PROSTAGLANDIN THERAPY FOR POST - PARTUM CLINICAL ENDOMETRITIS

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **PROSTAGLANDIN THERAPY FOR POST-PARTUM CLINICAL ENDOMETRITIS** 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 ther university or society.

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CERTIFICATE

I hereby declare that this thesis entitled **PROSTAGLANDIN THERAPY FOR POST-PARTUM NICAL ENDOMETRITIS** is a record of research work d independently by Dr. T.C. JACOB under my guidance and rvision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.

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We, the undersigned members of the Advisory Committee of Dr. T.C. JACOB, a candidate for the degree of Master of Veterinary Science in Animal Reproduction, agree that the thesis entitled **PROSTAGLANDIN THERAPY FOR POST-PARTUM CLINICAL ENDOMETRITIS** may be submitted by Dr. T.C. JACOB in partial fulfilment of the requirement for the degree.

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Introduction

INTRODUCTION

Bovine endometritis is the most common of all the periparturient diseases of dairy cattle with greatest impact on fertility. Though, precise information on the magnitude of this condition causing economic loss is scanty, there are reasons to believe that majority of cows culled and disposed off, for reasons of infertility, are due to uterine infections. There is consensus of opinion that endometritis does have a detrimental effect on subsequent fertility by extending the calving to conception interval and increasing the number of insemination per pregnancy and also resulting in sterility due to irrepairable changes of genital tract. Calving to conception interval has been reported to increase enormously with an increase in services per conception. Alarming figures of culling rate has also been reported from several countries due to uterine infection, especially, endometritis. In India authentic figures on the incidence of endometritis are not available, although reports indicate that a good percentage of cows are being culled and disposed off due to endometritis. In Kerala too, the prevalence of endometritis are on the increase even with modern husbandry practices.

Considering this grave situation on dairy economy, several attempts have been made in the recent past to

control this paradoxical situation. Intrauterine infusion of bewildering array of compounds had been the main stay of treatment of bovine endometritis for decades. Recent reports show that uterus is an anaerobic environment, making the aminoglycoside group of antibiotics ineffective because they require oxygen for their activity. During the post partum period many organisms in the uterus are capable of producing enzymes, that inactivate or degrade antibiotics. In addition, the presence of pus or organic debris in the uterine fluid could potentially inhibit drugs such as sulphonamides, aminoglycosides and nitrofurazone. More over, all intra uterine treatments result in some absorption of the drug, resulting in adulteration of milk with various degree depending upon the drug, vehicle and dosage used. There are also no official guidelines for minimum withholding time after intrauterine therapy. Systemic administration although, has potential advantages over intrauterine therapy, commercially available antibiotics that could be expected to be effective for uterine infection are not labelled properly. Further, the efficacy of systemic antibiotic therapy has not been thoroughly demonstrated. Uterine secretions are also reported to severely inhibit the antibacterial activity of antibiotics in aerobic and anaerobic conditions.

Increased recognition of the disappointing efficacy and disadvantages of antibiotics and other antibacterial agents, has focussed attention on alternative therapy for endometritis. Prostaglandins (PGF_2 alpha) and its structural analogues have been tried in the recent past for treatment of clinical and subclinical endometritis. The beneficial effect of prostaglandin is reported to be due to (1) induction of luteolysis if a functional corpus luteum is present (ii) a direct uterotonic effect (iii) stimulation of phagocytosis by uterine leucocytes (iv) ripening of cervix and (v) stimulation of uterine defence mechanism, consequent to a fall in circulating progesterone and a rise in oestrogen. Based on these, several trials were carried out to study the effectiveness of prostaglandins in the treatment of clinical endometritis and encouraging results were reported. In terms of fertility, the therapeutic use of PGF_2 alpha and its analogue appeared to show results better than antibacterial and antibiotic therapy. Further, it requires no milk withdrawal. The present work was, therefore, undertaken with the object of studying the effectiveness of administration of PGF_2 alpha in cross-bred cattle with the aim of evolving an effective non antibiotic therapy for clinical endometritis.

