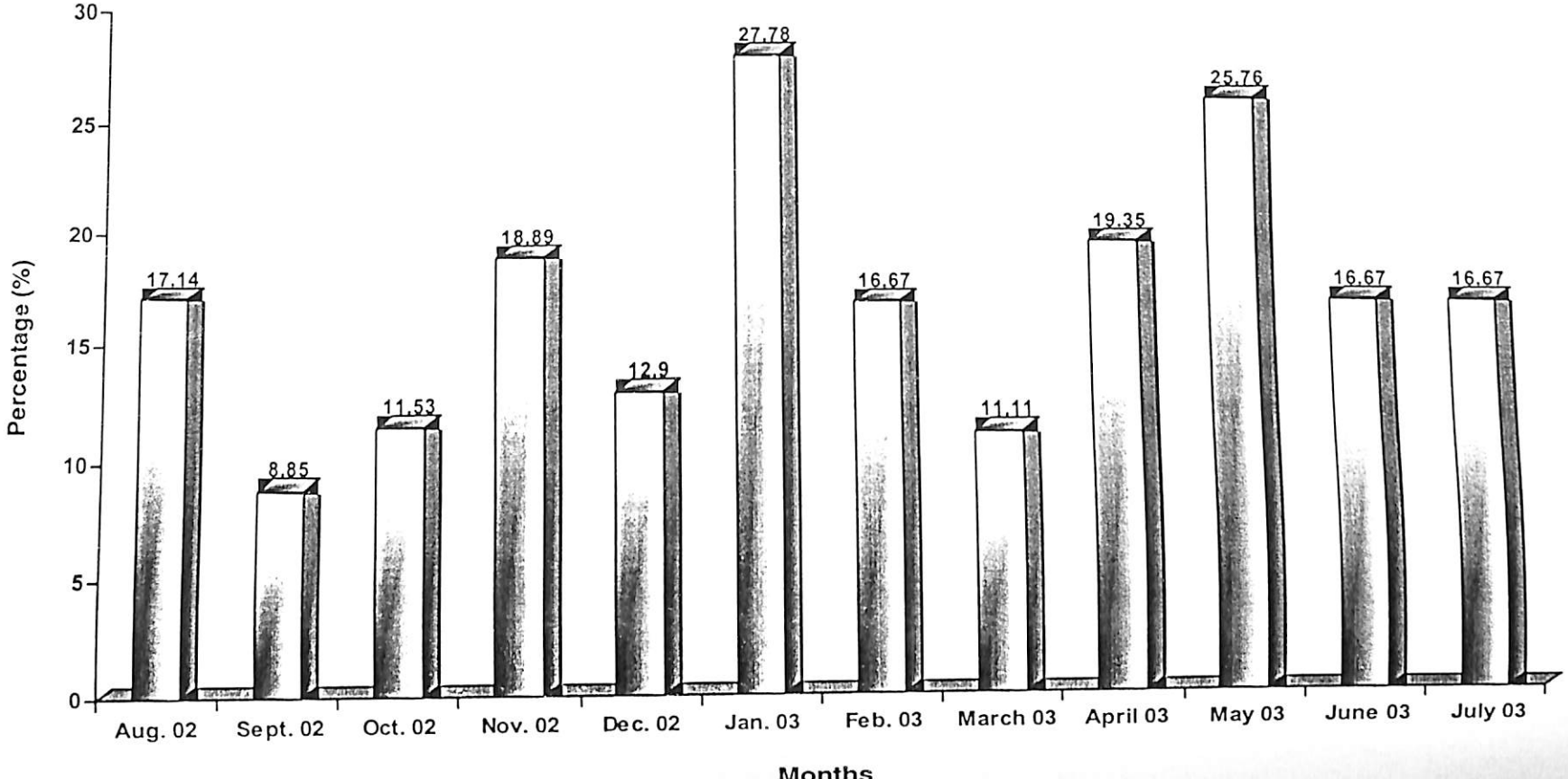


Table – 2

Seasonal prevalence of repeat breeding in cows

Season	Total no. of reproductive disorder	No. of repeat breeding cows	Seasonal prevalence of repeat breeding (%)	Calculated Chi-square value at 3 d.f.
Autumn (Aug. – Oct.)	296	37	12.50	7.81^{NS}
Winter (Nov. – Jan.)	219	39	17.80	
Spring (Feb. – April)	124	19	15.32	
Summer (May – July)	180	36	20.00	
Total	819	131	15.99	

NS : Non-significant

I.C. Parity wise prevalence: -

The parity wise prevalence of repeat breeding was analysed among outdoor clinics and outside clinical cases selected for the present study. Sequence of calving influenced repeat breeding prevalence that was maximum in cows of second calvers (27.59%) and lowest in fifth and onwards calvers (10.34%). Chi square test of significance (Table 3 and Fig. 3) indicates that calving sequence seemed to have no significant influence in the frequency of repeat breeding.

Table – 3

Parity-wise prevalence of repeat breeding cows.

Parity of cows	No. of repeat breeder cows	Parity wise prevalence of repeat breeding (%)	Calculated Chi-square value at 4 d.f.
1st Partum	18	20.69	7.06^{NS}
2nd Partum	24	27.59	
3rd Partum	20	22.99	
4th Partum	16	18.39	
5th Partum	9	10.34	
Total	87	100.00	

NS : Non-significant

II. BLOOD BIOCHEMICAL PROFILE

II.A. Blood glucose: -

The mean ± S.E. along with their CV% of blood glucose (mg%) in various groups have been depicted in Table 4.

Analysis of variance (Table 5) reveals significant effect of various groups on mean blood glucose (mg%).

On 0 day, the mean value of blood glucose (mg%) ranged from 52.42 to 65.66. The mean blood glucose of C_2, T_1, T_2, T_3, T_4 and T_5 groups decreased significantly ($p < 0.01$) by 12.92, 13.24, 12.78, 13.02, 12.88 and 12.83 mg% from the normal cycling control group. However, the mean blood glucose value did not differ significantly among repeat breeder control and treatment groups.

On 21st day, the mean blood glucose value decreased significantly ($p < 0.01$) in repeat breeder control and treated groups from normal cycling control group. The mean blood glucose values in C_2, T_1, T_2, T_3, T_4 and T_5, decreased by 10.43, 2.73, 4.95, 2.06, 4.08 and 4.18 mg% from normal cycling control group, respectively. There were significant differences between repeat breeding control to different treated groups. However, T_2, T_4 and T_5 did not differ significantly.

The trend on 42nd day was almost similar to that of 21st day. The mean blood glucose levels decreased significantly ($p < 0.01$) in repeat breeder control as well as treated groups except T_3 group from normal cycling control group. The mean blood glucose values of

Table – 4

Mean ± S.E. along with their CV% of blood glucose (mg%) in various groups

Groups	No. of cows	0 day	21st day	42nd day
C_1	20	$65.66^{a} \pm 0.09$ (0.63)	$63.39^{a} \pm 0.22$ (2.34)	$62.25^{a} \pm 0.02$ (1.48)
C_2	10	$52.74^{b} \pm 0.07$ (047)	$52.96^{b} \pm 0.14$ (0.88)	$52.71^{b} \pm 0.11$ (0.65)
T_1	20	$52.42^{b} \pm 0.20$ (1.74)	$60.66^{c} \pm 0.51$ (3.83)	$61.31^{c} \pm 0.38$ (2.81)
T_2	10	$52.88^{b} \pm 0.49$ (2.97)	$58.44^{d} \pm 0.52$ (2.80)	$58.38^{d} \pm 0.44$ (1.71)
T_3	10	$52.64^{b} \pm 0.04$ (2.80)	$61.33^{cd} \pm 0.73$ (3.77)	$61.22^{ac} \pm 0.50$ (2.62)
T_4	10	$52.78^{b} \pm 0.06$ (0.39)	$59.31^{d} \pm 0.18$ (0.98)	$60.44^{c} \pm 0.50$ (2.65)
T_5	10	$52.83^{b} \pm 0.10$ (0.63)	$52.2^{d} \pm 0.26$ (1.40)	$60.65^{c} \pm 0.44$ (2.29)

Figures in parenthesis indicate co-efficient of variation percentage. Mean with different superscripts taken columnwise differ significantly ($p<0.01$).

Table – 5

Analysis of variance showing the effect of blood glucose (mg%) in various groups

Sources of variation	Degree of freedom	0 day		21st		42nd day	
		M.S.S.	F-value	M.S.S.	F-value	M.S.S.	F-value
Between treatments	6	437.88		131.73		119.37	
			577.68**		47.90**		63.59**
Within treatment	83	0.758		2.75		1.877	

** Significant ($p<0.01$)

C_2, T_1, T_2, T_3, T_4 and T_5, decreased significant ($p < 0.01$) by 9.54, 0.94, 3.87, 1.81 and 1.60 mg% from the normal cycling control group, respectively. However, T_1,T_3,T_4 and T_5 groups did not differ significantly.

II.B. Serum total protein: -

The mean ± S.E. along with their CV% of serum total protein (gm%) in various groups have been depicted in Table 6.

Analysis of variance (Table 7) reveals significant effect of various groups on mean serum total protein (gm%).

On 0 day, the mean value of serum total protein ranged from 5.62 to 6.70 gm%. The mean serum total protein of C_2, T_1, T_2, T_3, T_4 and T_5 groups decreased significantly ($p < 0.01$) by 1.07, 1.08, 0.97, 1.05, 0.94 and 1.02 gm% from the normal cycling control group. However, the mean serum total protein value did not differ significantly among repeat breeder control and treatment groups.

On 21st day, the mean serum total protein value decreased significantly ($p<0.01$) in repeat breeder control and treated groups from normal cycling control group. The mean serum total protein values in C_2, T_1, T_2, T_3, T_4 and T_5 decreased by 1.06, 1.11, 0.93, 0.89, 0.87 and 0.89 gm% from normal cycling control group, respectively. However, no significant difference was observed between repeat breeder control and T_1,T_2, T_3 and T_5 treated group.

Table – 6

Mean ± S.E. their CV% of serum total protein (g%) in various groups.

Groups	No. of cows	0 day	21st day	42nd day
C_1	20	6.70^{a}± 0.06 (4.08)	6.79^{a}± 0.04 (2.88)	6.91^{a} ± 0.07 (4.72)
C_2	10	5.63^{b} ± 0.05 (2.65)	5.73^{bd} ± 0.03 (2.02)	5.67^{b} ± 0.04 (2.63)
T_1	20	5.62^{b} ± 0.04 (3.82)	5.68^{b} ± 0.04 (3.92)	5.89^{cd} ± 0.07 (5.42)
T_2	10	5.93^{b} ± 0.10 (5.50)	5.86^{de} ± 0.07 (3.79)	5.70^{bd} ± 0.07 (4.05)
T_3	10	5.65^{b} ± 0.08 (4.51)	5.90^{df} ± 0.07 (3.91)	5.82^{bde} ± 0.08 (4.84)
T_4	10	5.76^{b} ± 0.03 (1.89)	5.92^{cof} ± 0.03 (2.07)	6.01^{co} ± 0.06 (3.36)
T_5	10	5.68^{b} ± 0.04 (2.72)	5.90^{df} ±0.06 (3.38)	5.98^{ce} ± 0.05 (2.82)

Figures in parenthesis indicate coefficient of variation percentage. Mean with different superscripts taken column wise differ significantly ($p<0.01$).

Table – 7

Analysis of variance showing the effect of total serum protein (g%) in various groups

Sources of variation	Degree of freedom	0 day		21st		42nd day	
		M.S.S.	F. value	M.S.S.	F-value	M.S.S.	F-value
Between treatments	6	2.71		2.618		3.07	
			51.84**		67.13**		42.63**
Within treatment	83	0.524		0.039		0.072	

** Significant ($p<0.01$)

The trend on 42nd day was almost similar to that of 21st day. The mean serum total protein levels decreased significantly ($p < 0.01$) in repeat breeder control as well as treated groups from the normal cycling control group. The mean serum total protein values of C_2, T_1, T_2, T_3, T_4 and T_5 groups decreased significantly ($p < 0.01$) by 1.24, 1.02, 1.21, 1.09, 0.90 and 0.93 gm% from the normal cycling control group, respectively. However, significant difference was noted between repeat breeder control and T_1, T_4 and T_5 treated groups. T_2 and T_3 groups did not differ significantly from repeat breeder control group.

II.C. Serum calcium: -

The mean ± S.E. along with their CV% of serum calcium (mg%) in various groups have been shown in Table 8. Analysis of variance (Table 9) reveals non-significant effect of various groups on mean serum calcium (mg%).

On 0 day, 21st day and 42nd day, the mean values of serum calcium ranged from 8.79 to 8.93, 8.84 to 9.05 and 8.89 to 9.09 mg%, respectively, but these values did not differ significantly among control and various treated groups.

Table – 8

Mean ± S.E. with their CV% of serum calcium(mg%) in various groups.

Groups	No. of cows	0 day	21st day	42nd day
C_1	20	8.93 ± 0.03 (1.70)	8.94 ± 0.04 (2.27)	9.09 ± 0.05 (2.57)
C_2	10	8.85 ± 0.06 (2.45)	8.92 ± 0.06 (2.29)	8.95± 0.09 (3.46)
T_1	20	8.83 ± 0.05 (2.73)	8.95 ± 0.05 (2.77)	8.98 ± 0.05 (1.24)
T_2	10	8.86±0.06 (2.33)	8.84 ± 0.06 (2.14)	8.91 ± 0.05 (1.97)
T_3	10	8.82 ± 0.07 (2.76)	9.05 ± 0.11(3.87)	9.06 ± 0.09 (3.25)
T_4	10	8.84 ± 0.06 (2.45)	8.87 ± 0.08 (2.96)	8.89 ± 0.12 (4.25)
T_5	10	8.79 ± 0.09 (3.23)	8.86 ± 0.09 (3.41)	8.98 ± 0.08 (3.05)

Figures in parenthesis indicate co-efficient of variation percentage

Table – 9

Analysis of variance showing the effect of serum calcium (mg%) in various groups

Sources of variation	Degree of freedom	0 day		21st		42nd day	
		M.S.S.	F-value	M.S.S.	F-value	M.S.S.	F-value
Between treatments	6	0.025	0.51^{NS}	0.051	0.83^{NS}	0.068	0.92^{NS}
Within treatment	83	0.049		0.062		0.074	

NS = non significant ($p<0.05$).

II.D. Serum inorganic phosphorus: -

The mean ± SE along with their CV% of serum inorganic phosphorus (mg%) in various groups have been depicted in Table 10.

