TREATMENT OF ENDOMETRITIS FOR IMPROVING FERTILITY IN DAIRY COWS

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
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Department of Animal Reproduction
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
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DECLARATION

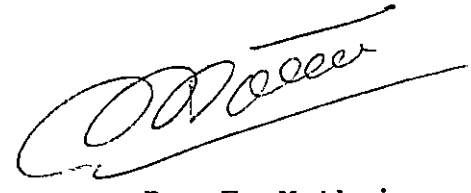
I hereby declare that this thesis entitled "TREATMENT OF ENDOMETRITIS FOR IMPROVING FERTILITY IN DAIRY COWS" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship or other similar title, of any other University or Society.

Mannuthy,

A. M. VAHIDA

CERTIFICATE

I hereby declare that this thesis entitled **"TREATMENT OF ENDOMETRITIS FOR IMPROVING FERTILITY IN DAIRY COWS"** is a record of research work done independently by **Dr.A.M. VAHIDA** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to her.



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A.M. VAHIDA

Dedicated to
my loving parents

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Introduction

INTRODUCTION

Infertility in cattle has been recognised as a global problem and the magnitude of loss sustained on account of this has been illustrated from different countries. Infertility has been and continues to be the major cause for disposal of cows in different parts of the world. However precise information on the magnitude of economic loss on account of infertility in cattle in India is not available, although, there are ample reasons to believe that majority of cows, that reach slaughter houses are disposed off due to reasons of various infertility conditions. Informations on various infertility conditions and their magnitude of prevalence are necessary to exploit the full production potential of cross-bred cows in India through adoption of better therapeutic measures and practices.

To be successful in dairy farming and to retain economic production, it is necessary to minimize losses from infertility of cows. Uterine affections inflict greater loss on dairy stock owners than do the affections of all other organs. Increased calving interval, longer intervals between first oestrus and

first service after calving and decreased conception at first service are some of the factors associated with infections of reproductive tract. Albrechtsen (1917) for the first time emphasized that the chief cause of infertility in cows was endometritis produced by bacterial infections. Among various etiological factors for repeat breeding in cows, Namboodiripad et al. (1976) reported that the infections of the uterus with non-specific organisms constituted 63.14 per cent.

A number of microbial agents have been incriminated as etiological agents for endometritis. Apart from the specific organisms, non-specific organisms constituted of Staphylococci, Streptococci, Escherichia coli, Corynebacterium pyogenes, Pseudomonas and Micrococci (Dawson, 1960).

Non-specific organisms cause infertility either by actual invasion of the tissues or by the production of metabolic byproducts, which are irritating and responsible for local tissue reaction preventing pregnancy (Hardenbrook, 1958).

Application of the methods proposed by Pasteur and Koch for curing of the genital diseases of cattle made the rational diagnosis and treatment of certain reproductive failures possible (Hardenbrook, 1958). The

success in the treatment of uterine infections depends upon susceptibility of the causative agent to the drug. When infection is caused by microorganisms belonging to a non-specific group, application of appropriate susceptibility tests is a must to determine the most effective, cheapest and safest therapeutic agent. Antibiotic sensitivity tests have been established as an important part of the clinical bacteriological routine. Standard disc diffusion assay is the simple and easy method to carry out sensitivity tests (Cowan, 1974).

For efficient antimicrobial treatment, an effective concentration of drug must be achieved and maintained at the site of infection for an adequate period. For this, local and systemic routes of administration are adopted in case of uterine infections. By systemic administration antibiotic concentration in uterine tissue and lumen becomes equal to that of blood plasma concentration. Antibiotic alone or in various combinations have been used by intrauterine infusion for the destruction of microorganisms present in the uterus (Gustafsson, 1984).

The present work was taken up to investigate on the incidence of uterine infections, causative organisms of endometritis and to find out suitable antimicrobial agents to counteract the infection.

Review of Literature

REVIEW OF LITERATURE

Infections of the bovine genital tract adversely affect fertility by altering its environment, thereby resulting in impaired transport and death of sperms, defective development of the conceptus, death of embryo or fetus, stillbirth or birth of weak calves (Arthur, 1989). It has been established that infection of the reproductive organs with non-specific organisms constituted the most important cause of impaired fertility in cattle (Azizuddin, 1954).

2.1 Incidence of endometritis

Hardenbrook (1958) reported that 80 per cent of breeding failures in bovines were due to non specific infections. In cyclic non-breeders Namboodiripad et al. (1976) reported an incidence of 63.14 per cent genital infections due to non-specific organisms. Rao and Kotayya (1976) reported an incidence of 30.77 per cent endometritis in infertile cows in Andhra Pradesh. In Southern Israel, endometritis was reported to an extend of 16 per cent, among 2745 cows during the period from

1965 to 1973, while in Northern Israel endometritis occured in 14 per cent among 281 cows from 1977 to 1979 (Francos, 1979).

In Karnataka Rao et al. (1983) reported an incidence of 32.86 per cent of endometritis. While in Kerala, Varadarajan (1985) observed an incidence of 9.66 per cent in the cows presented for Artificial Insemination, at the Artificial Insemination Centre attached to the Department of Animal Reproduction, College of Veterinary and Animal Sciences, Mannuthy.

In a study conducted in Brazil by Ferreira et al. (1987) in 50 herds showed that the incidence of uterine infections was 21.6 per cent. Field observation on the puerperal period in three herds showed an average incidence of metritis as 39.1 per cent (Zezula-Szpysa et al., 1988).

Iyer et al. (1992) reported an incidence of 20 per cent endometritis in cross-bred cows of Kerala.

2.2 Isolation and identification of bacterial organisms

Albrechtsen (1917) for the first time emphasised that the chief cause of infertility in cows was

endometritis which resulted from bacterial infections.

Though it is generally recognised that endometritis caused by bacteria is the most important etiological factor for sterility, very little information is available on the bacteria responsible (Azizuddin, 1954).

There are a number of non-specific bacterial organisms that are common in the reproductive tract of infertile cattle. These organisms included Staphylococci,Streptococci, Proteus, Escherichia coli and Corynebacteria (Hardenbrook, 1958). A high percentage of of cows that had normal heat periods and appear normal, but fail to reproduce because of uterine infections. Metritis was most commonly caused by Staphylococci, Streptococci, Corynebacteria and E. coli which are found in the normal surroundings of dairy herds (Hinze, 1959).

Staphylococci, especially haemolytic forms appeared to be the main pathogens in first and second degree cases of endometritis. Streptococci, haemolytic strains of E. coli, Corynebacterium pyogenes also play an important part in the third degree cases (Dawson, 1960). Van Waveren (1962) conducted a bacteriological study of mucopurulent vaginal discharge in 426 cows and found

C. pyogenes to be major pathogen. He also isolated other Corynebacteria spp., E. coli, haemolytic and non-haemolytic Streptococci, Staphylococci, Brucella abortus, Salmonella dublin, Proteus, Pseudomonas, Shigella and fungi. Savov and Dimitrov (1973) investigated on the bacterial flora of reproductive tract and isolated 24 species of pathogenic bacteria. Venkateswarlu et al. (1983a) observed that cultures from the uterine discharges of 180 cows and buffaloes which had second degree endometritis contained Staphylococci (15.00 per cent) Pseudomonas (13.80 per cent), Streptococci (1.60 per cent), E.coli (11.60 per cent), Corynebacteria (10.00 per cent), Bacillus Group (8.30 per cent), Proteus (5.50 per cent) Micrococcus (0.55 per cent), Klebsiella (0.55 per cent) and mixed infections (22.70 per cent).

In a study of bacterial isolates from uterine discharge in clinical endometritis Varadarajan (1985) could isolate Haemophilus (8 per cent), Enterobacteria (24 per cent) Pseudomonas (12 per cent), Staphylococci (20 per cent), Bacillus (4 per cent), Pasteurella (8 per cent), Actinobacillus (4 per cent) Necromonas (12 per cent), Lactobacillus (4 per cent) and Streptobacillus (4 per cent).

David (1986) in 421 dairy cows with endometritis observed that the common organisms involved were Streptococci and Actinomyces. Other organisms included were E. coli, Pseduomonas, Pasteurella, Salmonella, Acinetobacter, Moraxella and Absidia. Out of 370 mucus samples obtained from infertile cows, Malik et al. (1987) reported that the percentage of occurrence of Staphylococcus aureus, Streptococcus pyogenes, C. pyogenes Bacillus subtilis and E. coli were 22.98 per cent, 31.08 per cent, 3.24 per cent, 1.35 per cent and 2.43 per cent respectively.

Singh et al. (1989) described 178 isolates of bacteria obtained from cervical mucus swabs collected during oestrus periods of 86 cows, which included S.aureus (61), Haemolytic Streptococci (43), Corynebacteria (29), E. coli (18), Micrococci (19) and Pseudomonas (8). Mixed bacterial infections were present in 72 (83.7 per cent) cases while only 14 (16.3 per cent) showed single bacterial infection. It was further showed that common pyogenic bacteria S. aureus dominated in mixed bacterial infections.

Sirohi et al. (1989) during an investigation of the uterine samples in 120 animals, found that the common bacterial isolates were S. epidermidis, S. aureus,

Streptococci, C. bovis, C. pyogenes, E. coli, Klebsiella pneumoniae, K. aerogenes, Enterobacter spp., Pseudomonas aeruginosa, Proteus vulgaris, P. mirabilis, Citrobacter and some unidentified Coccobacillary rods.

2.3 Instrument for aseptic collection of Uterine discharge

An instrument developed by Minocha et al. (1964) for obtaining asceptic bacteriological and histological samples from bovine genital tract consisted of four telescoping metal tubes, the outer one served as the vaginal Speculum, the second tube for insertion upto the middle of cervix and the two inner units constituted the sampler. Sterile gelatine capsules protected the tips of tubes from contamination. However, the capsule used for protection was detained in the uterus and subsequently absorbed. The collection of samples for bacteriological examination using the instrument was satisfactory and there was no incidence of transfer of infection from vagina to uterus.

Ghosh et al. (1980) designed uterine biopsy equipment which was a partial modification of the model by Minocha et al. (1964). It consisted of four telescoping

units, the outer tube served as a vaginal speculum. The second tube during introduction was inserted to the middle of the cervix. The third tube had a window one cm short of its tip at the cranial end,the fourth tube acted as a cutter. The tip of the fourth unit was made sharp by a saw so that the endometrium could be cut easily.

2.4 Sensitivity of bacterial organisms and treatment

Several drugs are being used to treat endometritis. The use of different antibiotic preparations at therapeutic and subtherapeutic levels to counteract the infections in the uterus were practiced. Most of the antibiotics were rapidly absorbed from the uterine lumen and distributed to body fluids and tissues, hence they should always be infused at the recommended therapeutic level (Arthur, 1989).