Review of Literature

2.1. Incidence

Bovine endometritis has been recognised as a global problem and the worldwide figures for its prevalence are varied. Hardenbrook (1958) reported that 80 per cent of breeding failures in bovine were due to endometritis. In southern Israel, on a study of 2745 cows during the period from 1965-'73, endometritis was reported to an extent of 16 per cent; whereas in Northern Israel the incidence was reported to be 14 per cent during the period from 1977-'79 (Francos, 1979). In USA the incidence was reported to be 6.25 per cent in Jersey and 10.3 per cent among Holstein cows (Fonseca et al., 1983). Mazumdar et al. (1985) found that, among 835 cows, 200 animals showed presence of uterine infections as evidenced by gross lesions of metritis. In Brazil, Ferreira and SA (1987) reported, uterine infections to an extent of 21.1 per cent. Zezula-SzPysa et al. (1988) indicated an incidence of 39.1 per cent uterine infections in bovines. Maneta et al. (1990) reported that, out of 142 cows genitalia, 72 (50.7 per cent) had chronic inflammation of uterus and also found that catarrhal form being the most common. Reports on the incidence of endometritis in Indian cattle are scanty. However, Namboodiripad et al. (1976) reported an incidence of 63.14 per cent of genital infections. Rao and Kottaya (1976), on the other hand, found

an incidence of 30.77 per cent of endometritis among infertile cows in Andhra Pradesh. Nair and Raja (1977), on a study of 2250 genitalia of cow (150 gravid), found that 17 had inflammatory lesions of the uterus. This included necrotic metritis (1) chronic non suppurative metritis (1), acute suppurative endometritis (2), acute non suppurative endometritis (6), chronic non-suppurative endometritis (2), perimetritis (2) and hydrometra (3) and remarked that the extent of uterine pathology varied in their manifestation. In Karnataka, Rao et al. (1983) found an incidence of 32.86 per cent of endometritis. In Kerala, Varadarajan (1985) observed an incidence of 9.66 per cent among cross-bred cows, but Iyer et al. (1992) found the incidence as 20 per cent. However, Vahida (1992) noted this to the extent of 7.95 per cent only.

2.2. Impact of endometritis on fertility

Impact of endometritis on fertility, both short term and long term has been well documented. With regard to short term influence, Studer and Morrow (1978) found a significant correlation between the state of uterus and calving conception interval. Extension of calving to conception interval to the extent of 12 days (Tennant and Peddicord, 1968), 20 days (Erb et al., 1981), 10 days

(Bretzlaff et al., 1982) has been reported. An increase in the services per conception has been reported from 1.67 and 2.16 to 2.0 and 2.42 respectively by Tennant and Peddicord (1968) and Bretzlaff et al. (1982). Roberts (1971) pointed out that delay in conception of a single cow leads to a loss of 30 dollar per month in America. Dohoo and Martin (1984) reported economic losses due to uterine infections to the extent of 2 to 5 per cent in milk yield/kg/day. In terms of currency the loss on account of infertility due to endometritis has been enormous. However, precise information on the magnitude of economic loss due to endometritis in India is scanty.

2.3. Etiology

Considerable interest has been shown in the past on the etiological factors of endometritis. According to Hinze (1959), non specific opportunist pathogens are the most important causes of endometritis and opined that these organisms might have reached the uterus from vagina at oestrus or at parturition, although, haematogenous metastatic infection is possible in some circumstances. He further remarked that, most of these organisms would have been normal inhabitants in the surroundings of dairy herd. Elliot et al. (1968) identified 33 different species of

bacteria, which fluctuated as a result of spontaneous contamination, clearance and recontamination during the first seven weeks post-partum. Hartigan et al. (1974) found a transient uterine infection by Corynebacterium pyogenes during the puerperal period resulting in delayed involution of uterus in cows. Benjamin et al. (1982) isolated Staphylococcus aureus, Escherichia coli, Corynebacteria and Achromobacter, from cervical mucus of infertile cows and buffaloes. Eduvie et al. (1984) however, reported the puerperal uterine contamination by bacteria did not interfere with normal process of involution. It was further reported that the uterine contamination usually occurred between days 10 and 21 and declined thereafter, which has coincided with sloughing of caruncular tissue with increased discharge of lochia. Martinez and Thibier (1984) remarked that the epidemiological pattern of endometritis need not be generalised and reported no definite influence of calving seasons, milk yield and parity on the incidence of endometritis. However, Bartlett et al. (1986) found a definite association of age and season with endometritis. Navaryo (1986), on bacteriological examination of uterine exudate of 1520 Holstein cows, having placental retention and endometritis, isolated C. pyogenes, streptococcus viridans, Streptococcus pyogenes and E. coli, and observed that the incidence of endometritis was more during the rainy season when the frequency of placental retention was also