Analysis of variance (Table 11) shows significant effect of various groups on mean serum inorganic phosphorus (mg%).

On 0 day, the mean value of serum inorganic phosphorus ranged from 4.15 to 5.80 mg%. The mean serum inorganic phosphorus values of C_2, T_1, T_2, T_3, T_4, & T_5 groups decreased significantly ($p < 0.01$) by 1.49, 1.54, 1.61, 1.49, 1.65 and 1.56 mg% from normal cycling control group. However, the mean serum inorganic phosphorus value did not differ significantly among repeat breeder control and treatment groups.

On 21st day the mean serum inorganic phosphorus value decreased significantly ($p < 0.01$) in repeat breeder control and treated groups from normal cycling control group. The mean serum inorganic phosphorus values in C_2, T_1, T_2, T_3, T_4 and T_5 decreased by 1.61, 1.41, 1.48, 1.10, 1.51 and 1.42 mg% from the normal cycling control group, respectively. However, there was no significant difference between repeat breeder control to treated group T_2, T_4 and T_5. However, significant difference was noted in the mean values of serum inorganic phosphorus between repeat breeder control to T_1 and T_3 treated group.

Table – 10

Mean ± S.E. along with their CV% of serum inorganic phosphorus (mg%) in various groups

Groups	No. of cows	0 day	21st day	42nd day
C_1	20	$5.80^{a} \pm 0.04$ (3.01)	$5.95^{a} \pm 0.04$ (2.96)	$5.77^{a} \pm 0.06$ (4.64)
C_2	10	$4.31^{b} \pm 0.03$ (2.77)	$4.34^{b} \pm 0.05$ (4.09)	$4.41^{b} \pm 0.03$ (2.71)
T_1	20	$4.26^{b} \pm 0.05$ (5.12)	$4.54^{c} \pm 0.07$ (7.34)	$4.80^{c} \pm 0.09$ (8.66)
T_2	10	$4.19^{b} \pm 0.06$ (4.56)	$4.47^{bc} \pm 0.06$ (4.48)	$4.46^{b} \pm 0.09$ (6.45)
T_3	10	$4.31^{b} \pm 0.04$ (3.18)	$4.85^{d} \pm 0.10$ (6.54)	$5.05^{d} \pm 0.08$ (5.21)
T_4	10	$4.15^{b} \pm 0.05$ (3.80)	$4.44^{bc} \pm 0.05$ (2.57)	$4.52^{b} \pm 0.13$ (9.49)
T_5	10	$4.24^{b} \pm 0.04$ (3.72)	$4.53^{bc} \pm 0.03$ (2.55)	$4.64^{bc} \pm 0.04$ (2.90)

Figures in parenthesis indicate co-efficient of variation percentage. Means with different superscripts taken column wise differ significantly ($p<0.01$).

Table – 11

Analysis of variance showing the effect of serum inorganic phosphorus (mg%) in various groups.

Sources of variation	Degree of freedom	0 day		21st		42nd day	
		M.S.S.	F-value	M.S.S.	F-value	M.S.S.	F-value
Between treatments	6	6.29	153.57**	5.51	100.07**	3.69	38.57**
Within treatment	83	0.041		0.055		0.095	

** Significant ($P<0.01$)

Table – 12

Mean ± S.E. along with their CV% of serum alkaline phosphatase (KAU%) in various groups.

Groups	No. of cows	0 day	21st day	42nd day
C_1	20	6.56^{a}±0.04 (3.22)	6.77^{a} ± 0.05 (3.58)	6.81^{a} ± 0.08 (5.67)
C_2	10	7.85^{b} ± 0.04 (1.82)	7.98^{c} ± 0.05 (2.34)	8.11^{b} ± 0.06 (2.49)
T_1	20	7.83^{b} ± 0.05 (2.93)	9.39^{b} ± 0.04 (2.15)	9.53^{c} ± 0.03 (1.80)
T_2	10	7.96^{b} ±0.07 (2.85)	9.35^{be} ± 0.03 (1.15)	9.22^{d} ± 0.05 (1.76)
T_3	10	7.73^{b} ± 0.09 (3.70)	9.13^{de} ± 0.05 (1.79)	9.01^{d} ± 0.07 (2.48)
T_4	10	8.20^{c} ± 0.13 (4.93)	8.86^{f} ± 0.14 (5.16)	8.95^{d} ± 0.13 (4.75)
T_5	10	8.22^{c} ± 0.12 (4.61)	9.0^{df} ± 0.16 (3.06)	9.1^{d} ± 0.15 (5.55)

Figures in parenthesis indicate co-efficient of variation percentage. Means with different superscripts taken column wise differ significantly ($p<0.01$).

Table – 13

Analysis of variance showing the effect of serum alkaline phosphatase (KAU%) in various groups.

Sources of variation	Degree of freedom	0 day		21st		42nd day	
		M.S.S.	F. value	M.S.S.	F-value	M.S.S.	F-value
Between treatments	6	5.28	72.72**	15.5	185.18**	15.45	152.72**
Within treatment	83	0.072		0.083		0.010	

* Significant ($p<0.01$).

On 21st day, the mean serum alkaline phosphatase value increased significantly ($p < 0.01$) in repeat breeding control and also various treated groups from normal cycling control group. The mean serum alkaline phosphatase values in C_2, T_1, T_2, T_3, T_4 and T_5 groups increased by 1.21, 2.62, 2.58, 2.36, 2.09 and 2.23 KAU% from the normal cycling control group, respectively. Significant difference was also observed between repeat breeder control and T_1, T_2, T_3, T_4, and T_5 treated groups.

The trend on 42nd day was almost similar to that of 21st day. The mean serum alkaline phosphatase levels increased significantly ($p < 0.01$) in repeat breeder control as well as treated groups from the normal cycling control group. The mean serum alkaline phosphatase values of C_2, T_1, T_2, T_3, T_4, and T_5 groups increased significantly ($p < 0.01$) by 1.30, 2.72, 2.41, 2.20, 2.14 and 2.29 KAU% from the normal cycling control group, respectively. Repeat breeder control (C_2) differs significantly from that of different treatment groups viz., T_1, T_2, T_3, T_4, and T_5.

III. BACTERIOLOGICAL STUDIES OF REPEAT BREEDING COWS

Samples of uterine discharge of repeat breeding cows which were brought to the outdoor clinic of the Department of Animal Reproduction, Gynaecology and Obstetrics, Bihar Veterinary College, Patna-14, organised khatals in and around Patna and cattle, farm of RAU Pusa, Samastipur were subsequently examined.

An effort was made to isolate, identify and characterize the different species of bacteria, which were present in uterine discharge of repeat breeding cows. *In-vitro* sensitivity test was performed against each bacterial isolate to know the pattern of sensitivity towards the antimicrobial to be used.

III.A. Isolation of bacteria: -

The studies were conducted on 131 repeat breeding cows. Out of 131 samples, 78 (59.54%) were positive for infectious agents while remaining 53 (40.45%) samples were bacteriologically sterile. Out of 78 bacterial positive samples, 63 (80.76%) samples were single isolates and rest 15 (19.23%) samples were mixed culture. Bacteria from 78 samples were isolated and identified as per the method described under the chapter "Materials and Methods". Different bacteria obtained from uterine samples collected from repeat breeding cows are presented in Table 14 and Fig. 4.

It is evident from Table 14 and Fig. 4 that 63 (80.76 %) samples had single type of organism where as 15 (19.23%) of the samples were of mixed type. *Esch. coli* was found to be maximum i.e. 20 (25.64%) samples followed by *Staph. aureus* 14 (17.95%), *Strept. pyogenes* 12 (15.38%), *Corny. pyogenes* 6 (5.13%) and *K. pneumoniae* 2 (2.56%). Chi square test reveals that there is a significant difference in the percentage of occurrence of bacterial agents in repeat breeding cows. *Esch. coli* was the predominant organism followed by *Staph. aureus* and *Strept. pyogenes* and *K. pneumoniae* showed the least percentage of occurrence.

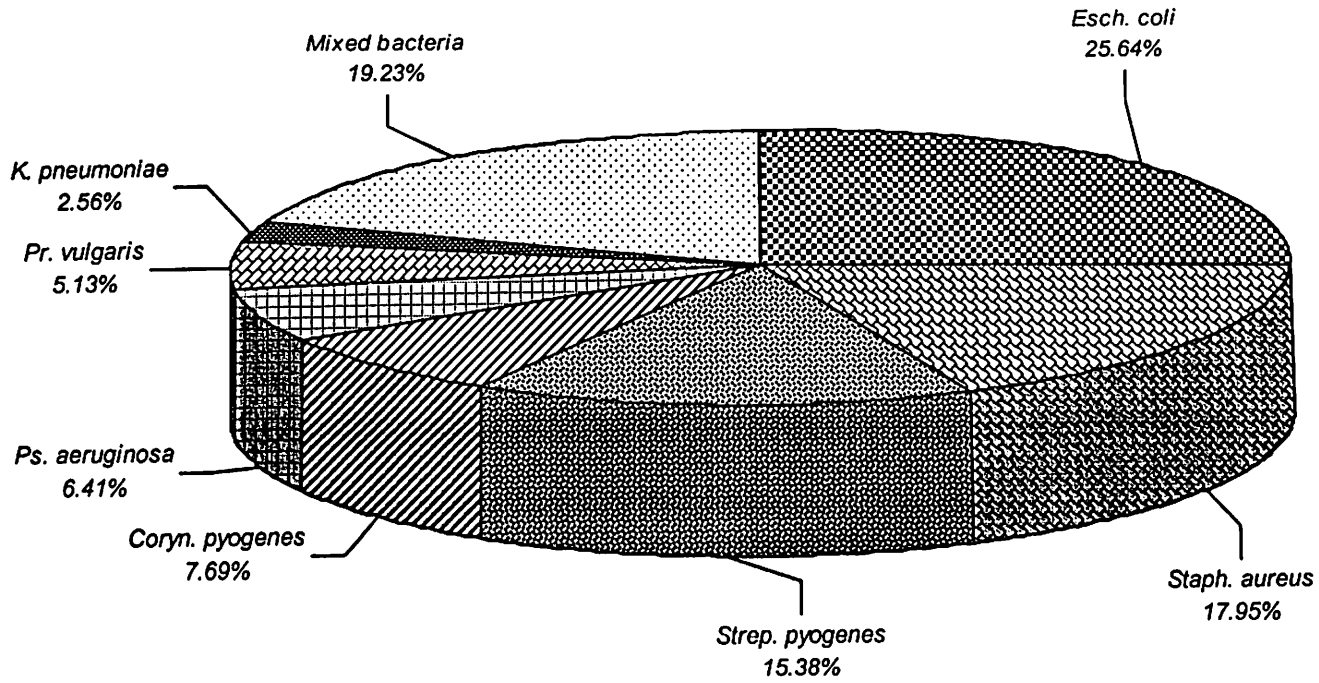
Table – 14

Different isolates obtained from uterine samples of repeat breeding cows.

Name of isolates	No of samples with pure and mixed isolates	Percentage	χ^2 at 7 d.f.
Esch. coli	20	25.64	29.28**
Staph. aureus	14	17.95	
Strep. pyogenes	12	15.38	
Coryn. pyogenes	6	7.69	
Ps. aeruginosa	5	6.41	
Pr. vulgaris	4	5.13	
K. pneumoniae	2	2.56	
Mixed bacteria	15	19.23	
Total	78	100.00	

** = significant, $p < 0.01$

Fig. 4. : Showing different isolates obtained from uterine samples of repeat breeding cows.



Bacterial isolates found in mixed cultures were separated in single form and grouped there after on the basis of their combination. Only two types of isolates were obtained from each of the mixed culture, which have been shown in Table 15.

Combination of *Esch. coli* and *Staph. aureus* was highest and found in 5 (33.33%) samples. *Esch. coli* with *Staph. pyogenes*, *Esch. coli* with *Coryn. pyogenes*, *Staph. aureus* with *Ps. aeruginosa* and *Staph. aureus* with *Pr. vulgaris* combination were found in 4 (26.67%), 3 (20%), 2 (13.33%) and 1 (6.67%) cases, respectively. Chi square test reveals non-significant difference among the combination of bacteria in mixed isolates in repeat breeding cows.

A total of 93 isolates (63 from single and 30 from mixed cultures) were obtained from 78 samples of cows, which has been depicted in Table 16 and Fig. 5. Numbers of different isolates in decreasing order were noted to be *Esch. coli* 32 (34.40%), *Staph. aureus* 22 (23.66%), *Strept. pyogenes* 16 (17.20%), *Coryn. pyogenes* 9 (9.68%), *Ps. aeruginosa* 7 (7.53%), *Pr. vulgaris* 5 (5.38%) and *K. pneumoniae* 2 (2.15%).

III.B. Sensitivity test: -

In-vitro sensitivity tests were performed by using different antimicrobial agents such as Enrofloxacin, Penicillin, Amikacin, Oxytetracycline, Gentamicin, Chloramphenicol and Ciprofloxacin. The antimicrobial discs were obtained from Hi-media,

Table – 15

Mixed isolates obtained from uterine samples of repeat breeding cows.