Sulphonamides infused into the uterus diffused within 48 hours. Therapeutic concentrations were maintained for more than 12 hours in the uterus with peak value reached in two hours. Effective blood concentrations were maintained after intrauterine administration as that of intravenous administration (Bierschwal et al., 1955). With the objective of freeing the uterus of bacteria, sulphadiazine suspension or Sulphamerazine solution could be used with good clinical

results either alone or in various combination with antibiotics (Hardenbrook, 1958).

Koleff et al.(1973) conducted sensitivity tests of mixed bacterial flora from the uterine contents of 100 cows with endometritis and showed that Ampicillin was the most effective antibiotic followed by Chloramphenicol and Tetracycline. They also opined that selection of a drug from the results of sensitivity test meant that fewer treatments were required and recovery was more rapid than after emperical therapy.

From the antibiogram of the isolates and the subsequent treatment practiced, Savov and Dimitrov (1973) showed that the best drugs for treatment in the order of preference were Chloramphenicol, Tetracycline, Oxytetracycline and Erythromycin. In repeat breeders, antibacterial treatment near the time of oestrus was reported to be more effective than the treatment during the luteal phase (Oxender and Seguin, 1976).

Sinha et al.(1977) reported that it would always be of great advantage if isolation and identification of organisms from the uterine discharge is done and their antibiotic sensitivity is determined. The recovery rate is rapid when specific antibiotic is used for specific type of organism in vivo. They also studied the effect of

treatment with Chloramphenicol, Terramycin, Streptomycin, Ledermycin and Penicillin and proved that Chloramphenicol was the most effective drug to inhibit 89.30 per cent of isolates.

Among the nine different antibiotics tested in vitro for sensitivity against organisms causing endometritis, none of the antibiotics was found fully sensitive against the organisms (Murty and Rao, 1979). Genital microflora of repeat breeders are highly resistant to most of the antimicrobial agents compared to the microflora of normal breeders. However, it is found that all isolates from both the groups were sensitive to Gentamicin and Kanamycin (Kharade and Kulharni, 1983).

Sensitivity of the bacterial isolates from infertile animals which had second degree endometritis was 26.1 per cent, 24.4 per cent, and 17.2 per cent respectively to Chloramphenicol, Gentamicin and Furacin (Venkateswarlu et al., 1983a).

Venkateswarlu et al. (1983b) in a study of 180 cows and buffaloes having second degree endometritis used six antibiotics after sensitivity tests on uterine discharges. Based on sensitivity pattern the drug of choice was infused into uterus with 15 ml distilled water three to five times on alternate days depending on

intensity of infection. Dose rate was Gentamicin 160 mg, Chloramphenicol 1 g and Furacin 5 g. Artificial Insemination was done in the subsequent heat when the uterine discharge was normal. The sensitivity of bacteria was found to be the highest for Chloramphenicol (21.10 per cent) and lowest for Tetracycline (8.30 per cent). The overall conception rate after treatment of endometritis was 71.93 per cent. The highest percentage of conception (88.00 per cent) was obtained in animals treated with Furacin. The other drugs in order of decreasing conception rate were Tetracycline, Chloramphenicol, Streptomycin, Ampicillin and Gentamicin. No significant difference in conception rate due to treatment with various drugs was observed.

Bacterial isolates from uterine discharge of 10 cows and seven buffaloes having endometritis were identified and sensitivity tests conducted by Dabas and Joshi (1984). The oestrous secretion of these animals were examined in the next heat for bacterial contamination, and then inseminated. Nine cows and five buffaloes conceived in the first heat itself. One buffaloe and one cow conceived in the subsequent heat.

Uterine discharges from 170 cross-bred cows and heifers with clinical evidence of endometritis were

cultured and antibiogram of the isolates to Gentamicin, Septran, Ampicillin, Terramycin and Chloramphenicol determined. Based on the sensitivity of isolates to Gentamicin 86 animals were given 250 mg of Gentamicin in 25 ml distilled water intrauterine, and 84 animals were administered Gentamicin (250 mg) stilboestrol (10 mg) combination in 25 ml distilled water. Animals showing clinical recovery with single infusion were inseminated in the succeeding heat. In the rest of the animals, a second intra uterine infusion was given and inseminated after clinical recovery. The percentage of conception with single insemination in Gentamicin treated and Gentamicin-stilboestrol treated were 56.45 and 40.9 respectively where as overall conception rates in the respective groups were 72.55 per cent and 63.63 per cent (Varadarajan,1985).

Sudhakar et al. (1986) conducted a study on bacterial cultures obtained from uterine discharges of 59 cows and 47 heifers with endometritis. The animals were inseminated after two normal oestrous periods. Sixty seven of the 106 animals (63 per cent) conceived. Isolates from 87 samples were highly or moderately sensitive to Gentamicin. Twenty five animals were treated with Gentamicin and 19 (76 per cent) animals conceived.

In an experiment conducted to determine the

disposition of Sulphamethoxazole (SMZ) and Trimethoprim (TMP) following intra uterine administration in experimentally induced metritis in buffaloes, both the drugs were absorbed quickly from the uterus and excreted quite rapidly from the blood circulation. Uterine tissue levels of drugs were found to be much higher than the corresponding plasma levels. Drugs were also detectable for a longer duration in uterine tissue (12-24 hours) than that in plasma (2-12 hours). Their effective concentration in uterine tissue was observed for 12 hours at both lower (0.4 g TMP + 2 g SMZ) and higher (0.8 g TMP + 4 g SMZ) dose levels, thereby indicating that the drug combination should be repeated at 12 hour intervals, irrespective of dose, for intra uterine therapy of genital tract infections (Chaudhary et al., 1987).

After culture and sensitivity tests of 520 samples of cervical discharges from repeat breeder cows, Dholakia et al. (1987) reported that Gentamicin was most effective (66.60 per cent) followed by Chloramphenicol (55.70 per cent).

Malik et al. (1987) studied the antibiotic sensitivity of the 397 mucus samples from the genital tract of infertile cattle and observed that highest sensitivity was for Gentamicin (93.67 per cent) followed

by Streptomycin (89.90 per cent), Kanamycin (88.61 per cent) and Chloramphenicol (87.33 per cent). When a study was conducted on 120 animals with history of reproductive disorders like endometritis, repeat breeders, metritis, cervicitis and prolapse of Vagina, Sirohi et al. (1989) reported that the highest number of bacterial isolates were sensitive to Gentamicin (88.80 per cent) and least to penicillin (17.46 per cent). In vitro antibiotic sensitivity tests of the isolates obtained from cervico-vaginal mucus samples from 50 repeat breeding cows and buffaloes revealed that Gentamicin was the most effective antibiotic followed by Penicillin, Chloromphenicol, Oxytetracycline, Triple Sulpha, Streptomycin, Nitrofurantoin and Ampicillin in the descending order. More than 40 per cent of the isolates showed multiple resistance to four or more drugs (Sharda et al., 1991).

Two hundred and fifty two swabs cultured in peptone water and incubated for 5 to 6 hours at 37°C were subjected to direct antibiotic sensitivity test by disc diffusion method. The antibiotic discs used were Nitrofurantoin, Oxytetracycline, Penicillin G, Ampicillin, Co-trimoxazole, Streptomycin and Chloramphenicol. Chloramphenicol was the drug of choice with 41.27 per cent sensitivity followed by Streptomycin 33.73 per cent. (Venkateswaran and Rajeswar, 1991).

Materials and Methods

MATERIALS AND METHODS

Cows and heifers brought to the Artificial Insemination Centre attached to the Department of Animal reproduction, and animals maintained at University Livestock Farm, College of Veterinary and Animal Sciences, Mannuthy during the period from Ist April, 1991 to 31st July, 1992 formed the material for the study. The animals were subjected to detailed clinico-gynaecological examinations, and those found to have clinical endometritis were selected for the present trial. Data on incidence of endometritis and conception rate were collected from the registers maintained for the same period at the Artificial Insemination Centre.

3.1 Collection of material

Aseptic collection of uterine discharge was carried out by using an instrument newly fabricated with modification of the design proposed by Minocha et al. (1964). The instrument used consisted of three telescoping metal tubes, the outer one was having a length of 30.5 cm, with outer diameter of 0.7 cm and thickness of 0.1 cm. This was having a circular base of 4 cm diameter

and 1 cm thickness, for holding the instrument conveniently at the time of collection of sample . The second tube (middle tube) had a length of 50.5 cm, with outer diameter of 0.5 cm, and thickness of 0.1 cm. The inner tube (sampler) was 58.5 cm long with an outer diameter of 0.3 cm and thickness of 0.1 cm. Sampler was having two holes, each of 0.5 cm in length and 0.2 cm in width and situated one at 0.4 cm and the other at 0.8 cm away from the tip, on opposite sides, and intended for aspirating the discharge from the uterus. The tip of the sampler was blunt and extending 0.1 cm outwards on all sides, so that this extension covered the tip of the middle tube to prevent entry of contamination during introduction. A stiletto (70 cm length and 0.20 cm diameter) was provided inside the inner tube (Fig.3.1, 3.2 and 3.3).

The instrument was cleaned well before use and dried in air drier. The outer tube, the inner assembly containing the second tube, and the sampler with the stiletto were wrapped separately in wrapping paper and sterilized at 160°C for one hour in hot air oven.

After cleaning the external genitalia of the selected animals and separating the vulval lips, the sterilized outer tube was introduced into the vagina, upto the external os and held in position. Then the second and

sampler with stiletto were introduced through the outer tube, until the tip of it reached the site of collection in the uterus. The stiletto was removed and the sampler alone was pushed 1.3 cm further forwards so that the two openings on either sides were exposed. The discharge from uterus was aspirated by applying negative pressure through an adaptor and syringe attached to the outside end of the sampler.

The sampler was then drawn back so that the openings, on it were closed, and the sampler with middle tube was taken out through the outer tube. The outer tube was also removed gently. The sample collected was transferred into a test tube containing peptone water (Cowan, 1974) through the holes exposed by drawing back the second tube. A portion of the sample was transferred to a sterile vial for isolation of the organisms. It was then incubated at 37°C for 5 to 7 hours. Before incubation the nature of discharge was grouped as purulent, cloudy, with flakes of pus and clear on the basis of appearance.

3.2 Isolation and identification of bacterial organisms

The portion of the sample was streaked on

Mueller-Hinton agar* / Tryptic Soy agar** by streak plate method in order to get well isolated colonies of bacteria present in the culture and incubated at 37°C for 18 to 24 hours. One half of a well isolated single colony was subjected to gram staining and other half was subcultured on Mueller-Hinton agar / Tryptic Soy agar for further purification of organism. Isolation and identification of organisms were attempted with 30 samples.