found to be more. David and Bonnier (1987) reported that the commonest bacteria with endometritis in France were streptococci and Actinomyces (corynebacterium) Pyogenes. Singh et al. (1989) and Sirohi et al. (1989) described the isolates from cervical mucus of cows as S. aureus, Haemolytic streptococci, E. coli, pseudomonas, Enterobacter, citrobacter and coccobacillary rods. According to Khan et al. (1990) the main bacteria identified from uterine discharge were S. pyogenes, S. aureus, Corynebacterium bovis, E. coli, and Bacillus megaterium. Biolatti et al. (1991) and Kudryavtsev et al. (1991) also isolated similar bacteria from the uterine samples of infertile cows. Vahida (1992), on a clinical study of endometritis, found coagulase negative staphylococcal species, S. aureus, corynebacterium spp., bacillus spp. and pseudomonas spp. as the common organisms in the uterine discharge.

Viral agents have also been isolated from the uterine discharges. A herpes virus was isolated in bovine foetal kidney cell culture from the uterine discharge of 13 cows with chronic metritis. Direct immunofluorescence tests showed that LVR 140 was antigenically related to Movar 33/63 and the herpes virus isolated from a bull with orchitis (Wellemans et al., 1983). Opdenbosch et al. (1984) also isolated a herpes virus (strain LVR 140) from cases of

puerperal endometritis. It was suggested that the viral agent had an immuno suppressive effect which predisposed the animals to other infections. Rose (1987), on a study of endometritis in Britany, found antibodies to Bovine herpes virus 1 in 32 per cent of cases tested, Q fever antibodies in 21 per cent of 127 cows screened; and antibodies to chlamydia in one case. IBR virus was also isolated by Misra and Mishra (1987) in cows which failed to conceive.

Association of mycotic organisms and endometritis was reported by Kremlev and Banakova (1979). They isolated Candida, Actinomycetes and Mucoraceous fungi in 18 of 22 cases of uterine discharges. It was also found that Iodinal (Iodine and Potassium Iodide in Polyvinyl alcohol) inhibited growth of cultures of Candida albicans, Proactinomyces lignieresi, Absidia ramosa, Mucor pusillus and Lichtheimia corymbifera in a concentration of 125 mic g/ml and it was successfully used to treat acute mycotic endometritis.

2.4. Aseptic collection of uterine discharge

For effective treatment of endometritis it is necessary to conduct bacterial sensitivity tests of the uterine exudate and for this a special equipment has been designed by Minocha et al. (1964). It consisted of four telescoping metal tubes, the outer one served as the vaginal

speculum, the second tube for insertion upto the middle of the cervix and the two inner units constituted the sampler. Sterile gelatin capsules protected the tip of the tubes from contamination. Which was detained in the uterus and subsequently absorbed. The collection of samples for bacteriological examination using the instrument was found satisfactory and there was no incidence of transfer of infection from the vagina to the 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 1 cm short of its tip at the cranial end and 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. Vahida (1992) fabricated a new instrument which was a modification of the design proposed by Minocha et al. (1964).

2.5. Antibiogram of Bacterial Isolates

Koleff et al. (1973) by conducting sensitivity test of bacterial flora of uterine contents of 100 cows, having endometritis found that the most effective antibiotic was ampicillin, followed by chloramphenicol and tetracycline.