Combination of isolates	No. of samples	Percentage	χ^2 at 7 d.f.
Esch. coli + *Staph. aureus*	5	33.33	3.33^{NS}
Esch. coli + *Staph. pyogenes*	4	26.67	
Esch. coli + *Coryn. pyogenes*	3	20.00	
Staph. aureus + *Ps. aeruginosa*	2	13.33	
Staph. aureus + *Pr. vulgaris*	1	6.67	
Total	15		

NS = Non-significant

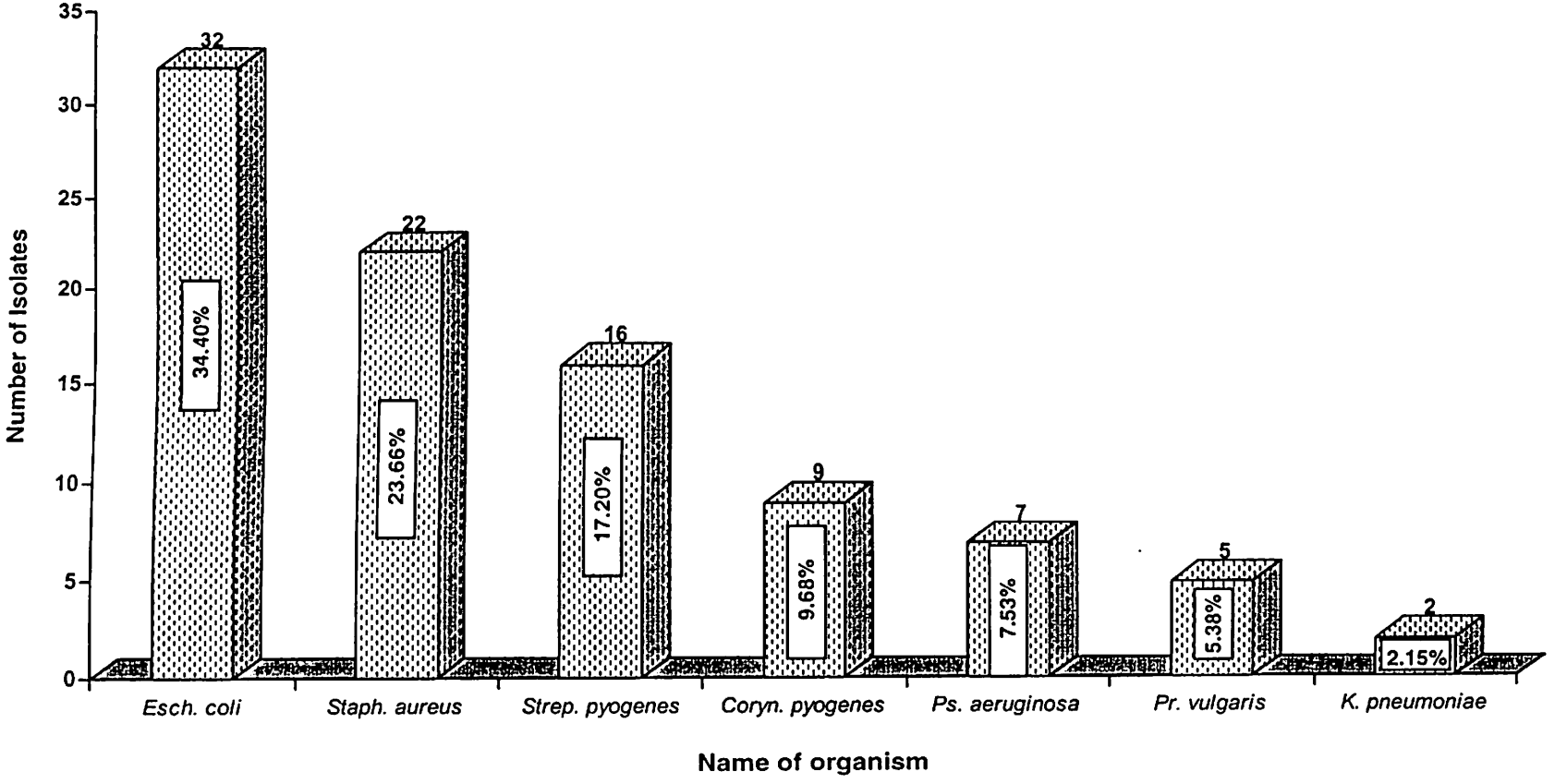
Table – 16

No. of isolates obtained from Uterine Samples of Repeat breeder cows.

No. of samples examined	No. of samples of single isolates	No. of samples of mixed isolates	Name of organism	Sample with single isolate	Sample with mixed isolates	Total no. of isolates both from single and mixed	X^2at 6 d.f.
78	63	15	*E. coli*	20	12	32(34.40)	51.76**
			Staph. aureus	14	8	22(23.66)	
			Strep. pyogenes	12	4	16(17.20)	
			Coryn. pyogenes	6	3	9(9.68)	
			Ps. aeruginosa	5	2	7(7.53)	
			Pr. vulgaris	4	1	5(5.38)	
			K. pneumoniae	2	—	2(2.15)	
			Total	63	30	93	

** $p < 0.01$

Fig. 5. : Showing number of isolates obtained from uterine samples of repeat breed cows.



Mumbai. The concentration of drug per disc was presented in Table II under "materials and methods". The results of antimicrobials sensitivity tests of different isolates from cows against the various antimicrobial agents have been depicted in Table 17 and Fig. 6. It is evident from the Table 17 that most of the isolates were found sensitive to more than one drugs and none of them was found completely resistant to all the drugs used. Amikacin (91.31%) was found to be most effective drug followed by Enrofloxacin (80.64%), Chloramphenicol (79.57%), Oxytetracycline (32.25%) and Penicillin (25.80%).

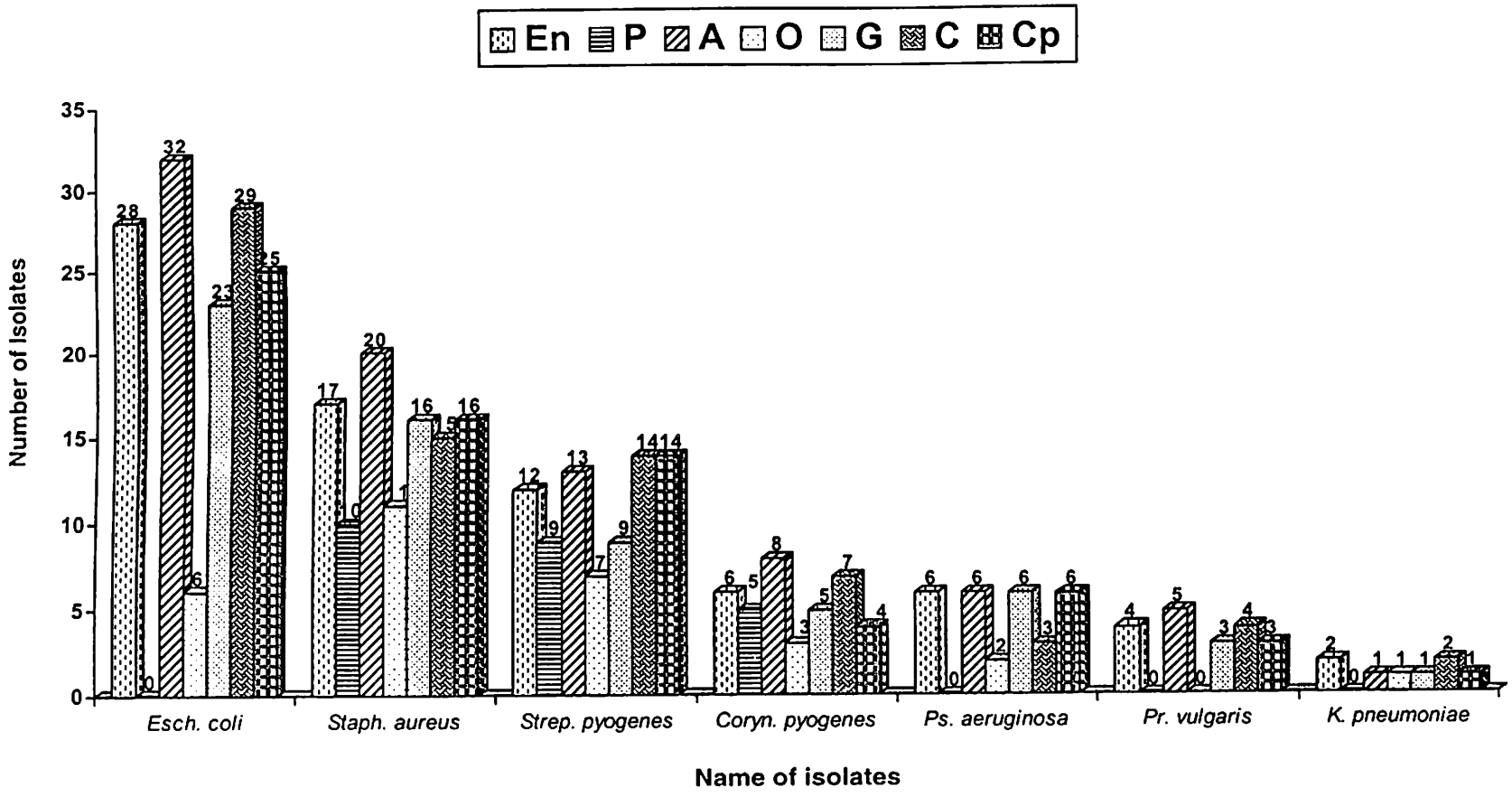
Amikacin showed the maximum sensitivity (100%) to *Esch. coli* and *Pr. vulgaris* while the minimum sensitivity (50%) was against *K. pneumoniae.* Enrofloxacin shows maximum sensitivity (100%) to *K. pneumoniae* and minimum (66.67%) to *Coryn. pyogenes.* The sensitivity pattern of Enrofloxacin against other organisms were 87.50% to *Esch. coli,* 85.71% to *Ps. aeruginosa,* 80% to *Pr. Vulgaris,* 77.27% to *Staph. aureus* and 75% to *Strept. pyogenes.* In case of Chloramphenicol, the highest sensitivity was against *K. pneumoniae* (100%) and lowest to *Ps. aeruginosa* (42.85%). Ciprofloxacin showed maximum sensitivity against *Strep. pyogenes* (87.50%) and minimum to *Coryn. Pyogenes* (44.44%). Maximum (85.71%) sensitivity against *Ps. aeruginosa* and minimum (50%) against *K. pneumoniae* was recorded for Gentamicin. Oxytetracycline showed maximum (50%) sensitivity against *Staph. aureus* and *K. pneumoniae* and resistant to

Table – 17

In-vitro sensitivity test

Name of isolates	No. of isolates tested	NO. OF ISOLATES SENSITIVE TO ANTIMICROBIALS						
		En	P	A	O	G	C	Cp
Esch. coli	32	28 (87.5%)	R(00.00)	32(100)	6(18.75)	23(71.87)	29(90.62)	25(78.13)
Staph. aureus	22	17(77.27)	10(45.45)	20(90.91)	11(50)	16(72.72)	15(68.18)	16(72.72)
Strept. pyogenes	16	12(75)	9(56.25)	13(81.25)	7(43.75)	9(56.25)	14(87.50)	14(87.50)
Coryn. pyogenes	9	6(66.67)	5(55.56)	8(88.89)	3(33.33)	5(55.56)	7(77.78)	4(44.44)
Ps. aeruginosa	7	6(85.71)	R(00.00)	6(85.71)	2(28.57)	6(85.71)	3(42.85)	6(85.71)
Pr. vulgaris	5	4(80)	R(00.00)	5(100)	R(00.00)	3(60)	4(80)	3(60)
K. pneumoniae	2	2(100)	R(00.00)	1(50)	1(50)	1(50)	2(100)	1(50)
	93	75(80.64)	24(25.80)	85(91.31)	30(32.25)	63(67.74)	74(79.57)	69(69.74)

Fig. 6. : Showing in-vitro sensitivity test



Pr. vulgaris. In case of penicillin, maximum sensitivity (56.25%) was shown against *Strep. pyogenes* and resistant to *Esch. coli*, *Ps. aeruginosa*, *Pr. vulgaris* and *K. pneumoniae* were noted.

IV. DISTRIBUTION STUDY OF ANTIMICROBIALS IN SERUM AND UTERINE FLUID

IV.A. Distribution study on healthy animal

1. Enrofloxacin: -

(a) Serum levels

Concentrations of Enrofloxacin in serum of healthy cows after i.m. administration (5 mg/kg) are presented in Table 18. The drug was present in Serum with a mean of 1.55 ± 0.124 µg/ml at 1 h. The drug reached its mean peak concentration of 3.58 ± 0.43 µg/ml at 4 h. The drug was detectable upto 24 h in two animals, with a mean of 0.076 ± 0.047 µg/ml. The mean therapeutic concentration (≥ 0.12 µg/ml) was maintained upto 12 h in all animals.

Table – 18

Concentrations of Enrofloxacin (µg/ml) in serum of healthy cows after its i.m. administration @ 5 mg/kg.

Time (hours)	Animal Numbers					Mean ± S.E.
	1	2	3	4	5	
1	1.68	1.85	1.66	1.12	1.46	1.554±0.124
2	3.55	3.25	3.85	2.56	2.22	3.086±0.304
4	3.12	4.55	2.16	3.98	4.12	3.586±0.425
8	1.55	2.12	1.48	1.56	1.88	1.718±0.122
12	0.68	1.06	0.88	0.92	1.02	0.912±0.066
24	ND	0.16	ND	ND	0.22	0.076±0.04

ND – Non Detectable

(b) Uterine fluid levels

Enrofloxacin appeared in uterine fluid of three out of five animals with the mean of 0.104 ± 0.045 µg/ml of 1 h where as it appeared in all animals at 2 h with the mean of 0.602 ± 0.093 µg/ml (Table 19). The drug reached its mean peak concentration at 2.424 ± 0.281 µg/ml at 8 h. The drug was detectable in three out of five animals with the mean concentration of 0.086 ± 0.037 at 36 h. The therapeutic concentration was (≥ 0.12 µg/ml) was maintained from 2 to 30 h.

Table – 19

Concentration of Enrofloxacin (µg/ml) in uterine fluid of healthy cows after its i.m. administration @ 5 mg/kg.

Time (hours)	Animal Numbers					Mean ± S.E.
	1	2	3	4	5	
1	0.12	0.22	0.18	ND	ND	0.104±0.045
2	0.68	0.82	0.75	0.36	0.40	0.602±0.093
4	2.88	3.12	1.96	0.78	0.82	1.912±0.493
8	1.65	1.85	3.05	2.82	2.75	2.424±0.281
12	0.85	0.90	1.25	1.12	0.92	1.008±0.075
24	0.52	0.65	0.78	0.72	0.68	0.67±0.043
30	0.25	0.32	0.34	0.22	0.18	0.262±0.030
36	ND	0.15	0.18	0.10	ND	0.086±0.037
48	ND	ND	ND	ND	ND	------

ND – Non Detectable

(c) Uterine fluid to serum ratio

Table 20 presents the uterine fluid to serum ratio of enrofloxacin in healthy cows. The data of this table shows ratio >1 from 8 to 24 h which denotes that the drug penetrates to a greater amount in uterine tissues of healthy cows. Thus, enrofloxacin crosses the uterine barrier easily in case of healthy animals.

Table – 20

Uterine fluid to serum ratio of Enrofloxacin in Healthy cows.