Identification of the isolates was done on the basis of morphology and staining reaction, oxidation-fermentation of glucose, growth on Mac-Conkey's media, catalase test, oxidase test, motility, haemolysis on blood agar, coagulase test, pigment production and further by a battery of biochemical tests (Cowan, 1974).

3.3. Antibiotic sensitivity test

3.3.1 Preparation of culture medium

The reconstituted Mueller-Hinton agar (38 grams of Mueller-Hinton agar in 1000 ml distilled water) was

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* Mueller-Hinton agar, Hi-Media Laboratory Pvt. Ltd., Bombay-400686 India.

** Tryptic Soy agar Span Biologicals, 174, New Industrial Estate, Udhina, Surat, India.

sterilized by autoclaving at 121°C under 15 lbs pressure for 15 minutes. It was then cooled to 45 to 50°C and poured into sterile glass petri dishes to a depth of 4 mm and kept in the incubator at 37°C for 18 hours for testing sterility.

3.3.2 Inoculation of plates

Adjusted the turbidity of actively growing broth culture with sterile saline / peptone water so as to obtain a turbidity usually comparable to half the density of a No.1 Mac Farland Standard. Within 15 minutes of adjusting the turbidity of inoculum a sterile cotton swab was dipped into the standardised suspension and excess of inoculum was removed from the swab by rotating the swab several times with a firm pressure on the inside wall of the test tube above the fluid level and inoculated the surface of a sterile Mueller-Hinton agar plate by streaking the swab over the entire surface. Repeated the streaking procedure two or three times by rotating the plate approximately 60° each time so as to ensure an even distribution of the inoculum (Barry, 1976).

3.3.3 Application of antibiotic discs

The antibiotic discs used were shown in Table 3.1.

Table 3.1 ZONE SIZE INTERPRETATIVE CHART

No.	Discs used	Strength (micro-gram)	Sensi-tive (mm or more)	Inter-mediate (mm)	Resis-tant (mm or less)
1.	Ampicillin	10	17	14-16	13
2.	Chloramphenicol	30	18	13-17	12
3.	Co-trimoxazole	25	16	11-15	10
4.	Furazolidone	100	17	15-16	14
5.	Gentamicin	10	15	13-14	12
6.	Oxytetracycline	30	19	15-18	14

Using sterile forceps, antibiotic discs were applied over the surface of the plate and pressed gently to ensure even contact with the medium. To avoid overlapping of the zones of inhibition, the discs were kept at a minimum of 15 mm away from each other and also from the edges of the plate.

3.3.4 Incubation

The plates after inoculation were incubated at 37°C for 18 hours.

3.3.5 Reading of the zones of inhibition

The diameter of zone of inhibition was measured by a millimetre ruler to the nearest millimetre and each zone was interpreted as sensitive, intermediate or resistant on the basis of zone interpretative chart.

3.4 Treatment groups

All the selected animals were divided into three groups based on the sensitivity tests.

Animals from which the discharge collected showed sensitivity to Gentamicin were divided at random into Group I A and Group II A.

Similarly animals from which the discharge collected showed sensitivity to Chloramphenicol were divided at random into Group I B and Group II B.

Animals in Group I A were given Gentamicin* 4 mg / kg body weight intramuscularly at 12 hour interval for three days.

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* Gentamicin (Inj) 30 ml (Alembic). Each ml contain as Gentamicin Sulfate I.P equivalent to 40 mg of Gentamicin base.

Animals in Group I B were given Chloramphenicol * 4mg/kg body weight intramuscularly at 12 hour interval for three days.

The animals in Group II A were treated by intrauterine administration of Gentamicin 400 mg dissolved in 30 ml distilled water at 12 hour interval for two days.

Animals in Group II B were treated with intrauterine administration of 1 gm Chloramphenicol** dissolved in 30 ml distilled water at 12 hour interval for two days. Animals from which the discharge collected showed sensitivity to Co-trimoxazole*** were included in Group III A and those showed sensitivity to Furazolidone**** in Group III B.

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* Enteromycetin - 8 ml injection of Chloramphenicol Each ml contains Chloramphenicol I.P 125 mg.

** Kemicetin 1 g (Mac Lab) powder form Chloramphenicol Sodium Succinate equivalent to 1 gm of Chloramphenicol.

*** Oriprim - 30 ml vial (Cadila). Each ml contains Sulphamethoxazole 400 mg, Trimethprim 80 mg.

**** Furacin Vet 30 ml (Eskayef) Nitrofurazone B.P. 0.2 per cent w/w in a water scluble base.

Animals in Group III A were treated with Co-trimoxazole 7.5 ml dissolved in 30 ml distilled water by intra-uterine route at 12 hour interval for two days.

Animals in Group III B were given intra-uterine administration of Furazolidone 30 ml at 12 hour interval for two days.

In the succeeding heat of the treated animals, uterine discharge was collected and culture and sensitivity tests were conducted to assess the success of the treatment. The animals which were found to be clinically normal were inseminated.

During the subsequent heat, those animals showing no improvement after the treatment were again treated based on the sensitivity of the discharge as described earlier.

Conception rate of animals in each treated group were assessed on the basis of pregnancy diagnosis at 90 days. The data were subjected to statistical analysis (Snedecor and Cochran, 1967).

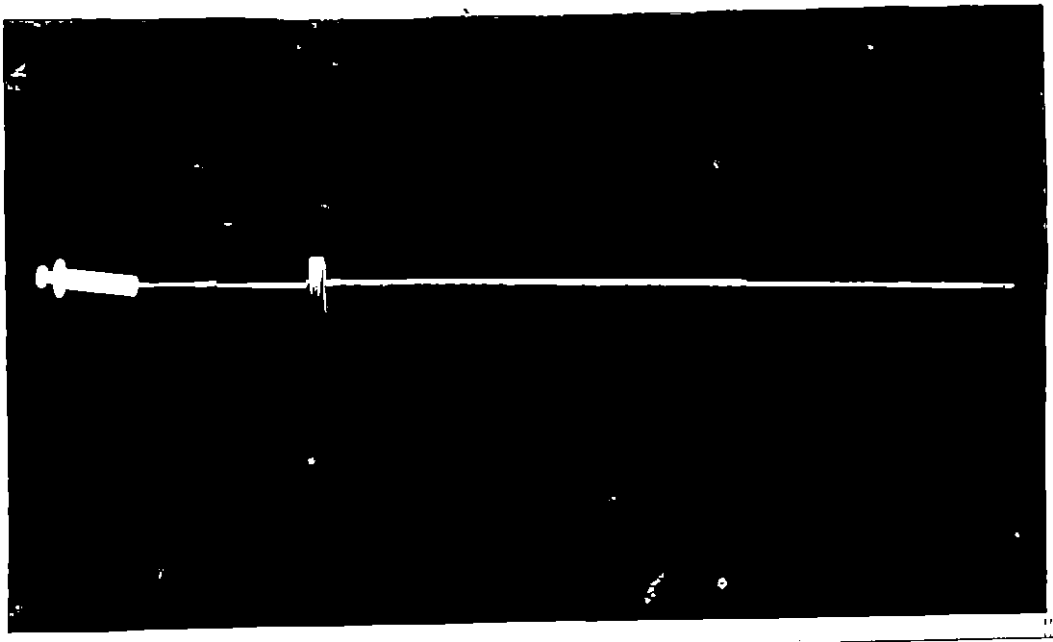


Fig. 3.1 INSTRUMENT FOR ASEPTIC COLLECTION OF UTERINE DISCHARGE

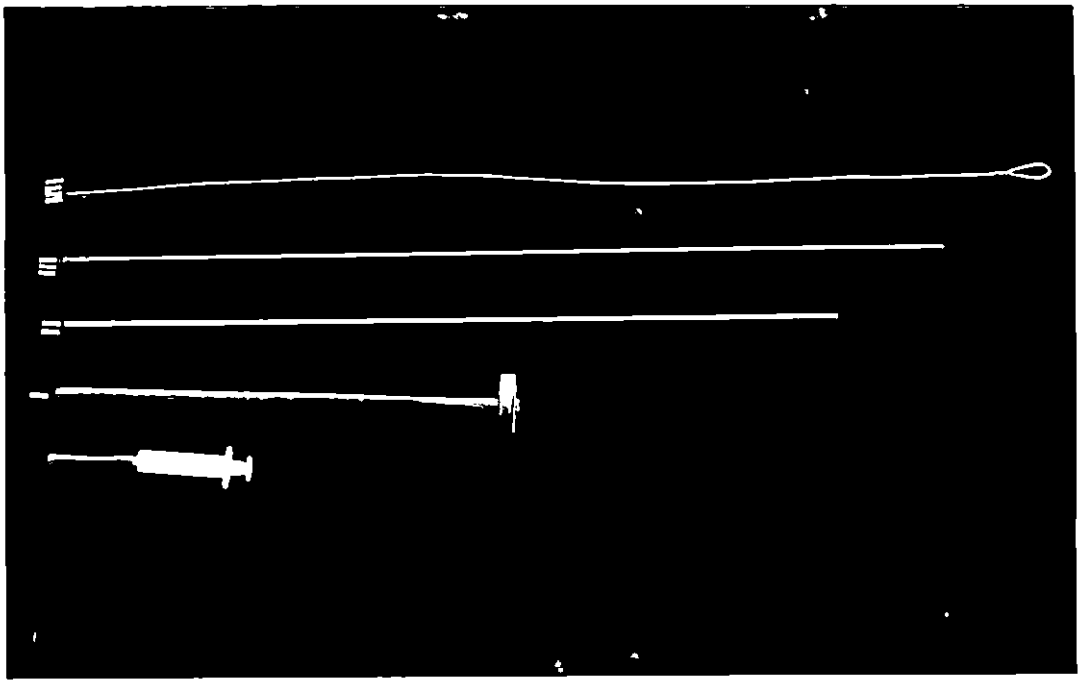
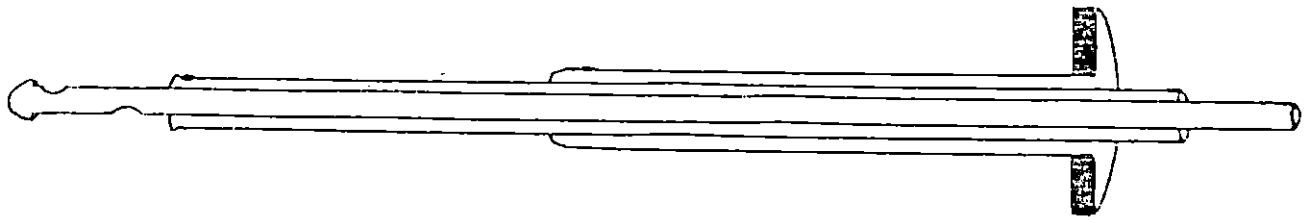


Fig. 3.2 PARTS OF THE INSTRUMENT FOR ASEPTIC COLLECTION OF UTERINE DISCHARGE

i. Outer tube ii. Middle tube
iii. Sampler tube iiii. Stylette
Syringe with adaptor

Fig. 3.3 DIAGRAMATIC REPRESENTATION OF LONGITUDINAL SECTION OF THE INSTRUMENT FOR ASEPTIC COLLECTION OF UTERINE DISCHARGE



Results

RESULTS

In the present study, the incidence of endometritis during the period from April, 1991 to July 1992 was noted in the animals brought to the Artificial Insemination Centre attached to the Department of Animal Reproduction, College of Veterinary and Animal Sciences, Mannuthy,Thrissur. Endometritis cases were identified on the basis of gynaecoclinical examinations and on the basis of *in vitro* sensitivity tests, treatment was attempted using Gentamicin, Chloramphenicol, Co-trimoxazole and Furazolidone. Identification of bacterial organisms was also carried out.