According to them the selection of a drug for treatment of endometritis, based on sensitivity test, resulted shorter duration of treatment and rapid recovery. Sinha et al. (1977) reported that chloramphenicol was the most effective remedy to inhibit the bacterial growth from uterine exudate. Murty and Rao (1979), however, reported that the genital microflora of repeat breeders were highly resistant to most of the antimicrobial agents. Kharade and Kulharni (1983) found that most of the uterine isolates were sensitive to gentamicin and kanamycin. Venkateswarlu et al. (1983a) observed sensitivity of the bacterial isolates from infertile cows, which had second degree endometritis, as 26.1 per cent, 24.4 per cent and 17.2 per cent respectively to chloramphenicol, gentamicin and Furacin. On a study of uterine discharge from 170 cross-bred cows and heifers having clinical evidence of endometritis and based on antibiogram of isolates, Varadarajan et al. (1985) observed that gentamicin was the most effective drug for treatment of clinical endometritis. Sudhaker et al. (1986) were also of the opinion that most of the bacterial isolates were sensitive to gentamicin. Malik et al. (1987) by studying the antibiotic sensitivity of 359 mucus samples from genital tract of infertile cows, found the highest sensitivity for gentamicin followed by streptomycin, kanamycin and chloramphenicol. Similar observations were also made by

Sirohi et al. (1989) and Sharda et al. (1991), who further reported that more than 40 per cent of isolates showed multiple resistance to four or more drugs. Venkateswaran and Rajeswar (1991), while screening 252 uterine swabs and subjecting to antibiotic sensitivity test, noticed that chloramphenicol was the most effective drug of choice followed by streptomycin. Iyer et al. (1992), found that majority of cows were resistant to common antibiotics, although gentamicin and kanamycin were more effective, compared to other antibiotics. Vahida (1992) 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.

2.6. Therapy for endometritis

2.6.1. Antibiotics and Sulphonamides

The use of various antibiotics for the treatment of endometritis have given variable results. The route of administration of these drugs and the time of administration have also been varied. Roberts (1956) remarked that antibacterial treatment at the time of oestrus was more effective than the luteal phase. According to Seguin et al. (1974), absorption and clearance of the systemically administered drugs is more rapid than from intrauterine

administration and opined that repeated doses two to three times a day may be required to maintain therapeutic levels of antibiotics. According to Oxender and Seguin (1976) the drugs infused into uterus would cause prolongation of the oestrous cycle or shortening depending up on the time of infusion. They recommended intrauterine treatment during oestrus and 24 to 48 hours after oestrus. Gustafsson (1984) observed apparent failures of intrauterine therapy in getting rid of uterine infection, due to altered leucocyte function, and remarked that all intrauterine drugs interfered with phagocytosis for several days. Ziv et al. (1983) and Jayappa and Loken (1983) recommended, systemic administration of antibiotics, to overcome this disadvantage. Paisley et al. (1986) observed that by intrauterine therapy there was risk of introducing new infection into uterus, injuring endometrium and also depressing uterine phagocytic activity. Vahida (1992), however, reported no significant difference between systemic and intrauterine treatment.

i. Ampicillin and Penicillin

Koleff et al. (1983) found that ampicillin was the most effective antibiotic for the treatment of endometritis. Singh et al. (1985) obtained a significant rise in conception rate by treating subclinical genital infections

with ampicillin 500 mg intrauterine, one hour before insemination. According to Gustafsson (1984), a systemic dose of 20,000 to 25,000 IU of sodium penicillin G per kg would result in genital tract tissue and lumen concentration in cow, which was sufficient to combat most pathogens, sensitive to penicillin, but the duration of desirable concentration was relatively short. He further opined that intrauterine infusion of natural penicillin during mid cycle would gain satisfactory concentration in lumen upto 48 hours. However, Haaland et al. (1984) found residues of penicillin upto 84 hours after intrauterine treatment, and therefore recommended that milk should be held until it was found negative for antibiotics. Similar observations were also made by Whitacre (1992), who opined that intra uterine therapy with penicillin should not be done in early post-partum period (before day 30), because mixed bacterial infections may render penicillin ineffective due to penicillinase production, by non pathogenic bacteria.