Time (hours)	Animal Numbers					Mean ± S.E.
	1	2	3	4	5	
1	0.07	0.12	0.11	0.00	0.00	0.06±0.026
2	0.19	0.25	0.19	0.14	0.18	0.19±0.018
4	0.92	0.68	0.91	0.19	0.20	0.58±0.16
8	1.06	0.87	2.06	1.81	1.46	1.45±0.221
12	1.25	0.85	1.42	1.22	0.90	1.13±0.11
24	ND	4.06	ND	ND	3.09	1.43±0.89

ND – Non Detectable

2. Benzathine Penicillin: -

(a) Serum levels: - Concentrations of Benzathine Penicillin in serum obtained at various time intervals in healthy cows are shown in Table 21. The mean concentration of Benzathine Penicillin in serum at 1 h was noted to be 0.26 ± 0.017 IU/ml. The drug was present upto 96 h with the mean of 0.11 ± 0.019 IU/ml. Out of five animals, the drug was detectable only in two animals at 120 h with the mean of 0.04 ± 0.022 IU/ml. The therapeutic concentration (≥ 0.03 IU/ml) was maintained upto 96 h in all animals.

Table – 21

Serum concentration of Benzathine Penicillin (IU/ml) in normal healthy cow after i.m. administration @ 12000 IU/kg

Time (hours)	Animal Numbers					Mean ± S.E.
	1	2	3	4	5	
1	0.28	0.20	0.30	0.25	0.26	0.26±0.017
4	0.45	0.36	0.46	0.35	0.38	0.40±0.023
8	0.52	0.40	0.55	0.44	0.50	0.48±0.027
12	0.46	0.45	0.48	0.50	0.44	0.47±011
24	0.35	0.32	0.36	0.38	0.32	0.35±0.012
36	0.26	0.20	0.28	0.30	0.22	0.25±0.018
48	0.24	0.16	0.25	0.26	0.18	0.22±0.020
60	0.20	0.12	0.18	0.24	0.16	0.18±0.02
72	0.15	0.10	0.16	0.20	0.15	0.15±0.016
96	0.10	0.05	0.12	0.16	0.14	0.11±0.019
120	ND	ND	ND	0.10	0.08	0.04±0.022

ND – Non Detectable

(b) Uterine fluid levels: - The concentrations of Benzathine Penicillin in uterine fluid of healthy cows after i.m. administration of 12000 IU/kg are presented in Table 22. No drug was detectable after 1 h of administration. The drug was present in uterine fluid with a mean concentration of 0.23 ± 0.011 IU/ml after 4 h of administration. The drug reached its mean peak concentration of 0.60 ± 0.023 IU/ml at 12 h. The drug was detectable in all animals upto 96 h with mean of 0.12 ± 0.013 IU/ml. The drug was not detectable after 120 h of administration. The therapeutic concentration of (≥ 0.03 IU/ml) was maintained from 4 to 96 h in healthy cows.

Table – 22

Concentration of Benzathine Penicillin (IU/ml) in uterine fluid of normal healthy cow after i.m. administration @ 12000 IU/kg

Time (hours)	Animal Numbers					Mean ± S.E.
	1	2	3	4	5	
1	ND	ND	ND	ND	ND	-------
4	0.24	0.22	0.26	0.20	0.25	0.23±0.011
8	0.45	0.40	0.50	0.38	0.46	0.44±0.021
12	0.62	0.56	0.68	0.55	0.60	0.60±0.023
24	0.50	0.40	0.55	0.38	0.42	0.45±0.032
36	0.35	0.30	0.42	0.32	0.36	0.35±0.020
48	0.30	0.25	0.35	0.26	0.32	0.30±0.019
60	0.26	0.20	0.30	0.22	0.25	0.25±0.017
72	0.20	0.16	0.24	0.18	0.20	0.20±0.013
96	0.12	0.08	0.16	0.14	0.12	0.12±0.013
120	ND	ND	ND	ND	ND	----

ND – Non Detectable

(c) ***Uterine fluid to serum ratio***: - Table 23 presents the values of uterine fluid to serum ratio of Benzathine Penicillin in healthy cows at various time intervals. A mean ratio of 0.59 ± 0.022 was obtained at 4 h. The maximum (Peak) mean ratio (1.42 ± 0.143) was noted at 60 h. The data of this table shows > 1 from 12 to 96 h, which denotes that the drug penetrates into a greater amount in uterine tissues of healthy cows. Thus, the drug crosses the uterine barrier easily in healthy animals.

Table – 23

Uterine fluid to serum ratio of Benzathine Penicillin in healthy cows.

Time (hours)	Animal Numbers					Mean ± S.E.
	1	2	3	4	5	
1	--	--	--	--	--	--
4	0.53	0.61	0.56	0.57	0.66	0.59 ±0.022
8	0.86	1.00	0.91	0.86	0.92	0.91±0.026
12	1.35	1.24	1.41	1.10	1.36	1.29±0.055
24	1.43	1.25	1.53	1.00	1.31	1.30±0.090
36	1.34	1.50	1.5	1.06	1.64	1.41±0.099
48	1.25	1.56	1.40	1.00	1.78	1.39±0.132
60	1.30	1.67	1.67	0.92	1.56	1.42±0.143
72	1.33	1.60	1.50	0.90	1.33	1.33±0.119
96	1.20	1.60	1.33	0.87	0.86	1.17±0.141
120	-	-	-	-	-	-

IV.B. Distribution study in repeat breeder cows

1. Enrofloxacin

*(a) **Serum levels***: - Serum drug concentrations of Enrofloxacin in repeat breeding cows after i.m. administration (5 mg/kg) have been presented in Table 24. The drug was present in serum with a mean of 0.982 ± 0.063 μg/ml at 1 h. The drug attained its mean Peak concentration (1.806 ± 0.234 μg/ml) at h, which was declined with time and was present in all animals upto 12 h. (0.334 ±0.038 μg/ml). Enrofloxacin was detectable in three animals among five with a mean of 0.092 ± 0.039 at 24 h. The mean therapeutic concentration (≥ 0.12 μg/ml) was maintained upto 12 h.

Table – 24

Concentration of Enrofloxacin (μg/ml) in serum of Repeat breeding cows after its i.m. administration @ 5 mg/kg.

Time (hours)	Animal Numbers					Mean ± S.E.
	1	2	3	4	5	
1	1.18	1.05	0.98	0.82	0.88	0.982±0.063
2	2.26	2.12	2.15	1.08	1.42	1.806±0.234
4	1.08	0.92	1.00	0.78	0.94	0.944±0.049
8	0.92	0.64	0.75	0.52	0.68	0.702±0.066
12	0.45	0.38	0.30	0.22	0.32	0.334±0.038
24	0.20	0.14	0.12	ND	ND	0.092±0.039

ND – Non Detectable

(b) Uterine fluid levels: - Table 25 reveals the concentrations of Enrofloxacin at various time intervals in uterine fluid of repeat breeder cows after i.m. administration of 5 mg/kg. The drug was detectable at 1 h in all animals with a mean concentration of 0.676 ± 0.062 μg/ml. The drug reached its mean peak concentration of 3.798 ± 0.299 μg/ml at 4 h. The drug was present in all animals upto 36 h with the mean concentration of 0.166 ± 0.016 μg/ml. Out of five animals, the drug was detectable in two animals only at 48 h and the mean concentration was noted to be 0.044 ± 0.027 μg/ml. The therapeutic concentration (≥ 0.12 μg/ml) was maintained from 1 to 36 h.

Table – 25

Concentration of enrofloxacin (μg/ml) in uterine fluid of Repeat breeding cows after its i.m. administration @ 5 mg/kg.

Time (hours)	Animal Numbers					Mean ± S.E.
	1	2	3	4	5	
1	0.65	0.78	0.70	0.45	0.80	0.676±0.062
2	1.78	3.92	3.56	1.62	4.00	2.970±0.526
4	4.18	3.10	4.68	3.85	3.18	3.798±0.299
8	2.16	2.05	2.45	2.12	1.95	2.146±0.083
12	1.66	1.12	1.28	1.06	1.00	1.224±0.118
24	0.85	0.78	0.82	0.68	0.60	0.746±0.046
30	0.45	0.34	0.42	0.33	0.28	0.36±0.031
36	0.22	0.16	0.18	0.15	0.12	0.166±0.016
48	0.12	ND	0.10	ND	ND	0.044±0.027

ND = Non Detectable

(c) ***Uterine fluid to serum ratio***: - Uterine fluid to serum ratio of Enrofloxacin in repeat breeding cows is shown in Table 26. A ratio > 1 was noted from 2 h to 24 h, which denotes that the drug penetrated into uterine tissues in greater amount in repeat breeding cows suffering from sub clinical uterine infections.

Table – 26

Uterine fluid to serum ratio of Enrofloxacin in repeat breeding cows.

Time (hours)	Animal Numbers					Mean ± S.E.
	1	2	3	4	5	
1	0.55	0.74	0.71	0.55	0.91	0.692±0.0672
2	0.79	1.85	1.65	1.5	2.81	1.72±0.325
4	3.87	3.37	4.68	4.93	3.38	4.046±0.325
8	2.35	3.20	3.27	4.08	2.87	3.154±0.282
12	3.69	2.95	4.27	4.82	3.12	3.77±0.350
24	4.25	5.57	6.83	ND	ND	3.33±1.419

ND – Non Detectable

2. Benzathine Penicillin

*(a) **Serum levels***: - Table 27 shows the mean concentration of Benzathine Penicillin in serum after i.m. administration at the rate of 12000 IU/kg. The mean concentration of the drug at 1 h was noted to be 0.16 ± 0.010 IU/ml. The drug reached its mean peak concentration of 0.41 ± 0.42 IU/ml at 12 h. The drug was present upto 72 h in all five animals with mean concentration of 0.08 ± 0.007 IU/ml. At 96 h, the drug was detectable only in two animals with a mean of 0.02 ± 0.012 IU/ml. The mean therapeutic concentration of ≥ 0.03 I.U/ml was maintained upto 96 h.

Table – 27

Concentration of Benzathine Penicillin (IU/ml) in serum of repeat breeding cows after its i.m. administration @ 12000 IU/kg.

Time (hours)	Animal Numbers					Mean ± S.E.
	1	2	3	4	5	
1	0.16	0.14	0.20	0.15	0.16	0.16±0.010
4	0.25	0.32	0.36	0.25	0.28	0.29±0.021
8	0.32	0.46	0.44	0.32	0.48	0.40±0.035
12	0.48	0.30	0.50	0.45	0.32	0.41±0.042
24	0.35	0.22	0.30	0.32	0.20	0.28±0.029
36	0.22	0.18	0.22	0.22	0.16	0.20±0.012
48	0.18	0.12	0.16	0.18	0.12	0.15±0.013
60	0.14	0.10	0.10	0.12	0.10	0.11±0.008
72	0.10	0.06	0.06	0.08	0.08	0.08±0.007
96	ND	ND	ND	0.05	0.05	0.02±0.012
120	ND	ND	ND	ND	ND	----

ND – Non Detectable

(b)Uterine fluid: - Concentrations of Benzathine Penicillin in uterine fluid of repeat breeding cows with sub-clinical infection after i.m. administration of 12000 IU/kg are presented in Table 28. The drug was detectable only in two animals with a mean concentration 0.44 ± 0.027 IU/ml at 1 h. The drug was present in all animals with the mean concentration of 0.30 ± 0.017 IU/ml at 4 h. The drug reached its mean peak concentration 0.78 ± 0.017 IU/ml at 12 h. The drug was detectable in all animals upto 96 h with a mean 0.15 ± 0.013 IU/ml. At 120 h, the drug was detectable in only one animal with mean concentration 0.02 ± 0.24 IU/ml. The mean therapeutic concentration of ≥ 0.03 IU/ml was maintained from 1 to 96 h in repeat breeding cows.

Table - 28

Concentration of Benzathine Penicilin (IU/ml) in uterine fluid of repeat breeding cows after i.m. administration @ 12000 IU/kg.

Time (hours)	Animal Numbers					Mean ± S.E.
	1	2	3	4	5	
1	ND	ND	0.10	ND	0.12	0.44±0.027
4	0.26	0.28	0.32	0.30	0.36	0.30±0.017
8	0.55	0.52	0.62	0.56	0.58	0.57±0.017
12	0.82	0.65	0.88	0.76	0.78	0.78±0.038
24	0.64	0.48	0.68	0.50	0.56	0.57±0.039
36	0.50	0.35	0.52	0.42	0.48	0.45±0.031
48	0.42	0.28	0.44	0.34	0.38	0.37±0.029
60	0.22	0.25	0.36	0.28	0.32	0.29±0.025
72	0.18	0.20	0.28	0.20	0.22	0.22±0.017
96	0.14	0.14	0.20	0.16	0.12	0.15±0.013
120	ND	ND	ND	ND	ND	0.02±0.024

ND – Non Detectable

(c) Uterine fluid to serum ratio: -

Table 29 shows the values of uterine fluid to serum ratio of Benzathine Penicillin in repeat breeding cows. The mean ratio of 0.25 ± 0.158 was obtained at 1 h that increases with time upto 72 h. The maximum ratio of 3.01 ± 0.482 was noted at 72 h. A ratio > 1 was noted from 4 to 96 h, which denotes that the drug may penetrate into uterine tissues in greater amount in repeat breeding cows. Thus, the drug is expected to cross the uterine barrier easily in repeat breeding cows.

Table – 29

Uterine fluid to serum ratio of Benzathine Penicillin in repeat breeding cows.

Time (hours)	Animal Numbers					Mean ± S.E.
	1	2	3	4	5	
1	-	-	0.50	-	0.75	0.25±0.158
4	1.04	0.87	0.89	1.2	1.28	1.06±0.082
8	1.72	1.13	1.40	1.75	1.20	1.44±0.128
12	1.71	2.17	1.76	1.69	2.44	1.95±0.149
24	1.83	2.18	2.27	1.56	2.80	2.13±0.210
36	2.27	1.94	2.36	1.90	3.00	2.29±0.198
48	2.33	2.33	2.75	1.89	3.17	2.49±0.217
60	1.57	2.50	3.60	2.33	3.20	2.64±0.353
72	1.80	3.33	4.67	2.50	2.75	3.01±0.482
96	-	-	-	3.20	2.40	1.12±0.697
120	-	-	-	-	-	-

IV.C. Comparison of distribution of antimicrobials between healthy and repeat breeding cows after its i.m. administration.

1. Enrofloxacin:

(a) Serum Levels: -

Comparative serum concentrations of Enrofloxacin in healthy cows and repeat breeding cows after its i.m. administration of 5 mg/kg are shown in Table 30. The drug was present in serum upto 24 h in both healthy and repeat breeding cows. The mean therapeutic concentration (0.12 μg/ml) of Enrofloxacin was maintained upto 12 h in healthy as well as repeat breeding cows. Significantly higher serum drug concentration were maintained from 1 to 12 h in healthy as compared to repeat breeding cows. The drug reached its peak concentration 3.59 ± 0.42 μg/ml at 4 h in healthy cows while peak concentration 1.81 ± 0.23 μg/ml was noted at 2 h in repeat breeding cows.