4.1 Incidence of endometritis

During the period of study, 6110 animals were brought to the Artificial Insemination Centre, of which 2541 were subjected to treatment for different infertility conditions including 202 (7.95 per cent) cases of clinical endometritis (Table 1).

4.2 Collection of uterine discharge

An equipment for aseptic collection of uterine

discharge was designed and fabricated after modification of the basic design proposed by Minocha et al. (1964).

4.3 Nature of uterine discharge

Uterine discharge was collected from a total of 127 cows with clinical endometritis. The nature of discharge before treatment is given in Table 2 and Graph 1. The incidence of purulent, cloudy and discharges with flakes of pus were seen in 16 (12.60 per cent), 31 (24.40 per cent) and 80 (63.00 per cent) cows respectively.

After the treatment of 127 cows with specific drug selected based on in vitro sensitivity tests,121 cases were followed up, of which 111 (91.75 per cent) animals showed clear uterine discharge, 4 (3.15 per cent) purulent discharge, 2 (1.57 per cent) cloudy discharge and 4 (3.15 per cent) with flakes of pus in the discharge (Table 3 and Graph 2).

4.4 Identification of bacterial organisms

Uterine discharges from 30 animals were subjected to isolation and identification of bacteria and the results were given in Table 4. Only single type of bacterial organisms was recovered from each sample. The organisms were coagulase negative Staphylococcus spp.

(40.00 per cent), Staphylococcus aureus (30.00 per cent), Corynebacterium spp. (16.66 per cent), Bacillus spp.(6.67 per cent) and Pseudomonas spp. (6.67 per cent).

Among the isolates of coagulase negative Staphylococcus spp. 83.40 per cent were sensitive to Gentamicin and 58.30 per cent to Chloramphenicol and Furazolidone and 91.66 per cent resistant to Co-trimoxazole. Percentage of sensitivity shown by Staphylococcus aureus was 33.30 to Gentamicin and they were not sensitive to Chloramphenicol, Co-trimoxazole and Furazolidone. Corynebacterium spp. showed cent per cent sensitivity to Chloramphenicol and Furazolidone and 80.00 per cent sensitivity to Gentamicin and 60.00 per cent to Co-trimoxazole (Table 5 and 6). Isolates of Bacillus were cent per cent sensitive to Gentamicin, Chloramphenicol and Furazolidone. They were not sensitive to Co-trimoxazole. Isolates of Pseudomonas were fully resistant to all the four drugs.

4.5 Sensitivity tests

It could be seen from Table 7 and Graph 3 that out of 127 samples 78 (61.41 per cent) were sensitive to Gentamicin, 75 (59.05 per cent) to Chloramphenicol, 59 (46.45 per cent) to Furazolidone and 30 (23.62 per cent)

to Co-trimoxazole. Intermediate sensitivity to Co-trimoxazole was seen in isolates from 12 (9.45 per cent) samples (Fig. 4.1, 4.2, 4.3, and 4.4)

4.6 Conception

Table 8 presents the number of animals conceived at first, second and third inseminations after treatment. Out of the total 127 animals treated, 98 animals were followed up after insemination, of which 73 were found pregnant. After first, second and third inseminations sixty three, eight and two animals respectively were found pregnant.

Conception rate after treatment with various drugs are listed in Table 9. Gentamicin was given intramuscularly to 20 animals with endometritis, of which 16 animals recovered and were inseminated, from which only 15 animals could be followed up and the percentage of conception was 66.60 per cent. Similarly intrauterine administration of Gentamicin was done in 20 animals and 19 animals recovered and were inseminated, of which 16 animals were followed up after insemination and 75.00 per cent conception wer recorded.

Chloramphenicol was given intramuscularly to 24 animals, of which 23 recovered and were inseminated. Follow up was carried out in 22 inseminated animals and

72.73 per cent conception rate was noted. Similarly intrauterine administration of Chloramphenicol was given in 20 animals and 17 animals recovered and were inseminated, of which 16 animals were followed up and conception rate was 75.00 per cent.

Co-trimoxazole was given as intrauterine infusion in 22 animals and 16 animals recovered and were inseminated. All the animals conceived in this group. In the Furazolidone group, 20 animals out of 21 recovered and were inseminated of which 17 animals were followed up. The conception rate observed was 64. 71 per cent.

The data on conception were subjected to statistical analysis (Table 10). The values of 'T' test showed no significant difference in conception rates among any of the groups except co-trimoxazole treated group (Group III) which showed significant difference in conception rate with all other groups.

Comparative effect of intramuscular and intrauterine routes of administration is shown in Table 11. On analysis the data revealed no significant difference in the percentage of recovery and conception rate.

Table 1. Incidence of endometritis (1991-'92)

S.No.	Months	Animals brought in A.I. Centre	Number of cases for treatment	Number of cases of endometritis	Incidence of endometritis (per cent)
1.	April	387	165	10	6.06
2.	May	373	146	7	4.79
3.	June	360	156	12	7.69
4.	July	407	159	15	9.43
5.	August	317	179	15	8.37
6.	September	368	185	12	6.49
7.	October	359	196	14	7.14
8.	November	305	219	12	5.48
9.	December	349	208	13	6.25
10.	January	317	174	12	6.9
11.	February	316	191	17	8.9
12.	March	317	195	12	6.15
13.	April	357	133	10	7.5
14.	May	452	115	7	6.09
15.	June	577	110	21	1.9
16	July	549	133	13	9.77
	Total	6110	2541	202	7.95

Table 2. Nature of discharge before treatment

S.No.	Drugs	Route	Purulent		Cloudy		Flakes of pus		Total
			Number	percent	Number	per cent	Number	per cent	
1.	Gentamicin	Intramuscular	2	10	4	20	14	70	20
		Intrauterine	3	15	4	20	13	65	20
2.	Chloramphenicol	Intramuscular	5	20.83	4	16.66	15	62.5	24
		Intrauterine	2	10	7	35	11	55	20
3.	Co-trimoxazole	Intrauterine	2	9.10	5	22.73	15	68.18	22
4.	Furazolidone	Intrauterine	2	9.52	7	33.33	12	57.14	21
	Total		16	12.60	31	24.40	80	63.00	127

Table 3. Nature of discharge after treatment

S.No	Drugs	Route	Clear		Purulent		Cloudy		Flakes of pus		Total
			Number	per cent	Number	per cent	Number	per cent	Number	per cent	
1.	Gentamicin	Intramuscular	16	80	2	10	..	..	..	..	18
		Intrauterine	19	95	1	5	..	..	..	..	20
2.	Chloramphenicol	Intramuscular	23	95.83	..	..	..	..	1	4.17	24
		Intrauterine	17	85	..	..	1	5	1	5	19
3.	Co-trimoxazole	Intrauterine	16	72.73	1	4.55	1	4.55	2	9.1	20
4.	Furazolidone	Intrauterine	20	95.23	..	..	..	..	..	..	20
	Total		111	91.75	4	3.30	2	1.65	4	3.30	121

Table 4. Identification of Organisms

S.No	Organisms identified	Number	Per cent
1.	Coagulase negative Staphylococcus spp.	12	40.00
2.	Staphylococcus aureus	9	30.00
3.	Corynebacterium spp.	5	16.66
4.	Bacillus spp.	2	6.67
5.	Pseudomonas spp.	2	6.67
	Total	30	100.00

Table 5. Bacterial isolates and their sensitivity

Sample No.	Bacterial Organisms	Genta-micin	Chloram-phenicol	Co-trim-oxazole	Furazo-lidone
1	2	3	4	5	6
1.	Staphylococcus aureus	R	R	R	R
2.	S. aureus	R	R	R	R
3.	Coagulase negative Staphylococcus spp.	S	S	R	S
4.	Bacillus spp.	S	S	R	S
5.	S. aureus	R	R	R	R
6.	S. aureus	S	I	R	R
7.	Corynebacterium spp.	S	S	S	S
8.	Coagulase negative Staphylococcus spp.	R	S	R	R
9.	Coagulase negative Staphylococcus spp.	S	S	R	S
10.	Coagulase negative Staphylococcus spp.	S	S	R	S
11.	Coagulase negative Staphylococcus spp.	S	R	R	S
12.	Pseudomonas spp.	R	R	R	R
13.	Bacillus spp.	S	S	I	S
14.	Staphylococcus aureus	R	R	R	R
15.	S. aureus	S	R	R	R
16.	Pseudomonas spp.	R	R	R	R

1	2	3	4	5	6
17.	Corynebacterium spp.	R	S	R	S
18.	Coagulase negative Staphylococcus spp.	S	S	I	S
19.	Coagulase negative Staphylococcus spp.	S	S	R	S
20.	Coagulase negative Staphylococcus spp.	S	R	R	R
21.	Coagulase negative Staphylococcus spp.	S	S	R	R
22.	Corynebacterium spp.	S	S	S	S
23.	S. aureus	R	R	I	I
24.	Corynebacterium spp.	S	S	I	S
25.	Coagulase negative Staphylococcus spp.	S	I	R	S
26.	S. aureus	S	R	R	I
27.	Coagulase negative Staphylococus spp.	R	R	R	I
28.	Coagulase negative Staphylococcus spp..	I	R	R	R
29.	Coagulase negative Staphyloccus spp.	R	R	R	I
30.	Corynebacterium spp.	S	S	S	S

S - Sensitive

R - Resistant

I - Intermediate

Table 6. Summary of Response of organisms to sensitivity tests

S.No	Organisms	Gentamicin						Chloramphenicol						Co-trimoxazole						Furazolidone					
		S	Per cent	I	Per cent	R	Per cent	S	per cent	I	per cent	R	Per cent	S	per cent	I	Per cent	R	Per cent	S	Per cent	I	Per cent	R	Per cent
.	Coagulase negative Staphylococcus spp.	8	83.40	2	8.30	2	8.30	7	58.30	1	8.3	4	33.3	-	--	1	8.30	11	91.66	7	58.30	1	8.30	4	33.33.
.	Staphylococcus aureus	3	33.30	-	--	6	66.70	-	-	1	11.10	8	88.30	-	--	1	11.10	8	88.80	--	--	3	33.30	6	66.70
.	Corynebacterium spp.	4	80.00	-	--	1	20.00	5	100.00	-	--	-	--	3	60.00	1	20.00	1	20.00	5	10000	-	--	-	--
.	Bacillus spp.	2	100.00	-	--	-	--	2	100.00	-	--	-	--	-	--	1	50.00	1	50.00	2	10000	-	--	-	--
.	Pseudomonas spp.	-	--	-	--	2	100.00	-	--	-	--	2	100.00	-	--	-	--	2	100.00	-	--	-	--	2	10000

= Sensitive

= Intermediate

= Resistant

Table 7. Response to sensitivity tests.