ii. Chloramphenicol

Based on antibiogram of isolates Savov and Dimitrov (1973) recommended chloramphenicol as the drug of choice for the treatment of endometritis. Sinha et al. (1977) could inhibit 89.30 per cent isolates using chloramphenicol. According to Ziv (1980), chloramphenicol was the most

effective available antibiotic for the treatment of uterine infections. Vahida (1992) could obtain better conception rates with chloramphenicol using it both intramuscular (71.73 per cent) and intrauterine (75.0 per cent) route.

iii. Oxytetracycline

Several workers have used oxytetracycline for treatment of endometritis both systemic and intrauterine. However, Masera et al. (1980) observed that intrauterine route did not result in detectable concentrations in genital tissue, apart from endometrium, 24 hours after administration and opined that, although this could be of advantage to disinfect the endometrium, many infections did not restrict to uterine cavity alone. Bretzlaff et al. (1983) recommended systemic dose of oxytetracycline as 11 mg/kg twice daily, which would maintain a concentration of 5 mic. g/g of genital tissues. This was recommended as the minimum inhibitory concentration level, for several uterine pathogens during the course of treatment. On the other hand, Jayappa and Loken (1983) recommended 0.5 to 5g as intrauterine dose and warned of the possible adverse influence on the uterine defence mechanism, because of the high concentration of the drug and injury caused by the repeated mechanical manipulations. Moore et al. (1984) even recommended that perinatal treatment with oxytetracycline at

a dose of 20 mg/kg would reduce the incidence of [illegible]
Haaland et al. (1984), however, pointed out the defect
intrauterine administration, because of the residues
found upto 84 hours after treatment and recommended
milk should be withheld until it is tested free
antibiotics. Whitacre (1992), was also of the opinion
if intrauterine therapy was instituted oxytetracycline
be the drug of choice especially in early post-
period.

iv. Gentamicin

Sande and Mandell (1980) observed that because of
anaerobic environment in the post partum bovine uterus,
aminoglycoside group of antibiotics like gentam
kanamycin, streptomycin and neomycin are ineffective bec
they require oxygen for their activity. Ziv (1980) repo
a good in vitro effect against uterine pathogens by u
gentamicin with minimum inhibitory concentration of 2-5 µ
g/ml. According to Guedawy et al. (1[illegible] 4 mg of gentami
per kg given intramuscular gave adequate blood and uter
concentration for 36 hours and for thi[illegible] total dose of 2
mg would be necessary. Varadarajan[illegible]85) obtained
conception rate of 56.45 and 40.9 per c[illegible] spectively wit
single insemination in gentamicin an[illegible]

stilboesterol treated animals. Sudhaker et al. (1986) isolated bacterial organisms which were highly or moderately sensitive to gentamicin. A good conception rate was also reported in animals treated with gentamicin. Dholakia et al. (1987) also opined that gentamicin was the most effective drug for treatment of uterine infection. Vahida (1992) also reported the efficacy of gentamicin and remarked that systemic administration was found to be more effective than intrauterine administration.

v. Nitrofurazone

Venkateswarlu et al. (1983 b) by comparing treatment of endometritis with furacin and othr/ antibiotics reported highest percentage of conception in animals treated with Furacin. Narasimhan (1987) reported + nitrofurazone was very effective against puerperal meti s. Vahida (1992) reported a conception rate of 64.7 per by intra uterine administration of nitrofurazone in ani affected with endometritis.

vi. Sulphonamides

According to Bierschwal et al. (1955) concentration of sulphonamides infused i erapeutic remained for more than 12 hours, with peak uterus within 2 hours. The therapeutic value of sulp aching

s

also reported by Ziv (1980) and Gustafsson and Ott (1981) and observed that most of the uterine pathogens appeared to be sensitive to sulphonamides. On the contrary, Ball et al. (1984) reported that the purulent material and debris in the post-partum uterus might antagonise the actions of sulphonamides. As an alternative, Gustafsson (1984) suggested systemic treatment with sulphonamides or in combination with trimethoprim. Chaudhary et al. (1987) indicated that the sulpha trimethoprim combination when used, should be repeated at 12 hours intervals, irrespective of dose, if it is given intrauterine. Vahida (1992), however, reported a comparatively poor result by the administration of co-trimoxazole.