(b) Uterine fluid levels: -

Table 30 reveals the uterine fluid concentrations of Enrofloxacin after i.m. administration (5mg/kg) in healthy and repeat breeding cows. The drug was detectable from 1 to 36 h in healthy cows whereas for a longer period (1 to 48 h) in repeat breeding cows. The drug reached its peak concentration of 2.42 ± 0.28 μg/ml at 8 h in healthy cows while 3.79 ± 0.29 μg/ml at 4 h in repeat breeding cows.

Figure - 7

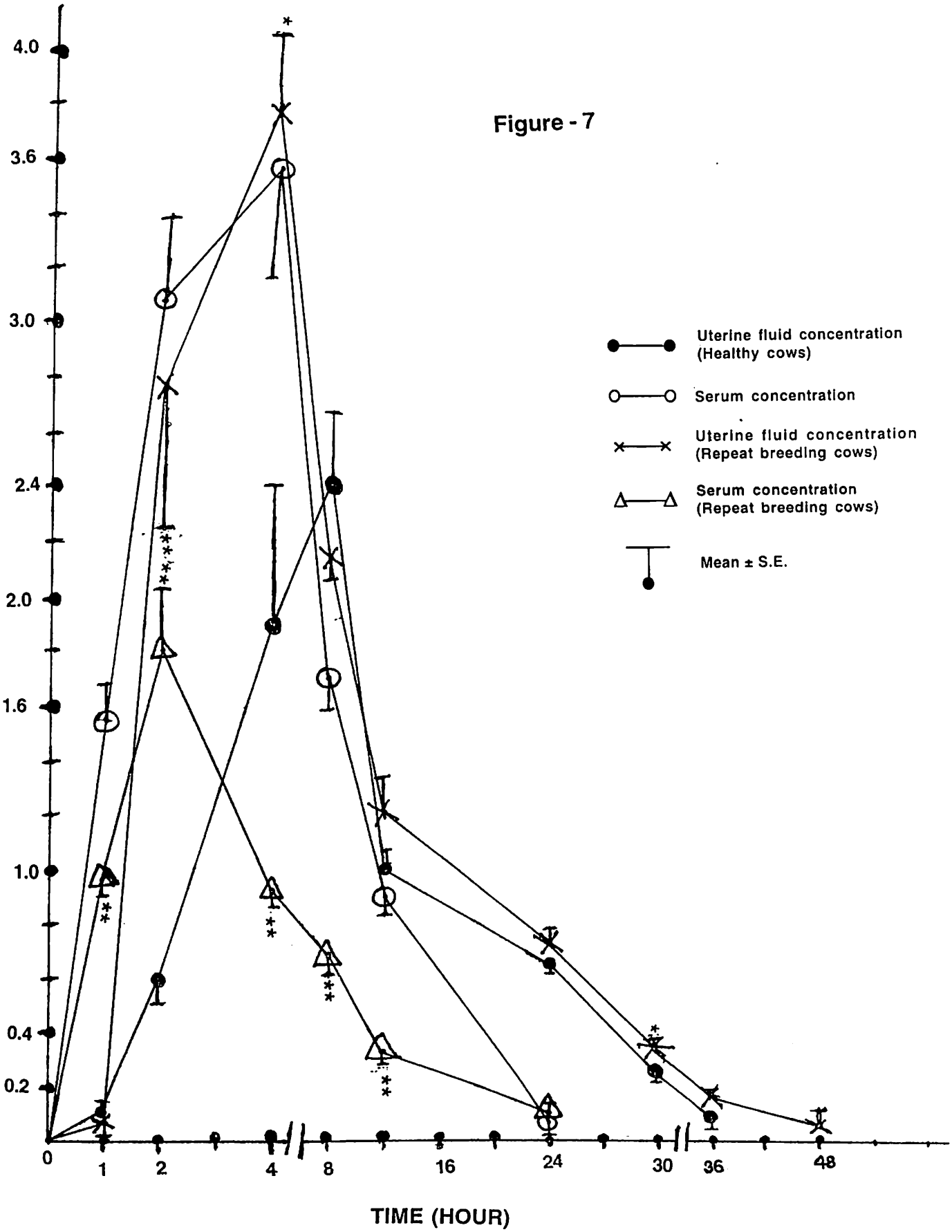

4.0
3.6
3.0
2.4
2.0
1.6
1.0
0.4
0.2
0
1
2
4
8
16
24
30
36
48
TIME (HOUR)
Uterine fluid concentration (Healthy cows)
Serum concentration
Uterine fluid concentration (Repeat breeding cows)
Serum concentration (Repeat breeding cows)
Mean ± S.E.

Table – 30

Comparison of distribution of Enrofloxacin between healthy cows and repeat breeding cows after a single i.m. administrations @ 5 mg/kg.

Time (hours)	HEALTHY COWS			REPEAT BREEDING COWS		
	Mean ± S.E.M.			Mean ± S.E.M.		
	Serum levels (µg/ml)	Uterine fluid levels (µg/ml)	Uterine fluid to serum ratio	Serum levels (µg/ml)	Uterine fluid levels (µg/ml)	Uterine fluid to serum ratio
1	1.55±0.12	0.10±0.04	0.06±0.03	0.98±0.06**	0.07±0.06^{NS}	0.69±0.067**
2	3.09±0.30	0.60±0.09	0.19±0.02	1.81±0.23**	2.78±0.53**	1.72±0.32**
4	3.59±0.42	1.91±0.49	0.58±0.16	0.94±0.05**	3.79±0.29*	4.05±0.32**
8	1.72±0.12	2.42±0.28	1.45±0.22	0.70±0.07**	2.15±0.08^{NS}	3.15±0.28**
12	0.91±0.07	1.01±0.07	1.13±0.11	0.334±0.04**	1.22±0.12^{NS}	3.77±0.35**
24	0.08±0.05	0.67±0.04	1.43±0.89	0.092±0.04^{NS}	0.75±0.05^{NS}	3.33±1.42^{NS}
30	----	0.26±0.03	---	----	0.36±0.03*	----
36	----	0.09±0.04	---	----	0.17±0.02^{NS}	----
48	----	----	----	-----	0.04±0.03^{NS}	----

* $p < 0.05$, NS = non-significant, ** $p<0.01$.

Significantly higher drug concentrations were noted only at 2, 4 and 30 h in repeat breeding cows as compared to healthy cows. The drug maintained its therapeutic concentration (≥ 0.12 μg/ml) from 2 to 30 h in healthy cows whereas from 2 to 36 h in repeat breeding cows.

C. Uterine fluid to serum ratio: -

Table 30 presents the uterine fluid to serum ratio of Enrofloxacin after i.m. administration of 5 mg/kg in healthy cows and repeat breeding cows. The ratio obtained at 1 h in healthy cows and repeat breeding cows were 0.06 ± 0.03 and 0.69 ± 0.067, respectively. This ratio in repeat breeding cows were significantly differed from healthy cows from 1 to 12 h. The maximum ratio of 1.45 ± 0.22 at 8 h was obtained in healthy cows while 4.05 ± 0.32 at 4 h was noted in repeat breeding cows. Highly significant ($p < 0.01$) increase in uterine fluid to serum ratio was noted at all time interval (except 24 h) in repeat breeding cows as compared to healthy cows, which denotes that Enrofloxacin penetrates to a greater amount in uterus of repeat breeding cows.

2. Benzathine Penicillin

(a) Serum levels: - Table 31 shows the comparative serum concentrations of Benzathine Penicillin in healthy cows and repeat breeding cows after its i.m. administration of 12000 IU/ml. The drug was present in serum from 1 to 120 h in healthy cows and 1 to 96 h in repeat breeding cows. The mean therapeutic concentration

Figure - 8

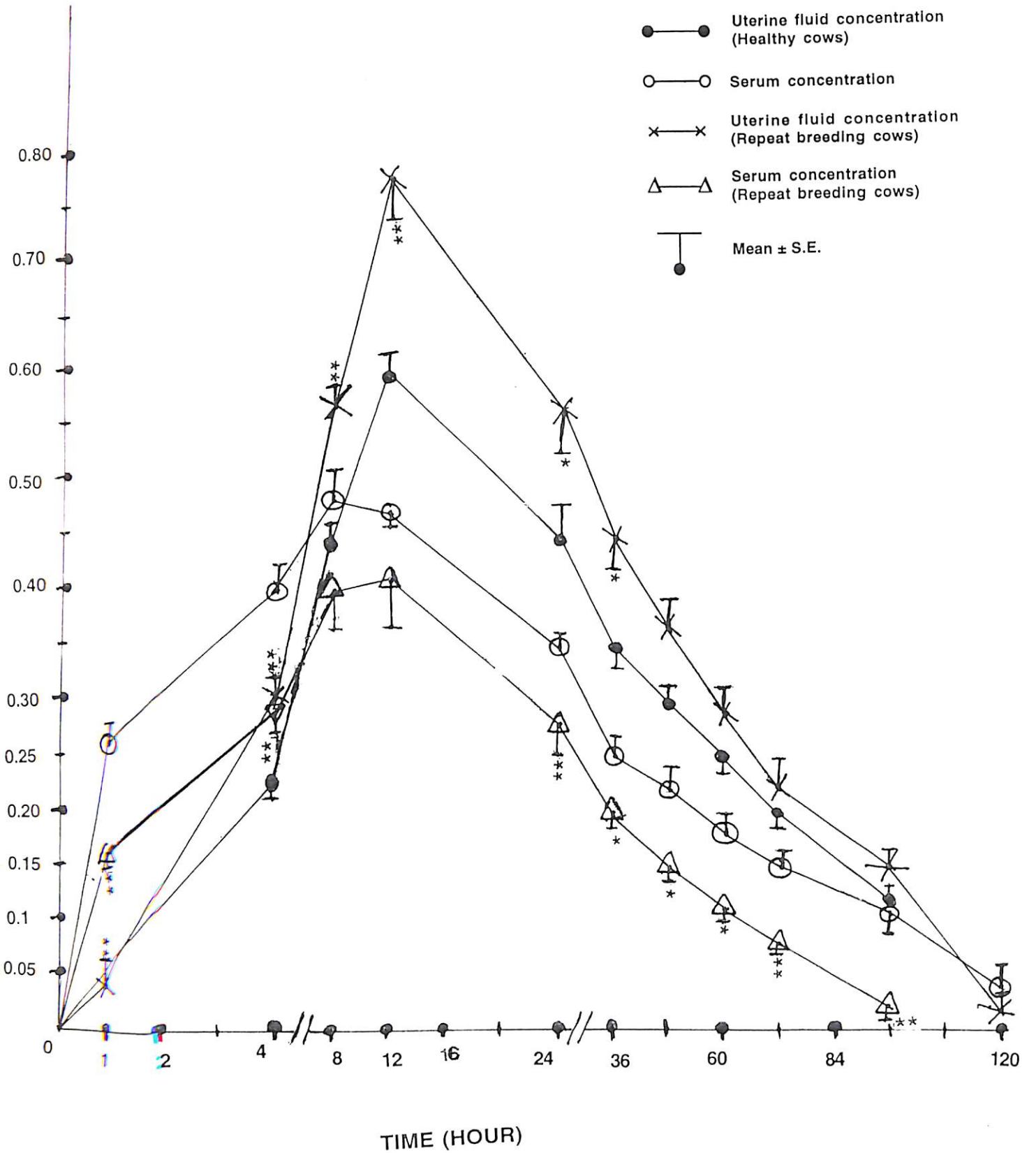

Uterine fluid concentration (Healthy cows)
Serum concentration
Uterine fluid concentration (Repeat breeding cows)
Serum concentration (Repeat breeding cows)
Mean ± S.E.
0.80
0.70
0.60
0.50
0.40
0.30
0.25
0.20
0.15
0.1
0.05
0
1
2
4
8
12
16
24
36
60
84
120
TIME (HOUR)

Table – 31

Comparison of distribution of Benzathline Penicillin between healthy and repeat breeding cows after a single i.m. administration at @ of 12000 IU/kg.

Time (hours)	HEALTHY COWS			REPEAT BREEDING COWS		
	Concentration µg/ml Mean ± S.E.M.			Mean ± S.E.M.		
	Serum (IU/ml)	Uterine fluid (IU/ml)	Uterine fluid to serum ratio	Serum levels (I.U/ml)	Uterine fluid levels (I.U./ml)	Uterine fluid to serum ratio
1	0.26±0.017	----	----	0.16±0.01**	0.04±0.027**	0.25±0.158 NS
4	0.40±0.023	0.23±0.011	0.59±0.022	0.29±0.021**	0.30±0.017**	1.06±0.082**
8	0.48±0.027	0.44±0.021	0.91±0.026	0.40±0.035NS	0.57±0.017**	1.44±0.128**
12	0.47±0.011	0.60±0.023	1.29±0.055	0.41±0.042 NS	0.78±0.038**	1.95±0.149**
24	0.35±0.012	0.45±0.032	1.30±0.090	0.28±0.029*	0.57±0.039*	2.13±0.210**
36	0.25±0.018	0.35±0.020	1.41±0.099	0.20±0.012*	0.45±0.031*	2.29±0.198**
48	0.22±0.020	0.30±0.019	1.39±0.132	0.15±0.013*	0.37±0.029NS	2.49±0.217**
60	0.18±0.02	0.25±0.017	1.42±0.143	0.11±0.008*	0.29±0.025 NS	2.64±0.353*
72	0.15±0.015	0.20±0.013	1.33±0.119	0.08±0.007**	0.22±0.017 NS	3.01±0.482**
96	0.11±0.019	0.12±0.013	1.17±0.141	0.02±0.012**	0.15±0.013 NS	1.12±0.697NS
120	0.04±0.022	---		----	0.02±0.024 NS	-----

* $p < 0.05$ NS – Non-significant ** $p < 0.04$.

(≥ 0.03 IU/ml) was maintained uptò 120 h in healthy cows and upto 72 h in repeat breeding cows. Highly significantly ($p < 0.01$) lower drug concentrations in serum were maintained 1 to 4 h and 72 to 96 h and significantly ($p < 0.05$) lower drug concentrations in serum were maintained from 24 to 60 h in repeat breeding cows.