Serial Number	Drugs	Sensitive		Intermediate		Resistant		Total
		Number	percent	Number	percent	Number	percent	
1.	Gentamicin	78	61.41	1	0.79	48	37.80	127
2.	Chloramphenicol	75	59.05	1	0.79	51	40.16	127
3.	Co-trimoxazole	30	23.62	12	9.45	85	66.93	127
4.	Furazolidone	59	46.45	1	0.79	67	52.76	127
	Total	242	47.64	15	2.95	251	49.41	508

Table 8. Result of Artificial Insemination after treatment.

S.No.	Drugs	Routes	Number Treated	No. of animals follow-ed up	No. of animals conceived after A.I			Total
					Ist A.I	2nd A.I	3rd A.I	
1.	Gentamicin	Intramuscular	20	15	10	..	..	10
		Intrauterine	20	16	10	2	..	12
2.	Chloramphenicol	Intramuscular	24	22	15	1	..	16
		Intrauterine	20	16	11	1	..	12
3.	Co-trimoxazole	Intrauterine	22	12	10	1	1	12
4.	Furazolidone	Intrauterine	21	17	7	3	1	11
	Total		127	98	63	8	2	73

Table 9. Effect of Drug administrations

S.No.	Drugs	Route	Number of animals treated	Animals recovered after treatment and inseminated		Animals not inseminated				Animals followed up after A.I.	Pregnant		Non pregnant	
						Treatment failed		Animals not traced						
				Number	per cent	No.	per cent	No.	Per cent		No.	Per cent	No.	Per cent
1.	Gentamicin	Intra-muscular	20	16	80	2	10	2	10	15	10	66.60	5	33.40
		Intra-uterine	20	19	95	1	5	..	..	16	12	75	4	25
2.	Chloramphenicol	Intra-muscular	24	23	95	1	..	..	5	22	16	72.73	6	27.27
		Intra-uterine	20	17	85	2	10	1	5	16	12	75	4	25
3.	Co-trimoxazole	Intra uterine	22	16	72.73	3	13.60	3	13.60	12	12	100	0	0
4.	Furazolidone	Intra uterine	21	20	95.24	1	..	..	4.76	17	11	64.71	6	35.29
	Total		127	111	87.40	10	7.87	6	4.72	98	73	74.49	25	25.51

Table 10. Values of 'T' Test

S.No	Drugs	Gentamicin IM	Gentamicin IU	Chloramphenicol IM	Chloramphenicol IU	Co-trimoxazole IU	Furazolidone IU
1.	Gentamicin IU	0.5155	..	..	..	..	..
2.	Chloramphenicol IM	0.3949	0.1597	..	..	..	..
3.	Chloramphenicol IU	0.5155	0.000	0.1597	..	..	..
4.	Co-trimoxazole IU	*2.7427	*2.3094	*2.8748	*2.3099	..	..
5.	Furazolidone IU	0.1130	0.6497	0.5338	0.6494	*3.0455	..

* $P < 0.05$

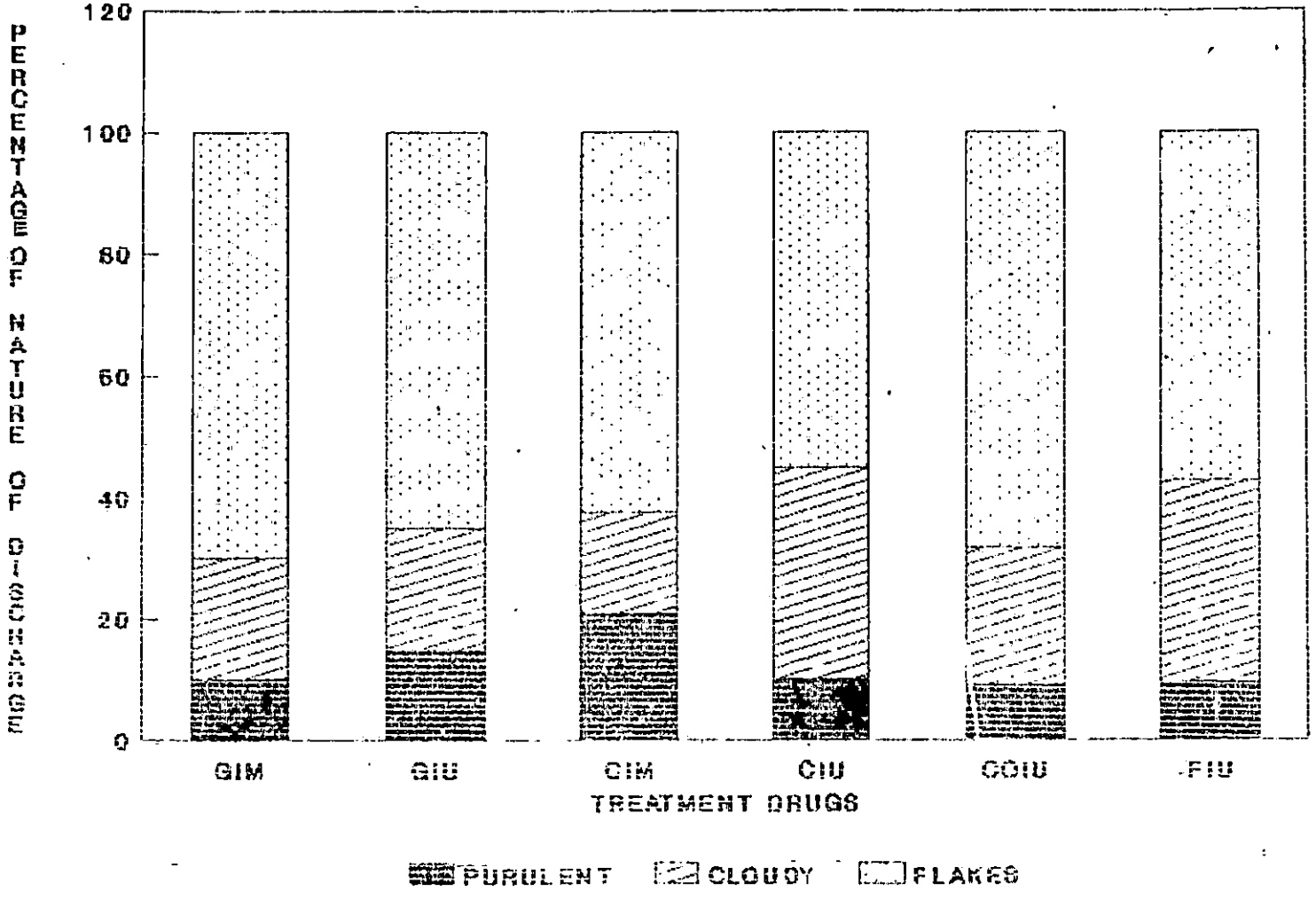
Table 11. Comparison of the effects of intramuscular and intrauterine routes of administration.

S.No.	Route.	No.of animals	Animals recovered		Number followed up	Conception	
			Number	per cent		Number	per cent
1.	Intramuscular group	44	39	92.86	37	26	70.27
2.	Intrauterine group	83	72	91.14	61	47	77.05
	Total	127	111	92	98	73	73.66
	T value			1.3158^{NS}			=0.7375^{NS}