2.6.2. Non antibiotic alternatives

Increased awareness of the risk of bacterial resistance and tissue residues and necessitating withholding meat and milk has resulted in evolving non antibiotic alternatives for treatment of uterine infections. Non antibiotic preparations which are capable of stimulating uterine contractibility (like prostaglandins, oxytocin and oestrogens) and or increase the uterine defence mechanisms (oestrogens, Gonadotrophin releasing hormone) have been widely used (Frank et al., 1983; Gustafsson, 1984; Kuz'mich et al., 1987).

i. Oxytocin

Gustafsson (1984) indicated that oxytocin has a uterotonic effect especially in post-partum period, and remarked that in mild to moderate cases of acute post-partum metritis, this could be used as the only treatment, especially if oestrogens have been used to sensitise the uterus.

ii. Ergonovine

Gustafsson (1984) also recommended use of ergonovine at a dose of 2-5 mg intramuscular, to treat uterine atony which is responsible for post-partum uterine infections.

iii. Oestrogens

The beneficial effect of oestrogens to improve uterine tonicity has been well established. Oestrogen has been used as the only treatment for mild to moderate post-partum uterine infections, with or without retention of placenta, at a dose of 3-10 mg of oestradiol benzoate, oestradiol-valerate or oestradiol cypionate intramuscularly.

iv. Disinfectants

Frank et al. (1983) recommended use of various disinfectants for treatment of post-partum infections as an

alternative to antibiotics. The possibility of suppression of uterine defence mechanisms has, however to be taken careof.

v. Magnetic field

Kuz'mich et al. (1987) reported the effectiveness of magnetic field impulses against uterine sub involution and endometritis syndrome in cows.

2.7. Prostaglandins (PGF_2 alpha)

Prostaglandins, a derivative of prostanoic acid is a closely related group of biologically active unsaturated fatty acid. The most dramatic action of PGF_2 alpha is on the reproductive system, because of its ability for luteolysis which may or may not be accompanied by a morphological degeneration of corpus luteum. This was first demonstrated in Rat (pharris and Wyngarden, 1969) and in Guinea pig (Blatchley and Donovan, 1969). This phenomenon prompted a flury of activity among reproductive scientists who were intrigued by the elusive nature of mechanism for the control of lysis of corpus luteum. The luteolytic effect of PGF_2 alpha was later confirmed by several scientists (Gutknecht et al. 1971; Labhsetwar, 1971; Bartke et al., 1972; Keyes and Bullock, 1974) in lab animals. Seguin et al.

(1974) and Lovie, et al. (1975) demonstrated that the agent responsible for luteal regression in cattle, at the end of oestrous cycle was prostaglandin. It was also postulated that a counter current transfer mechanism existed in the transfer of PGF_2 alpha from the utero ovarian vein to the utero-ovarian artery.

2.7.1. Mechanism of luteolysis

Mechanism of luteolysis brought about by PGF_2 alpha in cattle has been widely reviewed. Pharris and Wyngarden (1969) reported that the luteolytic activity was due to the constriction of ovarian vessels causing ischaemia and starvation, leading to death of luteal cells. Behrman et al. (1971) postulated that rather than vascular and central effect, intracellular changes, induced by a direct action of PGF_2 alpha, might also involve in luteolysis. Batta et al. (1974) and Sato et al. (1974) found a stimulatory effect of PGF_2 alpha on gonadotrophic secretion which could account for luteolysis, induced by PGF_2 alpha. According to Henderson and McNatty (1975), PGF_2 alpha would interfere with the coupling of adenyl cyclase and luteinising hormone, the latter being the luteotrophic substance responsible for maintaining the corpus luteum. Wiltbank and Niswender (1992), however, postulated that for luteolytic actions

(i) the PGF_2 alpha should bind a specific receptor present in the plasma membrane of the large luteal cells; (ii) activate phospho inositide specific phospholipase 'c' resulting in the production of inositol triphosphate (IP3) and Diacyl glycerol; (iii) increased free intracellular calcium concentration either due to increased concentration of IP3 or receptor activated calcium channel; activate protein kinase 'c', due to increase in concentration of free calcium and diacyl glycerol; (iv) decrease progesterone production through protein kinase 'c', effector system, apparently by inhibiting intracellular cholesterol transport; (v) cause degeneration and death of large luteal cells, due to activation of protein kinase 'c' and a sustained elevation of free intracellular calcium concentrations.