(b) Uterine fluid levels: - The comparative uterine fluid levels of Benzathine Penicillin after i.m. administration (12000 IU/kg) in healthy and repeat breeding cows are presented in Table 31. The drug was detectable from 4 to 96 h and 1 to 96 h in healthy and repeat breeding cows, respectively. The drug reached its mean peak concentrations (0.60 ± 0.032 IU/ml) in healthy and 0.78 ± 0.038 IU/ml in repeat breeding cows at 12 h. In both the conditions, highly significantly ($p < 0.01$) higher drug concentrations in uterine fluid were maintained from 1 to 12 h in repeat breeding cows. The drug maintained its therapeutic concentration of ≥ 0.03 IU/ml from 4 to 96 h and 1 to 96 h in healthy cows and repeat breeding cows, respectively.

(c) ***Uterine fluid to serum ratio***: - Table 31 presents the uterine fluid to serum ratio of Benzathine Penicillin after its i.m. administration (12000 IU/ml) in healthy and repeat breeding cows. The ratios in repeat breeding cows were significantly higher ($p < 0.01$) from 4 to 48 h and at 72 h as compared to healthy cows. The maximum ratio of 1.42 ± 0.14 at 60 h was noted in healthy cows while 3.01 ± 0.048 at 72 h was noted in repeat breeding cows. Highly significant difference between repeat breeding and healthy cows

shows that the Benzathine Penicillin penetrates to a greater amount in uterus of repeat breeding cows.

V. THERAPEUTIC MEASURES AND CONCEPTION RATE

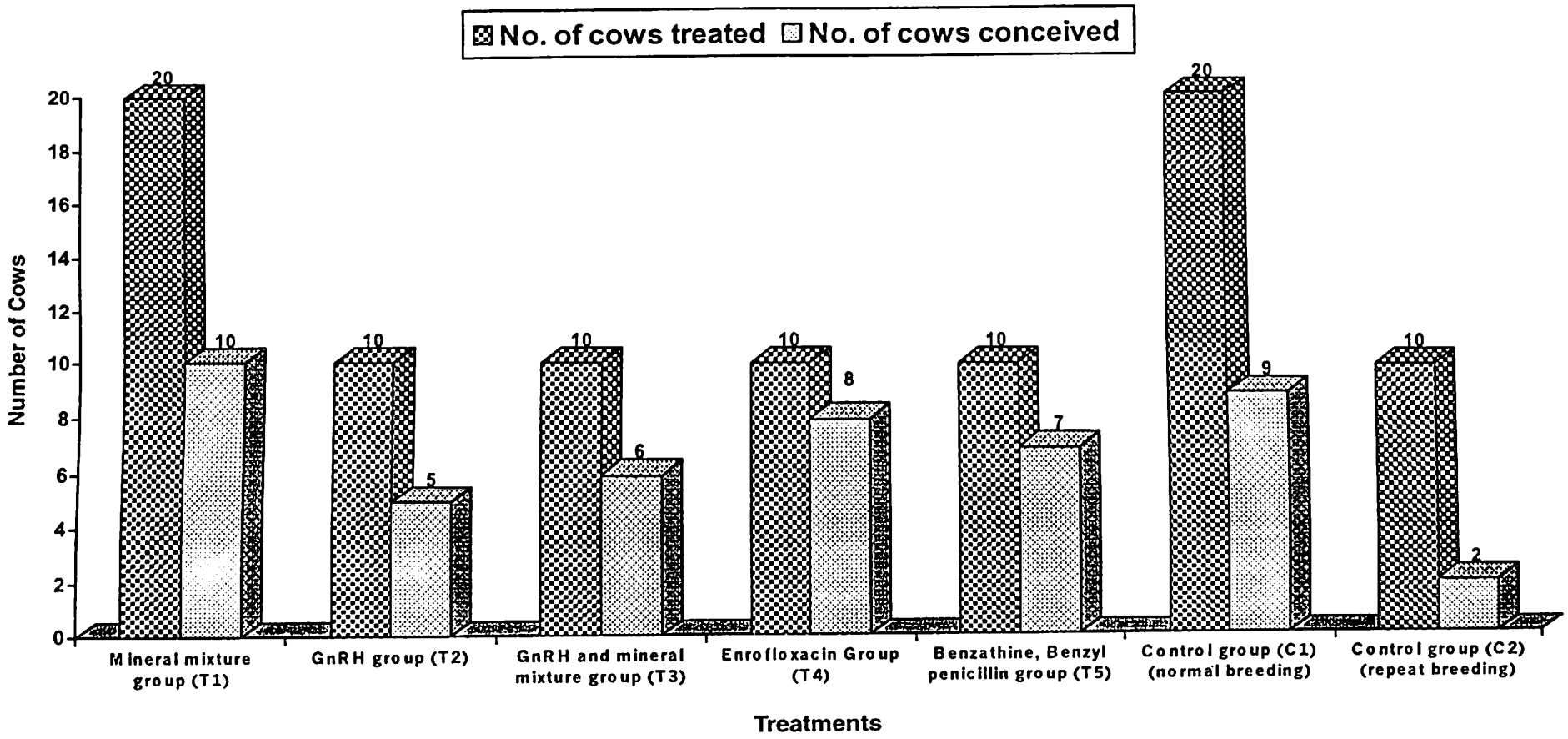
It is evident from Table 32 and Fig. 9, that the overall breeding efficiency in different treatment groups of repeat breeder cows were found to be higher than untreated controls. A total of 36 (60%) repeat breeder cows were found to be pregnant among 60 repeat breeder cows of different treatment groups whereas, 9 (45%) of 20 normal cycling cows and 2 (20%) of 10 repeat breeding cows kept in control groups were found to be pregnant. Among treatment groups, conception rate was maximum in T_4 group (80%) and minimum in T_1 and T_2 group (50%). In T_3 and T_5 groups, the conception rates were 60% and 70%, respectively.

Table – 32

Effect of treatments of conception rate in repeat breeding cows.

Treatments	Drug used	No. of cows treated	No. of cows conceived	Conception %
Mineral mixture group (T_1)	Mineral mixture feed supplement powder "KALMIN-L"	20	10	50
GnRH Group (T_2)	Buserelin – acetate inj. 0.0042 mg (GnRH analogue) "RECEPTAL Vet."	10	5	50
GnRH and mineral mixture Group (T_3)	Mineral mixture feed supplement powder "KALMIN-L" and GnRH analogue "RECEPTAL Vet."	10	6	60
Enrofloxacin Group (T_4)	Enrofloxacin (100 mg/ml) "QUININTAS"	10	8	80
Benzathine, Benzyl penicillin (Vet.) Group (T_5)	Long acting Penicillin "LONGACILLIN"	10	7	70
Total		60	36	60
Control group (C_1) (Normal breeding)	No treatment	20	9	45
Control groups (C_2) (Repeat breeding)	No treatment	10	2	20

Fig. 9. : Showing effect of treatments of conception rate in repeat breeding cows



Chapter - 5

Discussion

DISCUSSION

Repeat breeding in cattle is an important constraint for profitable running of dairy industry. It affects the calving interval as well as breeding efficiency. The present study was conducted in order to know the prevalence of repeat breeding in cows in and around Patna. Further, estimation of levels of some biochemicals and based on which treatment of repeat breeder cows were done. Since sub-clinical uterine infections is one of the causes of repeat breeding, culture and sensitivity test of uterine tissues/fluid and distribution studies of antimicrobial agents (Enrofloxacin and Benzathine Penicillin) in blood and uterine fluid in normal and repeat breeding cows were carried out. Based on the results of the study, treatment with antimicrobial agents in repeat breeding cows were carried out and the efficacy of treatment was evaluated on the basis of conception rate.

I. STUDIES ON PREVALENCE

The overall prevalence of repeat breeding in cows were found to be 15.99% in the present study. The present result is in close agreement with those of Zemjanis (1963), Shukla and Pandit (1989), Sreeramulu (1995) and Dhable *et al.* (1996) who reported 15%, 16.23%, 16.01% and 17.79%, respectively. However, Shah and Usmani (1984), Rahumathulla et al. (1986), Ben-Haj and Abdulmola (1988)

reported higher prevalence of 51.31%, 73.70%, 67%, respectively. In contrast, lower prevalence of repeat breeding of 10%, 8.2% and 8% were reported by Hewett (1968), Ronie and Saloniemi (1978) and Pargaonkar and Bakshi (1987), respectively. The variation in the results may possibly be due to differences in breeds of cow, agro-climatic conditions, calving sequences, nutrition and management.

A. Effect of season: -

In the present research work, the effect of season on repeat breeder cows was found to be non significant. This result is in agreement with those of Sinha (1978) and Rao and Kotaya (1980). The maximum cases of repeater (20%) were observed during summer and minimum (12.50%) during autumn season. It is well known that the majority of the cattle owners in India are poor and they depend mainly on grazing the animals. Plenty of green grass is available for grazing during autumn season; apart from that, it is a healthy season and there is least chance of nutritional deficiency and hence, lowest percentage (12.50%) of repeat breeding in cattle. In contrast, during summer season, there is scarcity of green grass as well as other fodders available for animals. Further, due to hot climatic condition, the animals are under stress. Due to above factors, there is the possibility of increase in cases of repeat breeding in cattle as noted by highest incident of repeat breeding (20%) in cows as noted in the present study.

B. Parity wise prevalence: -

The maximum incidence of repeat breeding was observed during second partum (27.59%) and minimum during fifth and onward partum (10.34%). This observation is in close agreement with the findings of Hafez (1987), Dhable (1996). But, the present finding is different from that of Hewett (1968).

Maximum incidence of repeat breeding in second partum may be due to lactation stress and metabolic stress as well as hormonal imbalances because cows have been observed to have higher milk yield during this period.

II. BLOOD BIOCHEMICAL PROFILE

A. Blood glucose: -

Blood glucose levels in repeat breeder and normal cycling control were found to be 52.71 ± 0.11 to 52.96 ± 0.14 mg% and 62.25 ± 0.02 to 65.66 ± 0.09 mg%, respectively. The blood glucose levels in normal cycling and repeat breeders differed significantly in the present study. This result is similar to the findings of Nair *et al.* (1987) and Dutta *et al.* (1991) who observed 50.11 mg% and 52.90 ± 0.51 mg% in repeat breeder and 66.70 mg% and 70.83 ± 0.82 mg% in normal cycling cows, respectively. It was also observed that in every treatment group (T_1 to T_5), blood glucose levels increased significantly from 0 to 42nd day. This is in accordance with the findings of Ramkrishna (1996) and Kumar (2000). Kumar and Sharma (1991)

also reported significantly low serum glucose levels in non-fertile (51.11 ± 2.08 mg%) as compared to fertile cow (59.00 ± 3.39 mg%). The result in T_3 (i.e GnRH) group is in close agreement with that of Kumar (2000) who reported blood glucose level to be 53.71 ± 0.23 mg % on 0^{th} day and 56.02 ±0.31mg% on 45^{th} days.

In contrast to the result of the present study, Parmar *et al.* (1986) reported significantly higher levels of blood glucose in repeat breeder cows (97.73 ± 9.36 mg%) than that of normal cycling (71.89 ± 8.33 mg%) control cows. Similarly, Islam *et al.* (1994) also reported higher serum glucose levels in repeat breeders than normal breeder cows on day 0 and 13^{th} day of the estrus cycles.

Low levels of glucose in repeaters may be an indication of sub-normal energy status. Hypoglycemia has been reported as one of the major causes of infertility in lactating cattle (Downie and Gelman, 1979). McClure (1965) reported that the secretion of gonadotropin might have reduced or stopped due to hypothalamic failure to utilize glucose. Elden *et al.* (1988) also established positive correlation between the level of glucose and conception.

B. Total serum protein: -

Serum protein levels in normal breeders and repeat breeders cows were found to be 6.70 ± 0.06 to 6.91 0.07g% and 5.63 ± 0.05 to 5.73 ± 0.03 g%, respectively. The result were significantly higher ($p < 0.01$) in normal cycling control as compared to repeat

breeding cows. This result is similar to those observed by Agrawal *et al.* (1982) and Burle *et al.* (1995). It was observed that in various treatment groups (T_1 to T_5) the total serum protein did not show any significant difference from 0 day to 42nd day. This result is in accordance with that of Kumar (2000). Ramkrishna (1996) found significantly lower serum protein levels in repeat breeders with uterine infections as compared to normal breeders without uterine infections which are in agreement with the present findings. However, in contrast to the present study, Enkhia *et al.* (1982) reported non significant difference in serum total protein levels between normal and repeat breeding cows. Srivastava (1995) also showed non significant difference between these two groups.

Herrrick (1977) and Patil and Despande (1979) reported that optimal protein level is necessary for the development of body, sex organ and for expression of estrus sign, respectively. Low serum protein levels affect the process of implantation (Roberts, 1971). Lower levels of total serum proteins in repeat breeder animals might have caused deficiency of particular amino-acids required for the synthesis of various releasing hormones and pituitary hormones, which in turn might have caused reproductive disturbances. Thus, the cause of failure of fertilization may be due to low level of serum proteins. Hence, it may be concluded from the present observation that low levels of total serum protein may be associated with repeat breeding syndrome.

C. Serum calcium: -

The levels of calcium in serum of repeat breeders and normal cycling control were found to be 8.85 ± 0.06 to 8.95 ± 0.09 mg% and 8.93 ± 0.03 to 9.09 ± 0.05 mg%, respectively.

On the day of estrous (i.e. 0 Day), the serum calcium levels in repeat breeder and normal cycling control were found to be 8.85 ± 0.06 mg% and 8.93 ± 0.03 mg%, respectively. However, serum concentrations of calcium did not differ significantly. Singh and Pant (1998) and Das et al. (2002) have also observed similar results in repeat breeder cows. In contrary to these findings, Umashankar *et al.* (1983) in repeat breeding buffaloes, Kumar *et al.* (1986) in repeater cows and heifers and Jayanthi *et al.* (2003) in repeat breeder cows found lower values of serum calcium. However, Srivastava and Kharche (1986) and Awasthi and Kharche (1987) reported higher serum levels (9 - 16.0 mg% and 8.93 ± 0.63 mg%) in repeat breeder cows as compared to normal cycling cows (10.50 - 15.0 mg% and 7.86 ± 0.42 mg%), respectively. Ramkrishna (1996) found 9.77 ± 0.52 mg% serum calcium levels in repeat breeders with uterine infections and 9.85 ± 0.21 mg% in repeat breeders without uterine infection and these values do not differs significantly which is similar to the present findings as noted in T_4 and T_5 treated groups.