NS = Not significant

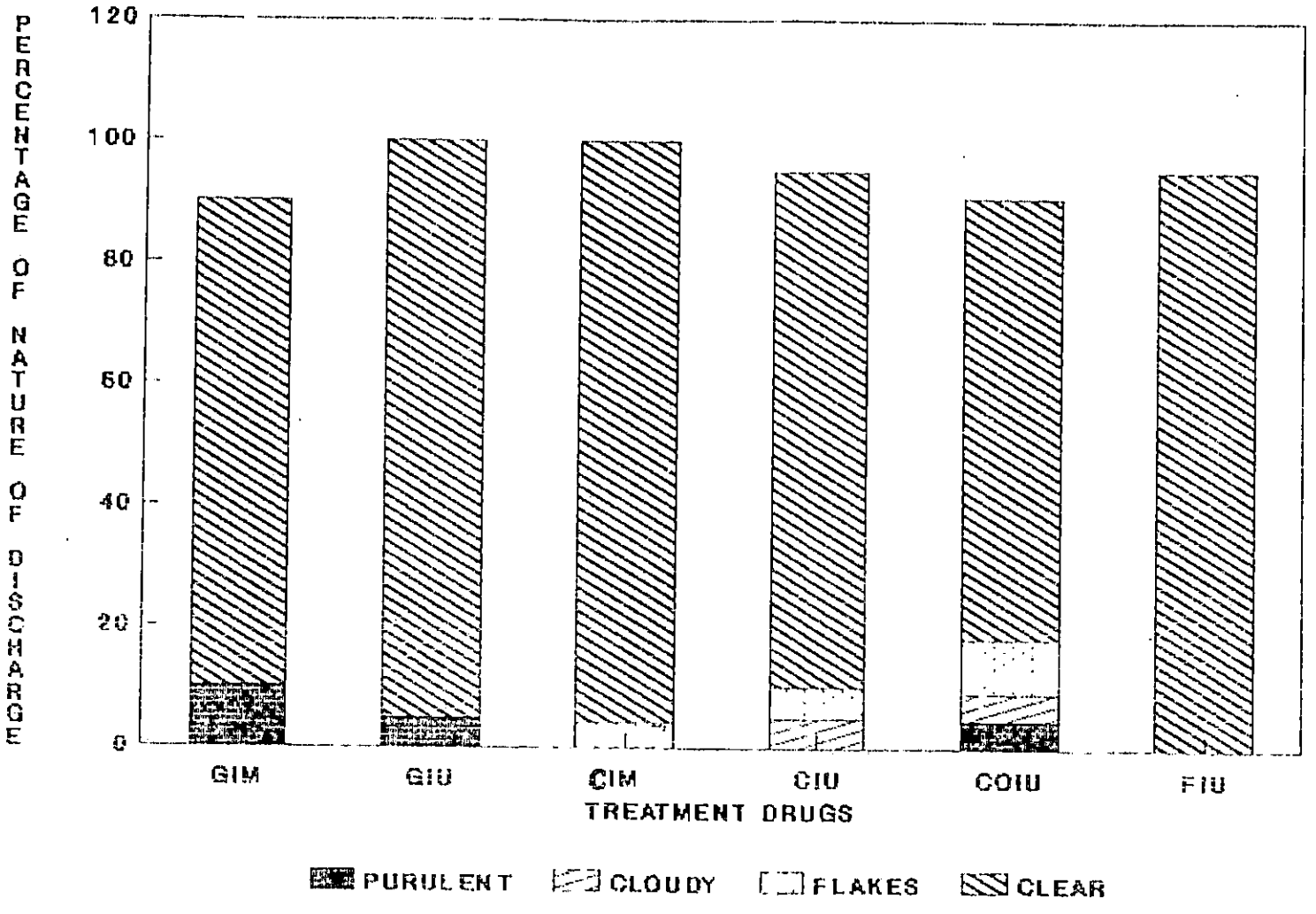
Graph 1

NATURE OF UTERINE DISCHARGE BEFORE TREATMENT



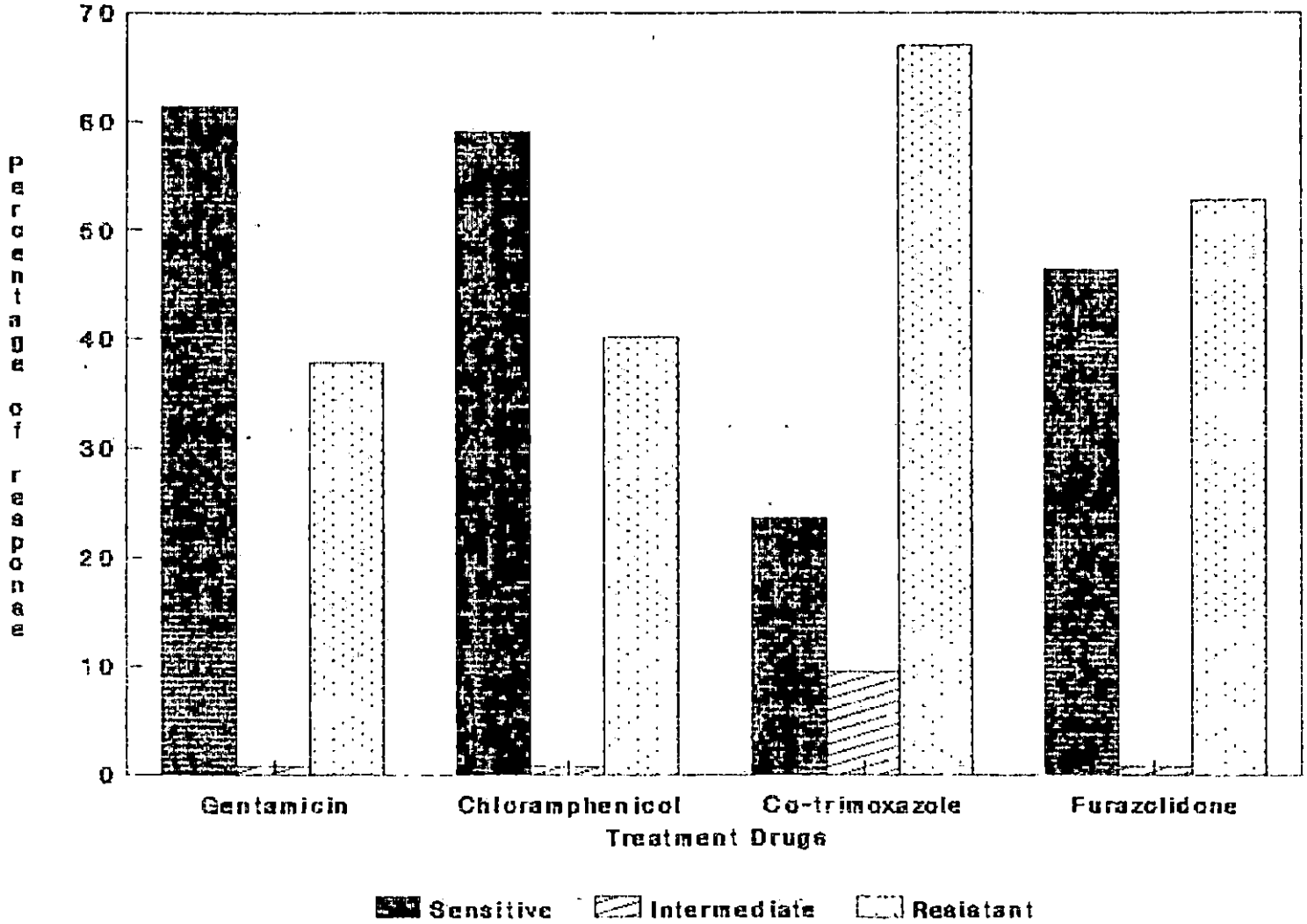
tamicin C - Chloramphenicol CO - Co-trimaxazole F - Furazolidone IM - Intramuscular IU - Intrauterine

Graph 2 NATURE OF UTERINE DISCHARGE AFTER TREATMENT



tamicin C - Chloramphenicol CO - Co-trimoxazole F - Furazolidone IM - Intramuscular IU - Intrauterine

Graph 3 RESPONSE TO SENSITIVITY TESTS



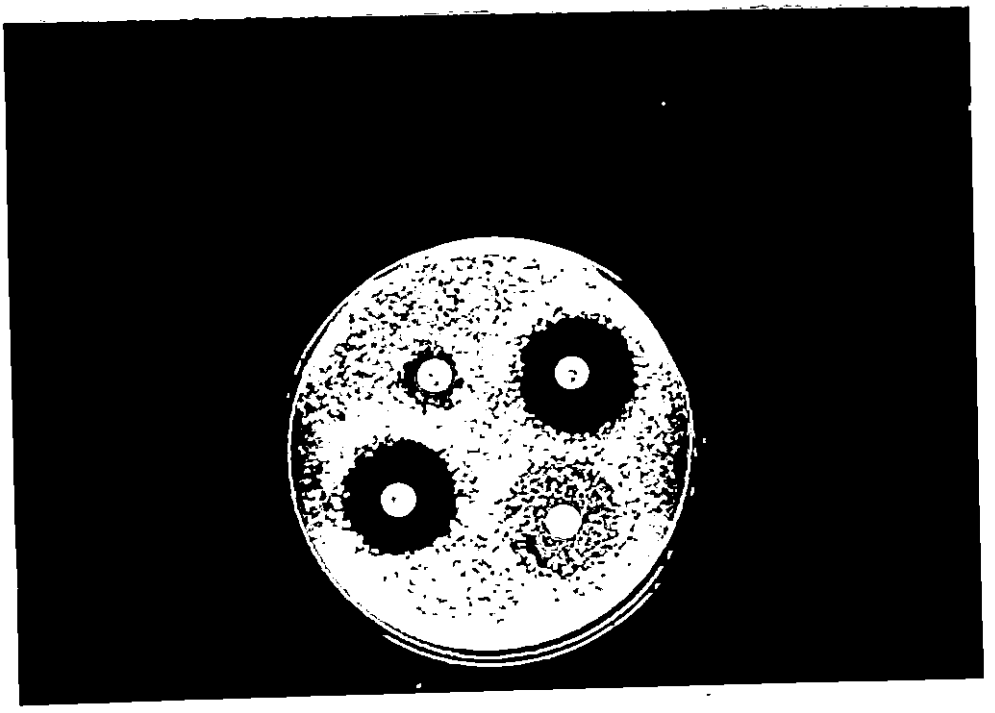


Fig. 4.1 ISOLATES SHOWING SENSITIVITY TO GENTAMICIN AND CHLORAMPHENICOL AND RESISTANCE TO CO-TRIMOXAZOLE AND AMPICILLIN

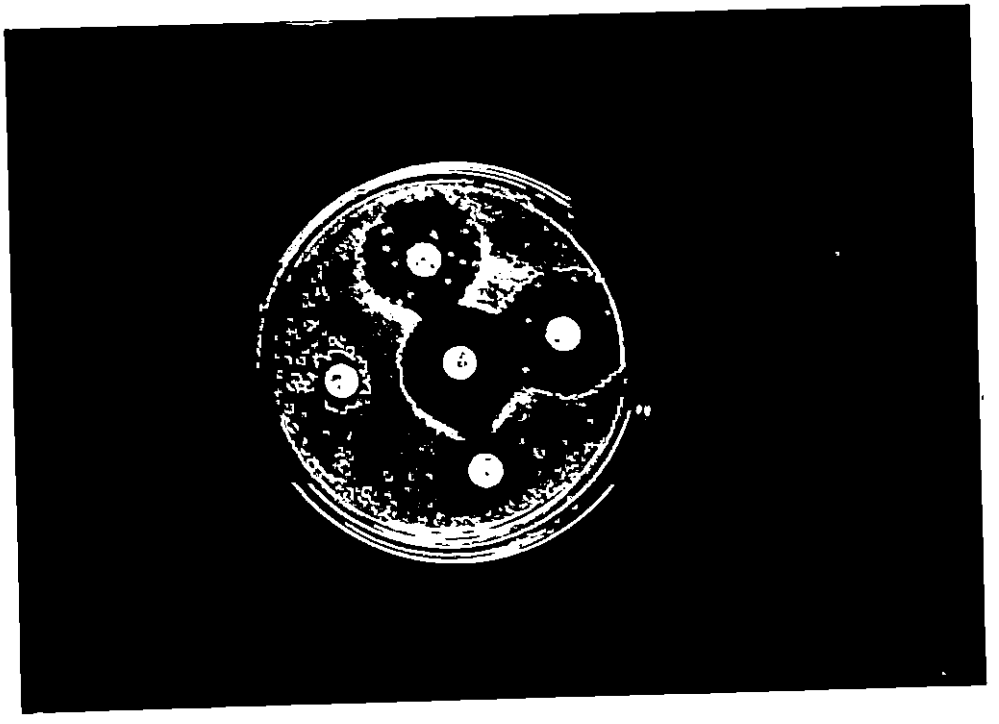


Fig. 4.2 ISOLATES SHOWING SENSITIVITY TO GENTAMICIN, CHLORAMPHENICOL AND FURAZOLIDONE AND RESISTANCE TO CO-TRIMOXAZOLE AND AMPICILLIN