The variations in serum calcium levels may be due to the difference in age, stage of lactation and nutritional status to the animals at the time of blood collection. Morrow (1969) reported that

calcium deficiency did not affect the reproductive performance of the cows. Roberts (1971) stated that calcium deficiency doesn't cause reproductive failure in cattle. Almost similar findings were also reported by Sane *et al.* (1982).

D. Serum inorganic phosphorus: -

The levels of serum inorganic phosphorus in normal and repeat breeder cows were found to be 5.77 ± 0.06 to 5.95 ± 0.04 mg% and 4.31 ±0.03 to 4.41 ± 0.03 mg%, respectively.

On the day of estrous (i.e. 0 Day), the serum inorganic phosphorus levels in normal cycling and repeat breeder control were found to be 5.80 ± 0.04 mg% and 4.31 ± 0.03 mg%, respectively. In the present observation, the serum inorganic phosphorus level was high in normal cyclic control as compared to repeat breeder cows. This result is also in close agreement with Enkhia *et al.* (1983) Awasthi and Kharche (1987), Das *et al.* (2002) and Jayanthi *et al.* (2003) who found 5.76 ± 0.28 and 3.16 ± 0.11 mg%, 5.06 ± 0.19 and 3.73 ± 0.29 mg%, 5.513 ± 0.265 and 4.729 ± 0.150 mg% and 5.27 ± 0.04 and 4.12 ± 0.03 mg% in normal and repeat breeder cows, respectively. In contrast to the present finding, Rao *et al.* (1981) reported higher serum inorganic phosphorus levels in normal (6.11 ± 0.39 mg%) than repeater cows (6.82 ± 1.13 mg%), respectively, but these values do not differ significantly. Rupde *et al.* (1993) also reported increased levels of phosphorus after mineral mixture treatment as in case of T_1 group. This result is in close agreement

with that of Kumar (2000). The treatment group T_4 (Enrofloxacin) and T_5 (Benzathine Penicillin) showed lower levels of serum inorganic phosphorus in pretreatment than post treatment. Ramkrishna (1996) also observed lower levels of serum inorganic phosphorus in repeaters with uterine infections than that of cows without uterine infections. Bhaskaran and Abdulla Khan (1981) reported that marginal deficiency of phosphorus is sufficient to cause disturbance in pituitary ovarian axis without manifestation of specific deficiency symptoms. Mufarrege *et al.* (1986) also found that blood phosphorus concentration was higher in cows which conceived than that in those which did not conceive, as phosphorus is essential for transfer of biological energy particularly through ATP and its deficiency may interfere with fertilization and may cause early embryonic death.

E. Alkaline phosphatase: -

The alkaline phosphatase (AKP) values in repeat breeder and normal cycling control cows were found to be 7.85 ± 0.04 to 8.11 and 6.56 ± 0.04 to 6.81 ± 0.08 KAU%, respectively.

On estrus day (or 0 Day), the serum alkaline phosphatase levels in repeat breeder and normal cycling control were 7.85 ± 0.04 and 6.56 ± 0.04 KAU%, respectively. A significantly higher ($p < 0.01$) level of AKP was observed in repeat breeders than normal cycling cows. Mahmood *et al.* (1991) and Ganmdotra *et al.* (1993) also observed high levels of AKP in repeat breeder cows. In contrast to the above and the result of the present study, Parmar and Mehta (1989)

observed lower levels of alkaline phosphatase in repeat breeders as compared to control cows.

Higher levels of AKP might be responsible for matabolization of glycogen much earlier than required time. When the fertilized ova reach the uterus and develop to blastocysts, energy stock is already exhausted in the emdometrium and may lead to implantation impairment. AKP is a widely distributed enzyme, which acts as a catalyst for the release of inorganic phosphate from many organic phosphomonoesters and thus provides energy in the form of phosphate for normal reproductive functions.

In normal cycling cows, decreased concentration of AKP may enhance the folliculogenesis and further may increase the pace of conception (Devraj, 1983).

Serum AKP levels increase during 2^{nd} and 3^{rd} trimester of pregnancy and the extra increase of AKP is due to additional secretion AKP by placenta (Anagostopoulos and Matsudaria, 1958; Yvonn *et al.*, 1964).

III. BACTERIOLOGICAL STUDIES

A. *Isolation of Bacteria:* -

A total of 131 uterine samples of 131 repeat breeding cows were taken into consideration for isolation and identification of various microorganisms. Out of 131 samples, 53 (40.45%) were found to be negative for bacterial growth and 78 (59.54%) were found

positive for bacterial growth. Single isolates were obtained from 63 (80.76%) where as rest 15 (19.23%) isolates are mixed type. Mixed infections revealed the association of only two types of bacterial isolates. The result of present study is in agreement with the findings of Sharda *et al.* (1991) who reported 64% bacterial positive and 36% bacterial sterile. However, the result differs with the findings of Krishnamurthy *et al.* (1974), Dhable *et al.* (1996), Mutiga (1978), Singh *et al.* (1983) and Dabas *et al.* (1995) who reported comparatively higher percentages of positive samples. The percentages of positive and negative samples were 81.87 & 18.13, 83.33 & 16.66, 90 & 10, 95 & 5 and 76.3 & 23.7, respectively.

It is evident from (Table-16) that the highest percentage of isolate (34.40%) was *Escherichia coli* out of the total samples (including single & mixed isolates). The other organisms obtained were *Staph. aureus* (23.66%), *Strep. pyogenes* (17.20%), *Corynebacterium pyogenes* (9.68%), *Pseudomonas aeruginosa* (7.53%), *Proteus vulgaris* (5.38%) and *Klebsiella pneumoniae* (2.15%). Similar findings were also observed by Leclerc *et al.* (1972), Bora (1984), Singla *et al.* (1991), Sarmah (1994), Verma *et al.* (1994), Das *et al.* (1996), Baishy *et al.* (1998), Arora *et al.* (2000) who also recorded the presence of *Escherichia coli* in highest percentage. However, Panangala *et al.* (1978) & Singh *et al.* (1989) found *Staphylococcus*, Das *et al.* (1996), *Pseudomonas* spp., Mohanrana *et al.* (2000) *Staphylococcus* and *Streptococcus* and Chandrakar *et al.* (2002) *Staphylococcus aureus* as the commonest organisms.

Next to *Esch. coli,* the *Staphylococcus aureus* (23.66%) was the major cause of uterine infection in repeat breeding cows. The result of Arora *et al.* (2000) also supports the present findings. The other isolates were *Streptococcus pyogenes*, *Corynebacterium pyogenes, Ps. aeruginosa, Pr. vulgaris* and *K. pneumoniae* which are present in 17.20%, 9.68%, 7.53%, 5.38% and 2.15%, respectively. Singla et al. (1991), Sarmah et al. (1994) and Arora et al. (2000) with minor variations also reported similar findings.

Non-specific infection introduced during natural or artificial insemination or at the time of previous calving on account of poor hygienic and managemental conditions may be responsible for repeat breeding. These non-specific organisms becomes pathogenic under favourable condition, which render the female genital tract more harmful to the viability of the sperms and this in turn may result into failure of fertilization (Bora, 1984 and Maurya *et al.*, 1992).

B. In-vitro sensitivity: -

In the present work, an attempt was made to study the antibiogram of the isolates to institute an effective rational therapy by proper use of drugs. Selection of proper antibiotics is very essential for the treatment of repeat breeding in cows. Since indiscriminate and prolonged uses of antimicrobials in absence of drug sensitivity test w resulted in emergence of drug resistant strains of bacteria. The evaluation of chemotherapeutic agents against genital tract infections

based on their *in-vitro* sensitivity result has received considerable attention in recent years mainly due to their reliability and economy.

A total of seven antimicrobial agents were tested against the 93 isolates obtained from 78 repeat breeder cows (Table 17). Amikacin was found to be highly sensitive (91.31%) followed by Enrofloxacin (80.64%), Chloramphenicol (79.57%), Ciprofloxacin (69.74%), Gentamicin (67.74%), Oxytetracycline (32.25%) and Penicillin (25.80%). Ramaswamy *et al.* (1992) observed that Amikacin is sensitive to 58.07%, which is different from the present finding. Verma *et al.* (1991) showed maximum sensitivity (100%) to Enrofloxacin, which is in close agreement with the present finding. Krishnan *et al.* (1994), Ramkrishna (1996) and Arora *et al* (2000) observed 84%, 73.9% and 74.43%, respectively, which are in close similarity to that of the present findings. Ramaswamy *et al.* (1992) showed the none of the organism is sensitive to Chloromphenicol, which is different to the present finding. Penicillin is the least sensitive in present finding because of its narrow spectrum and indiscriminate use for longer periods. Venkateshwaram and Rajeswar (1991) showed 23.4% sensitivity to Penicillin which is in close agreement to that of the present result. Krishnan *et al.* (1994) and Sharda *et al.* (1991) showed sensitivity of 44% and 68.12%, respectively to this antibiotics which is higher than that of the result of the present finding. Dabas *et al.* (1995) and Chandrakar *et al.* (2002) found that no organism is sensitive to Penicillin.

IV. DISTRIBUTION STUDIES OF ANTIMICROBIAL AGENTS

An antimicrobial agent may become effective only when it reaches the site of infection (Tan, 1978). In repeat breeding, usually the uterus may be infected with microorganisms, which may need therapy with antimicrobials. Therefore, it is essential to find out the distribution of antimicrobials in uterine tissues so that the infected organisms may be removed ultimately and conception may be attained. In the present study, an attempt has been made to know the distribution of the following antimicrobials by the easy, convenient and most popular i.m. route so that the effective dosage regimen can be suggested for the treatment of repeat breeding cows, which are mostly infected with microorganisms.

A. Enrofloxacin: -

It is a recently introduced fluroquinolone that is exclusively used in veterinary practice. It is rapidly acting bactericidal agent with a broad spectrum of activity against aerobic and facultative anaerobic bacteria including strains resistant to many other antimicrobials such as β-lactum antibiotics, aminoglycosides tetracyclines, macrolids etc.

Distribution studies of Enrofloxacin in serum and uterine fluid were carried out in repeat breeder cows after single i.m. administration at the dose rate of 5 mg/kg. The data was compared

with that of healthy cows to know the pattern of distribution. The study revealed that significant decrease in serum concentrations at most of time interval while significant increase in uterine levels at all time intervals were noted in repeat breeding cows with uterine infections as compared to healthy animals (Table 30). It is well known that inflammatory changes may cause increase in higher permeability of a drug, which may be due to increase in pore size of the membrane. The serum levels of the drug decreased due to higher penetration of the drug in the uterus. This leads to significant increase in uterine fluid to serum ratio in repeat breeding cows as compared to healthy cows. Very little studies have been conducted with regard to distribution of Enrofloxacin in repeat breeding animals, particularly in cows. Similar observation was obtained by Gigure *et al.* (1996) who conducted pharmacokinetic study of enrofloxacin in adult horses. They observed that the endometrial tissue concentrations exceeded that of plasma concentration by as much as three folds. Gatne *et al.* (1997) noted variation in the persistence of Enrofloxacin in serum between 6 to 8 h and suggested that dose of enrofloxacin should be repeated 12 h and in acute cases every 8 h. Kumar (2002) noted higher distribution of Enrofloxacin in case of endometritic cows. He observed that the drug reached its peak plasma concentration of 4.67 ± 0.23 μg/ml and 3.47 ± 0.21 μg/ml at 0.5 h in healthy and endometric cows, respectively. Peak concentration in uterine fluid was 2.70 ± 0.08 μg/ml at 4 h and 9.00 ± 0.73 μg/ml at 3 h in healthy

and endometritic cows, respectively. The drug maintained its therapeutic concentration in uterine fluid from 2 to 12 h in healthy and 1 to 24 h in cows suffering from endometritics.

In the present study, the mean therapeutic concentration of Enrofloxacin (0.12 μg/ml) was maintained upto 30 h in uterine fluid of healthy cows while it was maintained upto 36 h in repeat breeder cows after i.m. administration (5 mg/kg). Thus, the present observation clearly established that Enrofloxacin can be effectively used at the usual recommended therapeutic dosage of 5 mg/kg daily by parenteral route for the treatment of repeat breeding cows.

B. Benzathine Penicillin: -

Benzathine Penicillin is a β-lactum antibiotic. The drug has been widely used in infections of human as well as veterinary practice since its availability. It has a narrow range of activity, which acts mostly on gram positive bacteria. Benzathine Penicillin is absorbed very slowly from intramuscular route and maintains the longest duration of therapeutic concentration. Its action is bactericidal.

In the present study, distribution of Benzathine Penicillin in serum and uterine fluid were conducted in repeat breeder cows as well as healthy cows after its single i.m. administration at the dose rate of 12000 IU/kg. The study revealed that significant decrease in serum drug concentrations while significant increase in uterine fluid

levels at most of the time intervals were noted in repeat breeder cows as compared to healthy cows (Table 31). This has led to highly significant ($p < 0.01$) increase in uterine fluid to serum ratio in repeat breeding cows as compared to healthy cows. The higher permeability of the drug in repeat breeding cows may be due to inflammatory changes of the uterine membrane caused by microorganisms. Inflammation of any tissue may increase the size of the pores. Such increase in permeability was noted by Ames *et al.* (1983) who noted increase in the concentration of Oxytetracycline in pneumonic lungs of cows as compared to healthy animals. The therapeutic concentration (≥ 0.03 IU/ml) was maintained in serum from 1 to 120 h and in uterine fluid from 4 to 96 h in healthy cows whereas it was maintained from 1 to 72 h in serum and 1 to 96 h in uterine fluid of repeat breeding cows. By going through the above facts, Benzathine Penicillin can be administered by i.m. route at the dose rate of 12000 IU/kg every 96 hourly. Only very few studies have been done with regard to distribution of Benzathine Penicillin in serum and uterine fluid/tissues in animals. Hagdrup *et al.* (1986) studied the serum levels of syphilis patient after 1.44 gm (2.4×10^6 I.U.) Benzathine Penicillin administration and found concentrations below the recommended levels (0.018 μg/ml) were found 7 days after first or second injections. Which is similar to the present study. Kaplan *et al* (1989) studied the serum levels of Benzathine Penicillin G in young

human given at dose 1200000 units i.m. The mean serum Penicillin levels remained greater than or equal to 0.02 μg/ml for 21 days, which also showed that the Benzathine Penicillin might absorb and excrete slowly and maintain the therapeutic concentration for longer period. But, this result differ from repeat breeding cows of the present study where therapeutic concentration was maintained upto 4 days only.