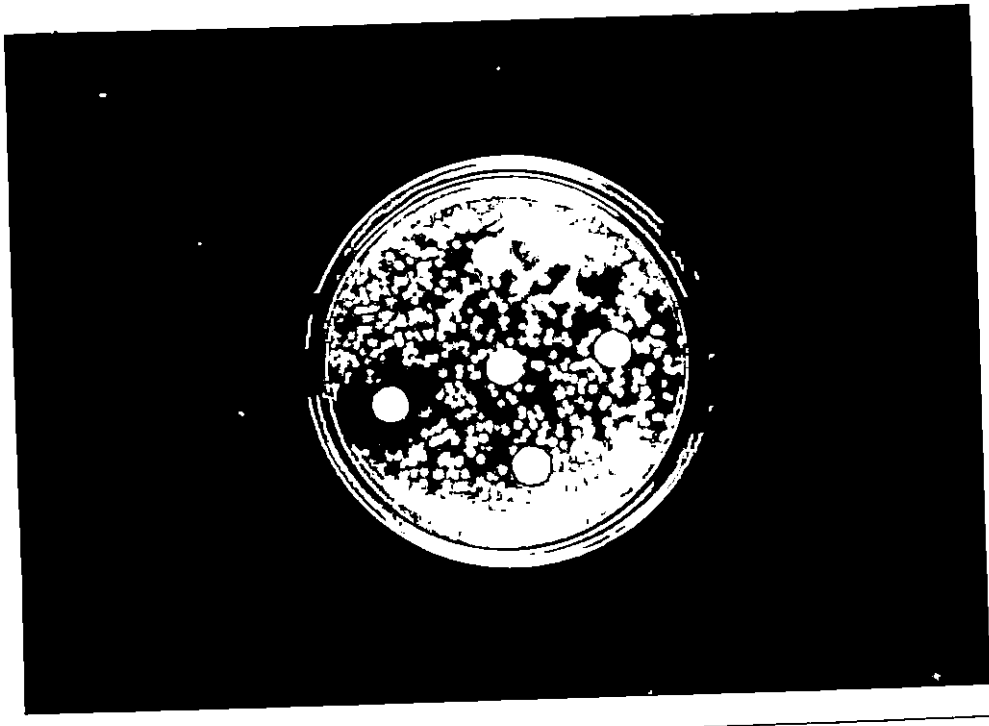


Fig. 4.3 ISOLATES SHOWING RESISTANCE TO GENTAMICIN, CHLORAMPHENICOL, FURAZOLIDONE, CO-TRIMOXAZOLE AND AMPICILLIN

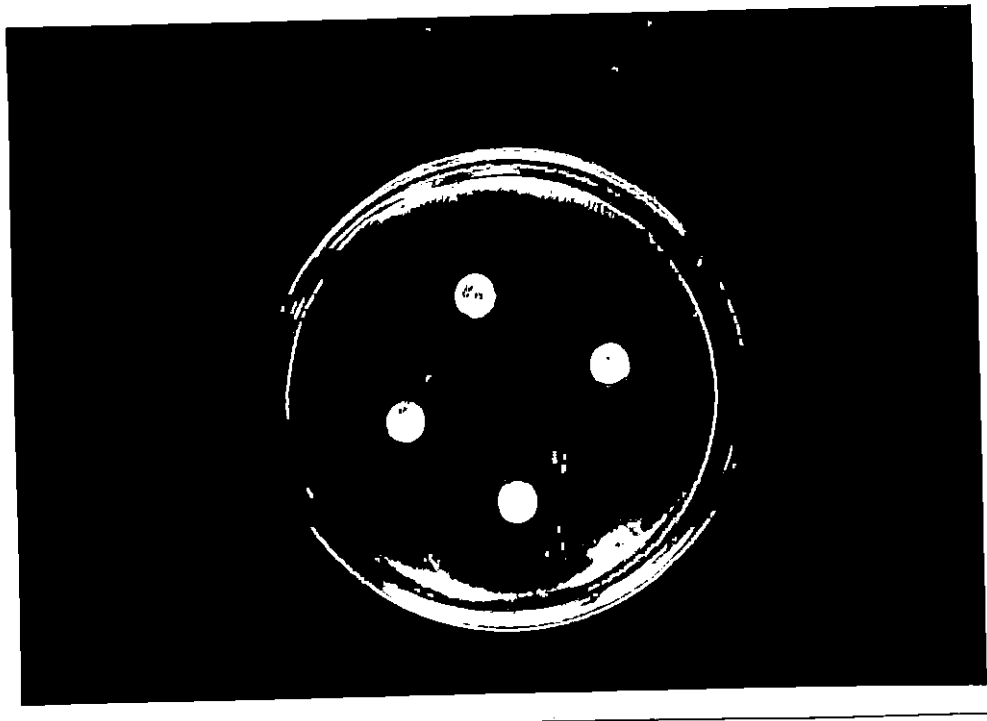


Fig. 4.4 ISOLATES SHOWING SENSITIVITY TO GENTAMICIN AND RESISTANCE TO CHLORAMPHENICOL, CO-TRIMOXAZOLE AND AMPICILLIN

Discussion

DISCUSSION

Endometritis is one of the common causes of infertility in cattle.The wide use of antibiotics against infecting organisms causing endometritis was started by the first half of the twentieth century. Treatments have been aimed at destruction of microorganisms responsible for the disease and thus to improve conception rate. The choice of drugs used for treatment depends on the type of organisms present and the duration and extent of tissue damages. The present study was undertaken to gather more information on drug sensitivity of bacterial isolates in cases of endometritis and to study the effect of treatment on conception rate.

5.1 Incidence of endometritis

The incidence of clinical endometritis in the present study was 7.95 per cent. Rao and Kotayya (1976) reported an incidence of 30.77 per cent and Francos (1979) noted 16.00 per cent incidence of endometritis. The present observation is lower than these values. However. Varadarajan (1985) reported an incidence of 9.66 per cent which was nearly similar to the present observations.

5.2 Collection of uterine discharge

The instrument presently developed for aseptic collection of uterine discharge with the modification of the design proposed by Minocha et al. (1964), served successfully the purpose throughout the period of study. Contamination of the uterine discharge from outside was almost nil during collection and there was no evidence of extension of infection from vagina to uterus.

5.3 Nature of discharge

Out of 127 samples collected before treatment, the discharge was purulent in 16 samples, cloudy in 31 samples and with flakes of pus in 80 samples (Table 2). Considerable improvenent in the nature of discharge was noted after the treatment. It was clear in 111 cases, purulent in four cases, cloudy in two cases and with flakes of pus in four cases. This showed that the treatment with selected drugs was very effective in destroying the organisms causing the disease. Similarly a high success of treatment based on nature of discharge was recorded by Sinha et al. (1977) and Venkateswarlu et al. (1983).

5.4 Identification of bacterial organisms

The bacterial isolates obtained from the uterine discharges of clinical endometritis were; coagulase negative Staphyloccus spp. (40.00 per cent), Staphylococcus aureus (30.00 per cent), Corynebacterium spp. (16.67 per cent) Bacillus spp. (6.67 per cent) and Pseudomonas spp. (6.67 per cent). Isolation and identification of pathogens involved in endometritis were also carried out by earlier workers and organisms involved were similar to the organisms identified in the present study (Hardenbrook, 1958; Hinze, 1959; Venkateswarlu, 1983a; Malik et al. 1987; Singh et al. 1989)

The causative organisms might have reached the uterus from vagina at oestrus or at parturition, although, it is possible in some circumstances for infection to arrive by blood circulation. Most of these organisms would have been normal inhabitants in the surroundings of dairy herd (Hinze, 1959; Arthur, 1989).

5.5 Sensitivity

In vitro drug sensitivity tests of the organisms causing endometritis showed that 61.41 per cent of the isolates were sensitive to Gentamicin, 59.05 per cent to Chloramphenicol, 46.45 per cent to Furazolidone and 23.62 per cent to Co-trimoxazole (Table 7). Venkateswarlu et al.(1983 b) recorded similar results with

Summary

Chloramphenicol showing maximum sensitivity, followed by Gentamicin and Furazolidone.

In the present study, sensitivity of organisms to Co-trimoxazole was the least (23.62 per cent). However, Varadarajan (1985) recorded a higher degree of sensitivity (44.00 per cent) in Co-trimoxazole. The reduced sensitivity of isolates to Co-trimoxazole and Furazolidone observed in the present study may be due to the frequent use of these drugs in animal practices. Susceptibility to antibiotics vary between any two species of bacteria and strains of any given species. Some species and groups of microorganisms are inherently resistant to certain antibiotics and resistance may also develop in normally susceptible species due to induction, mutation and transfer of resistance. The rate of antibiotic resistance is seen generally increasing among almost all bacterial species. Transferable drug resistance is universal in distribution and involves all antibiotics in common use. Incidence of drug resistance is directly proportional to the frequency of use of antibiotics in the area. (Williams, 1990).

5.6 Result of Artificial Insemination after treatment

Out of the total 98 animals followed up after treatment and Artificial Insemination , most animals

(63 No.) became pregnant at the first insemination itself. A few (8 No.) needed a second insemination and only two needed third insemination (Table 8). There was no significant difference among treatment groups in number of insemination required for conception. The average number of inseminations per conception was 1.16. While Varadarajan(1985) recorded 1.91 insemination per conception after intrauterine administration of Gentamicin in the treatment of endometritis. Hence it could be seen that the results of the present study show a better result.

5.7 Effect of treatment

For antimicrobial action to be more efficient, an effective effective concentration of drug must be achieved and maintained at the site of infection for an adequate period. Two main routes of administration of drugs are commonly used for uterine affections. They are local (intrauterine) and systemic (intramuscular).

A comparative study of the results obtained with intrauterine and intramuscular administration of Gentamicin, the former route was found more encouraging than the latter. The conception rates were 75.00 per cent and 66.66 per cent respectively (Table 9).

Systemic administration of antibiotics was reported to have better advantage over local (intrauterine) administration (Gustafsson, 1984). In the present study intrauterine administration of Gentamicin was found to be more efficient. In the case of intrauterine administration of antibiotics, to exert their full effect, they should not be confined to the uterine cavity and surface of endometrium, but therapeutic levels of drug should be achieved in the deeper layers of the genital tract. Absorption of drugs was generally diminished in endometritis, and with severity of inflammation there was further reduction in absorption of drug (Gustafsson, 1984). Perhaps, in the present work, animals received Gentamicin locally would have had a lesser degree of inflammation enabling the drug to act more effecitvely. Encouraging results after Gentamicin administration were reported by previous workers. Varadarajan (1985) and Sudhakar et al. (1986) reported an overall conception rate of 72.58 per cent and 76.00 per cent respectively, after intrauterine administration of Gentamicin and these results are in full agreement with the results of the present study. On the contrary lower conception rate (61.54 per cent) was reported by Venkateswarlu et al. (1983).