V. THERAPEUTIC MEASURES AND CONCEPTION RATE

The therapeutic measures are essential for the treatment of repeat breeding animals. The efficacy of treatment was evaluated based on conception rate. Very few reports are available in repeat breeders such therapeutic measures. The majority of the studies were mostly conducted on experimental basis or in the laboratory. The present study was carried out in field condition. Hence, the studies on conception rate after treatment will be helpful to cattle owners.

A. Oral feeding of mineral mixture (conventional therapy): -

Oral feeding of mineral mixture as feed supplement was tried by some workers in repeat breeder cows to improve fertility.

In present findings, very encouraging results (50% conception) were obtained in treatment of repeat breeder cows by feeding mineral mixture, which seems possible because of improved nutrition and farmer consciousness. Similar result was obtained by Ranjan (1991) after feeding of 5 gm iodized salt for one month and by Kumar (2000) after feeding of Supplevite-M and both found 50%

conception rate in repeat breeding cows. Some better results were obtained after feeding of organic iodine compound orally for 8 to 12 days by Mc Donald *et al.* (1961) and Triple sulphate mixture ($CuSO_4$ + $CoSO_4$ + $FeSO_4$) orally for ten days by Ranjan *et al.* (1991) and found 58% and 66.66% conception rate, respectively.

However, the study of treatment of repeat breeder cow on scientific manner is very much important for poor Indian farmers because a cheap sources of feed supplements are available everywhere.

B. Gonadotropin releasing hormone (GnRH) treatment: -

In this treatment group, five cows were pregnant out of ten. The conception rate for the treatment group was 50%. In repeat breeding control group, 2 cows were pregnant out of ten cows. The conception rate in control group was 20%. Thus, the conception rate in treatment group was boosted by 30% than control group. This result was resembling with the findings of Stevenson *et al.* (1988), Rao (1991) and Senthil Kumar and Rajasekar (1998) who reported 54%, 54.2% and 53.35% conception, respectively, in GnRH treated repeat breeder cows. Shelar *et al.* (2002) reported 20% higher conception in repeat breeder than control group. The conception rates higher than the present result were reported by Lee (1983), Majumdar (1989), Roy *et al.* (1995), Sonwane *et al.* (2001) and Rangnekar *et al.* (2002) who reported conception rate of 72.97%, 60%, 73.6%, 65% and 70%, respectively.

Synchronization of time of ovulation with estrus, development of more viable corpus luteum and enhanced progesterone production that improve the rate of embryonic survival may result in improved conception rate. The improved pregnancy rate with GnRH treatment followed by A.I. was associated with increased progesterone concentration following ovulation. Treatment with GnRH also increases the proportion of large luteal cells in developing corpus luteum (Mee *et al.*, 1993). The mechanism of GnRH work will remain be unresolved because there are multiple causes of repeat breeding. This type of treatment is extremely used in field condition during last few years and the cost involved is moderate. The scientific research on this line will help the cattle owners to get maximum benefits.

C. Mineral mixture and Gonadotropin releasing hormone (GnRH) treatment: -

In this group, six were pregnant out of ten cows taken in treatment group of mineral mixture and gonadotropin releasing hormone. The result showed the highest conception (60%) among treatment group of T_1 to T_3 (Table 32). The report on the observation of mineral mixture and GnRH treatment was scanty. Blood glucose levels is known to affect the pituitary gland function (Arthur, 1975). Before treating with GnRH (Hormonal), it is essential to maintain the basic requirement of body system for maximum action of hormone. Moddie (1965) reported that calcium has found to sensitize the female

genitalia for the action of hormone. The highest conception rate observed with combination of mineral mixture and GnRH as compared to alone treatment (T_1 and T_2) is probably due to correction of imbalance in nutrition, if any by mineral mixture.

D. Antimicrobial treatments: -

In the present study, attempts were made to treat the repeat breeder cows with antimicrobials based on *in-vitro* sensitivity test on different bacteria isolated from repeater. Enrofloxacin (T_4 group) by i.m. route showed the highest efficacy as noted by conception in 8 cases (80%) out of 10 cases followed by i.m. administration of Benzathine Penicillin (T_5 group) 7 cases (70%) out of 10 cases. It is also correlated with the bacterial sensitivity as shown in Table 17. The overall conception rate by antimicrobial is noted to be 75%. The conception rate observed in the present study is in agreement with that of Dabas *et al.* (1995) and Chandrakar *et al* (2002) who reported conception rate of 79.31% and 75%, respectively. On the other hand, higher conception rate of 86.6%, 84.80% and 86.41% were reported by Sharma et al (1978), Maurya et al (1992) and Roy et al. (2002), respectively. Antimicrobial agents were used by intrauterine route and lower conception rate (51.94%) was recorded by Saini *et al.* (1989) who used *Strepto-penicillin* i.u. 24 h after insemination. Kumar (2002) used Enrofloxacin i.v. in endometritis cases and reported 81.25% conception which is also in agreement with that of the present finding.

The result of the present study may lead to the conclusion that the treatment of repeat breeder with Enrofloxacin and Benzathine Penicillin can be carried out by parentral route apart from its conventional i.u. route. The variations in results might be due to differences of doses schedule of treatment and quality of semen used.

❑❑❑❑❑

Chapter - 6

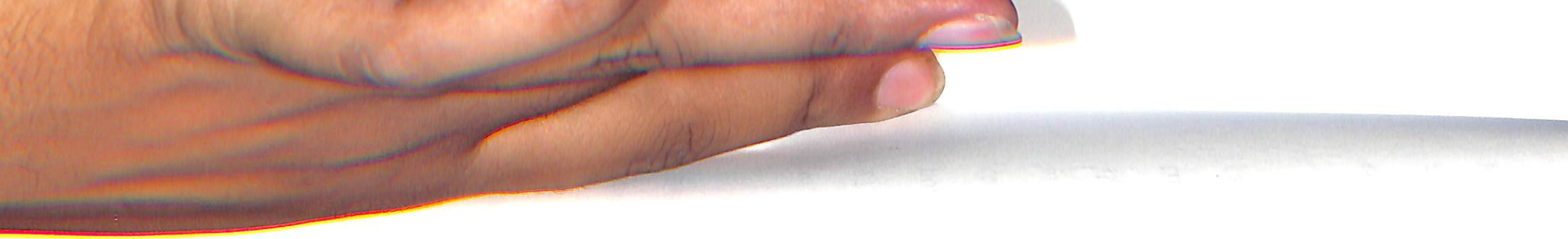
Summary

SUMMARY

The present study was undertaken to find out the prevalence of repeat breeding in 819 cows during the period of August 2002 to July 2003. Further, in the present study, bio-chemical profile, isolation & identification, antibiogram, distribution of antimicrobials (Enrofloxacin, Benzathine Penicillin) in serum and uterine fluid and treatment with minerals, hormones and antimicrobials in repeat breeding cows were carried out.

The prevalence of repeat breeding in cows was recorded to be 15.99 percent. The highest prevalence was found in January 27.78% and lowest in September 8.85%. Though statistically there is non significant influence of season in the incidence of repeat breeding cows. The maximum prevalence was observed in summer (20.00%) and minimum in autumn (12.50%). Sequence of calving also has non-significant effect on the prevalence of repeat breeding cows. However, the 2^{nd} calvers shows the maximum (27.59%) and 5^{th} and onward calvers the minimum (10.34%) prevalence.

Blood glucose levels in repeat breeder and normal cycling controls were found to be 52.71 ±0.11 to 52.96 ± 0.14 mg% and 62.25 ± 0.02 to 65.66 ± 0.09 mg%, respectively. On estrus day (Day 0), the blood glucose levels in repeat breeder and normal cycling control was



found to be 52.74 ± 0.07 and 65.66 ± 0.09 mg%, respectively. The blood glucose values were significantly higher in normal control as compared to repeat breeder cows.

The serum total protein levels in repeat breeders and normal cycling controls were found to be 5.63 ± 0.05 to 5.73 ± 0.03 g%, and 6.70 ± 0.06 to 6.91 ± 0.07 g%, respectively. On the day of estrus (Day 0), the serum total protein levels in repeat breeders and normal cycling control were found to be 5.63 ± 0.05 and 6.70 ± 0.06 g%, respectively. The serum total protein levels were significantly higher ($P < 0.01$) in normal cycling controls as compared to repeat breeder cows.

Serum calcium levels in repeat breeder and normal cycling controls were found to be 8.85 ± 0.06 to 8.95 ± 0.09 mg% and 8.93 ± 0.03 to 9.09 ± 0.05 mg%, respectively. On the day of estrus (Day 0), the serum calcium levels in repeat breeders and normal cycling control were found to be 8.85 ± 0.06 and 8.93 ± 0.03 mg %, respectively. However, serum concentrations of calcium did not differ significantly among various groups.

The serum inorganic phosphorus levels in repeat breeders and normal cycling control were found to be 4.31 ± 0.03 to 4.41 ± 0.03 mg% and 5.77 ± 0.06 to 5.95 ± 0.04 mg%, respectively. On estrus day (Day 0), serum inorganic phosphorus levels in repeat breeders and normal cycling control were found to be 4.31 ± 0.03 and 5.80 ±

0.04 mg%, respectively. In the present study, serum inorganic phosphorus was significantly higher in normal cyclic control as compared to repeat breeder cows.

The AKP values in repeat breeders and normal cycling control were found to be 7.85 ± 0.04 to 8.11 ± 0.06 KAU % to 6.56 ± 0.04 to 6.81 ± 0.08 KAU%, respectively. On the day of estrus (Day 0), serum alkaline phosphatase levels in repeat breeder and normal cycling control were found to be 7.85 ± 0.04 and 6.56 ± 0.04 KAU%, respectively. A significantly higher ($p < 0.01$) level of AKP was observed in repeat breeders than that of normal cycling cows.

A total of 131 repeat breeding cows were taken into consideration for isolation and identification of various microorganisms. Out of 131, 78 (59.84%) samples were positive for bacterial infection whereas 53 (40.45%) samples were bacteriologically sterile. Single type of organisms were isolated in 63 (80. 76%) whereas mixed isolates were present in 15 (19.23%) samples.

The overall frequencies of different isolates recovered from cervical mucus of repeat breeder cows were *Esch. coli* 20 (25.64%), *Staph. aureus* 14 (17.95%) *Strept. pyogenes* 12 (15.38%) *Coryn. pyogenes 6* (5.13%) and *K. pneuimoniae* 2 (2.56%).

The isolates were subjected to antibiogram studies against seven different antimicrobial agents. Wide variations of sensitivity pattern with different antimicrobials were obtained. The

percentages of sensitivity in descending order were 91.31, 80.64, 79.57, 69.74, 64.74, 32.25 and 25.80 for Amikacin, Enrofloxacin, Chloramphenicol, Ciprofloxacin, Gentamicin, Oxytetracycline and Penicillin, respectively.

The distribution studies of antimicrobial agents revealed that Enrofloxacin showed increase in uterine fluid levels at all time intervals (1 to 48 h) though significantly increased uterine fluid concentration at 2 and 4 h as compared to healthy cows. The therapeutic concentration ($\geq$ 0.12 μg/ml) was maintained from (2 to 36 h) and hence, the drug can be effectively used in repeat breeding cows. Similarly, the study on distribution of Benzathine Penicillin revealed significantly increased concentrations of the drug in uterine fluid from 1 to 36 h as compare to healthy cows. The drug was detectable in therapeutic concentration ($\geq$ 0.03 IU/ml) upto 96 h in repeat breeder cows. Thus, the drug can be used effectively in the treatment of bacterial sensitive organisms causing repeat breeding in cows.

The repeat breeder cows were treated with conventional (Mineral mixture), hormonal (GnRH) and antimicrobials (Enrofloxacin & Benzathine Penicillin) drugs. The overall breeding efficiency in different groups of repeat breeder cows were found to be higher than the untreated controls. Among treatment groups, conception rate was maximum 80% in T_4 (Enrofloxacin) and 70% in

T_5 (Benzathine Penicillin) group. Minimum conception (50%) was recorded in T_1 (Mineral mixture) and T_2 (GnRH) group. The highest conception rate obtained with antimicrobials may be due to presence of bacterial infections in uterus, one of the major causes of repeat breeding in cows as reported by various workers. The highest conception obtained among (T_1 to T_3 treatment group) in combination of mineral mixture and GnRH may be due to the nutritional deficiency along with the hormonal imbalance of repeat breeding cows.

Based on the above findings the following salient features were noted below:

(i) Calcium, phosphorus, total protein, glucose and alkaline phosphatase have definite role in reproduction. Hence, their levels in feed and fodder used in breeding practice needs to be maintained for effective breeding.

(ii) The reproductive efficiency could be achieved by application of antibiogram of cervical mucus (uterine fluid). For better results, the culture and sensitivity test should be carried out essentially before treatment of repeat breeder cows with uterine infection.

(iii) The cows suffering from repeat breeding syndrome should not be neglected until 5^{th} and onwards partum as it leads to poor conception and drop in milk yield.

(iv) Causes of repeat breeding are possibly microbial infections, nutritional deficiency and hormonal imbalance or combination of the above. Hormonal and non-hormonal and antimicrobial therapy should be used in field practices to increase pregnancy as per need.

(v) Close observations of repeat breeding are necessary to avoid managerial defects.

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Appendix

APPENDIX

Ingredients of

I. **Nutrient Broth :**

Beef extract	-	3 gm
Peptone	-	5 gm
Potassium nitrate	-	1 gm
Distilled water	-	1 lit.
pH	-	6.8 ± 0.2

II. **Nutrient Agar**

Beef extract	-	1.5 gm
Peptone	-	5 gm
Sodium chloride	-	5 gm
Yeast extract	-	1.5 gm
Agar	-	15 gm
Distilled water	-	1 lit.
pH	-	7.0 ± 0.2

III. Mac. Conkey's Agar

Peptone	-	17 gm
Proteose	-	3 gm
Lactose	-	10 gm
Sodium Chloride	-	5 gm
Bile Salt	-	1.5 gm
Agar	-	15 gm
Distilled water	-	1 lit.
pH	-	7.1 ± 0.2

IV. Blood Agar

10% sterile sheep blood added to sterile nutrient agar at 50^0C

V. Antibiotic assays media for enrofloxacin and penicllin

Peptone	-	6.0 gm
Tryptone	-	4.0 gm
Yeast extract	-	3.0 gm
Beef extract	-	1.5 gm
Dextrose	-	1.0 gm
Agar	-	15 gm
Distilled water	-	1 lit.
pH	-	7.9 ± 0.1

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