Conception rate in animals treated with Chloramphenicol both intramuscular (72.73 per cent) and Intrauterine (75.00 per cent) is encouraging. The spectrum of Chloramphenicol included Streptococci, Staphylocci, gram positive rods including Corynebacterium and small aerobic gram negative rods. In the present study, results obtained showed that Chloramphenicol was comparatively more successful in treating endometritis and thus to improve conception rate. Most of the organisms isolated in this study were Staphylococcus spp. (40.00 per cent) and Corynebacterium spp. (16.66 per cent) which come under the spectrum of this drug. The results obtained in the present study are in agreement with results obtained by Venkateswarlu et al. (1983 b) where conception rate after treatment with Chloramphenicol by intrauterine method was 72.00 per cent. Pharmacological studies suggest that Chloramphenicol should be the most effective available antibiotic for treating uterine infections (Paisley et al 1986).

Conception rate obtained by intrauterine administration of Nitrofurazone was 64.71 per cent. Better results (88.00 per cent conception) were reported by Venkateswarlu et al.(1983 b) in animals treated with furacin. Nitrofurzone is active against gram negative rods and gram positive cocci including coagulase negative

Staphylococci. In the present work 40.00 per cent isolates obtained were coagulase negative Staphylococci which come under the spectrum of activity of Nitrofurazone.

Although recovery recorded after the intrauterine administration of Co-trimoxazole was comparatively low (72.73 per cent), the conception rate of cases followed up was cent per cent. The relatively low level of recovery (72.73 per cent) obtained with Co-trimoxazole may be due to the fact that purulent discharge present in the uterine lumen decreasing its action.

Statistical analysis of the data on conception rate was carried out and there was no significant difference in conception rate among the treated group except Co-trimoxazole treated group. The conception rate in the Co-trimoxazole treated group was significantly higher ($P < 0.05$) than that in other groups.

Comparative effect of intramuscular and intrauterine routes of administration of all the four drugs was studied (Table 11). Out of 44 animals that received intramuscular administration, 39 (92.86 per cent) recovered and were inseminated and the conception rate obtained was 70.27 per cent; whereas out of 83 animals that received intrauterine administration, 72 animals (91.14 per cent) recovered with a conception rate of 77.05

per cent. On analysis, the data showed no significant difference between the intramuscular and intrauterine routes of administration with respect to recovery and conception rate. As far as recovery of endometritis and subsequent conception rate in cows are concerned, the effects of both intramuscular and intrauterine administration of antibiotics are similar.

SUMMARY

Endometritis is a major cause of infertility in dairy cattle and hence study was undertaken to assess the magnitude of prevalence of endometritis to elucidate the causative organisms and to select suitable antimicrobial agents for treatment to improve fertility of dairy cows. Isolation and identification of organisms were carried out and *in vitro* antibiotic sensitivity tests were done for understanding specific effective antimicrobial agents.

Among 6110 cows and heifers examined in the Artificial Insemination Centre, attached to the Department of Animal Reproduction, College of Veterinary and Animal Sciences, Mannuthy during the period of study from April, 1991 to July 1992; incidence of endometritis was found to be 7.95 per cent. For the purpose of aseptic collection of uterine discharge, an instrument was devised by modifying the pattern proposed by Minocha *et al.* (1964).

In Vitro sensitivity tests showed that out of 127 samples, maximum number of isolates 78 (61.41 per cent) were sensitive to Gentamicin followed by Chloramphenicol 75 (59.05 per cent), Furazolidone 59 (46.45 per cent) and Co-trimoxazole 30 (23.62) per cent). Intermediate sensitivity to Co-trimoxazole was seen in isolates from 12

samples (9.45 per cent).

The bacteria identified from uterine discharges were coagulase negative Staphylococcus spp. (40.00 per cent), Staphylococcus aureus (30.00 per cent), Corynebacterium spp. (16.66 per cent), Bacillus spp. (6.67 per cent) and Pseudomonas spp. (6.67 per cent).

Out of the total 127 animals treated, 98 animals were followed up after insemination and 73 animals found pregnant, with a conception rate of 74.49 per cent. After first, second and third inseminations sixty three, eight and two animals respectively were found pregnant. Forty animals from which the uterine discharge collected showed sensitivity were divided at random into Group I A and Group II A. Animals in Group I A (20 animals) were given Gentamicin 4 mg/kg body weight intramuscularly at 12 hour interval for three consecutive days. Animals in Group II A (20 animals) were treated by intrauterine administration of Gentamicin 400 mg dissolved in 30 ml distilled water at 12 hour interval for two days.

Similarly 44 animals from which the uterine discharge showed sensitivity to Chloramphenicol was divided at random into Group I B and group II B. Animals in Group I B (24 animals) were given Chloramphenicol 4 mg/kg body weight intramuscularly at 12 hour interval

for three days. Animals in Group II B (20 animals) were given intrauterine administration of 1 gm Chloramphenical dissolved in 30 ml distilled water at 12 hour interval for two days.

Animals (22 No.) from which discharge collected showed sensitivity to Co-trimoxazole were included in Group III A and another 21 animals those showed sensitivity to Furazolidone in Group III B. Animals in Group III A were given Co-trimoxazole (Sulphamethoxazole 3000 mg + Trimethoprim 600 mg) dissolved in 30 ml distilled water by intrauterine administration at 12 hour interval for two days. Animals in Group III B were subjected to intrauterine administration of Furazolidone 30 ml at 12 hour interval for two days.

In the succeeding heat of the treated animals, those found to be clinically normal were inseminated. Animals showing no improvement after treatment were again treated based on the result of sensitivity tests conducted with the discharge.

Out of 20 animals in Group I A, 16 animals recovered and were inseminated. After treatment 15 animals could be followed up and the percentage of conception was 66.60 per cent. Similarly in Group II A (20 animals), 19 animals recovered and were inseminated after intrauterine administration of Gentamicin, out of

which 16 animals were followed up and 75.00 per cent conception was recorded.

When Chloramphenicol was given intramuscularly 23 animals out of 24 animals recovered and were inseminated. Follow up was carried out in 22 inseminated animals and 72.73 per cent conception was noted. After intrauterine administration of Chloramphenicol in 20 animals, 17 animals recovered and were inseminated, of which 16 animals were followed up and conception rate was 75.00 per cent. In 22 animals administered with Co-trimoxazole as intrauterine infusion 16 animals recovered and were inseminated, of which 12 animals were followed up and conception rate was cent per cent. Out of 21 animals treated after intrauterine administration of Furazolidone, 20 animals recovered and were inseminated, of which 17 animals were followed up and conception rate was 64.71 per cent.

The data on conception rate was subjected to statistical analysis and there was no significant difference in conception rates among any of the groups except Co-trimoxazole treated group, which showed significantly higher conception rate from all other groups. Comparative effect of intramuscular and

intrauterine routes of administration of drugs revealed no significant difference in the percentage of recovery and conception rate. Following conclusions were made from the results of the study.

1. Treatment following *in vitro* drug sensitivity tests on uterine discharge is an efficient method for improving fertility in cows with endometritis.

2. Both intrauterine and intramuscular routes of of administration were efficient in making recovery of endometritis.

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TREATMENT OF ENDOMETRITIS FOR IMPROVING FERTILITY IN DAIRY COWS

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ABSTRACT OF A THESIS

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ABSTRACT

A study was undertaken to assess the incidence of endometritis in cows and to find out the effect of intramuscular and intrauterine administration of different antimicrobial agents in improving the conception rate in cows during the period from April 1991, to July 1992. The study was conducted in the animals brought to the Artificial Insemination Centre attached to the Department of Animal Reproduction, College of Veterinary and Animal Sciences, Mannuthy, Thrissur.

In vitro sensitivity test was done on 127 samples of uterine discharge collected aseptically by the use of a newly designed Catheter. Sensitivity tests showed that 78 isolates (61.41 per cent) were sensitive to Gentamicin, 75 (59.05 per cent) to Chloramphenicol, 59 (46.45 per cent) to Furazolidone and 30 (23.62 per cent) to Co-trimoxazole. Intermediate sensitivity to Co-trimoxazole was seen in 12 (9.45 per cent) isolates.

Identification of bacterial organisms were also done in the study. Isolates obtained were Coagulase negative Staphylococc spp. (40 per cent), Staphylococcus aureus (30.00 per cent) Corynebacterium spp. (16.66 per

cent) Bacillus spp. (6.67 per cent) and Pseudomonas spp. (6.67 per cent). Animals were grouped into 3 treatment groups based on result of sensitivity tests. Animals in I A and II A received intramuscular and intrauterine administration of Gentamicin respectively. Dose rate was 4 mg/kg body weight for intramuscular and 400 mg for intrauterine administration at 12 hour interval for three days and two days respectively.

Animals in I B received Chloramphenicol at the rate of 4 mg/kg body weight intramuscularly at 12 hour interval for three days and animals in II B got intrauterine administration of 1 g Chloramphenicol at 12 hour interval for two days.

Group III A consisted of animals in which discharge showed sensitivity to Co-trimoxazole and received Co-trimoxazole (Sulphamethoxazole 3000 mg + Trimethoprim 600 mg) as intrauterine infusion at 12 hour interval for two days. Group III B animals were given Furazolidone 30 ml as intrauterine infusion at 12 hour interval for two days.

After treatment animals with clear discharge in the subsequent heat were considered as recovered and were inseminated.

The recovery obtained was 80.00, 95.00, 95.83, 85.00, 72.73 and 95.24 percentages respectively in Group IA , IIA, IB, IIB, IIIA and IIIB. Conception rate was calculated on the basis of actual follow up. The percentages of conception were 66.60, 75.00, 72.73, 75.00, 100.00 and 64.71 per cent in Group IA, IIA, IB, IIB, IIIA and III B respectively.

Out of 127 animals treated, 98 animals were followed up and 73 animals were found pregnant. Sixty three animals were found pregnant at first insemination, eight after second insemination and two after third insemination. The average conception rate was 74.49 per cent.

Data were subjected to statistical analysis and there was no significant difference among treatment groups in conception rates except for animals which received Co-trimoxazole as intrauterine infusion. These animals showed a significantly higher rate of conception.

The data on comparative effect of intramuscular and intrauterine routes of administration on analysis showed that both routes are equally effective in counteracting endometritis.