2.7.2. Management of oestrous cycle with PGF_2 alpha

The luteolytic effect of PGF_2 alpha has been advantageously used in the management of oestrous cycle in cattle. There are several reports to indicate that PGF_2 alpha or its analogues given to cattle between days 5 and 16 of cycle would cause luteolysis with induction of oestrus and ovulation within 3 days (Lauderdale,1972; Rowson et al., 1972; Louis et al., 1972; Cooper and Rowson, 1975;

Lauderdale, 1975; Philipsen and Rasbech, 1974; Hearnshaw, 1976; Cumming et al., 1977; Jackson et al., 1979). Although, the general route of administration is intramuscular, intrauterine treatment has also been tried with satisfactory results. Shelton (1973) tried intrauterine treatment and found better return to oestrus. Cummins et al. (1974) induced oestrus in 85 per cent of cattle with intrauterine administration of 500 mic.g of Estrumate (Cloprostenol) to the horn ipsilateral to the ovary containing a corpus luteum. This was also demonstrated by Nakahara et al. (1974) with promising results. Cooper and Furr (1974) tried intramuscular injection of Estrumate in 175 heifers by giving double spaced injections, 11 days apart, at a dose of 500 mic. g each and found that 171 heifers were in oestrus between 48 and 96 hours with ovulation occurring normally. Oxender et al. (1974) found that plasma progesterone fell from 4.0 ± 0.4 ng ml^{-1} to 1.5 ± 0.2 ng ml^{-1} at 12 hours and 0.8 ± 0.2 ng ml^{-1} at 48 hours after an injection of 30 mg PGF_2 alpha THAM salt to cows on days 11 of the oestrous cycle. It was also found that plasma LH peaked at 64 ± 4 hours and oestrus began at 74 ± 3 hours with ovulation occurring 104 ± 6 hours after the injection. Oestradiol concentration was more than double by 24 hours and increased to 15.5 ng ml^{-1} by 72 hours after the PGF_2 alpha treatment. Nakahara et al. (1975), Day (1977),

Peters et al. (1977), Barnabe et al. (1978), Seguin and Gustafsson (1978), Singh et al. (1979) and Swensson (1979) recommended 500 mic.g of Estrumate as the most effective dose. Hafs and Manns (1975) tried different doses of 20, 30 and 40 mg of PGF_2 alpha and found that all these doses were equally effective in inducing oestrus in heifers. Barnabe (1975) demonstrated that the injection of the drug into the uterine horn ipsilateral to the active corpus luteum was most effective, whereas administration into the body of uterus was less effective and into the cervix least effective. Moore (1976), proved that the dosage for intrauterine infusion was 4 or 5 times lesser than the intramuscular route. Greve (1976) reported, induction of heat by an intra uterine infusion of 6 mg PGF_2 alpha, into the uteri of 21 cows, with in 48-70 hours, as 95 per cent. The subsequent pregnancy rate was, however, only 20 per cent. The interval between the administration of PGF_2 alpha and the ovulation was reported to be 93.0 ± 18 hours (Elving et al., 1975) and 82.00 ± 5.4 hours (Hoffman et al., 1976). Cumming et al. (1977) reported that 90 per cent of the Fluprostenol treated animals, ovulated within 92 hours of the treatment. Coulson et al. (1979) observed that the preovulatory LH peak averaged 48.6 ± 9.2 ng per ml, which occurred about 70 hours after the second injection of PGF_2 alpha THAM salt. A number of analogues of PGF_2 alpha were

also shown to be luteolytic in cattle. These included Alfa prostol (Jochle et al., 1982; Schames and Karg, 1982), Cloprostenol (Cooper, 1974; Cooper and Rowson, 1975; Stotts et al., 1987) and Fenprostalene (Kelton, 1989).

2.7.3. Fertility in induced oestrus

Cooper (1974) reported a marginal decrease in fertility of cows inseminated at fixed time after the induction of heat. However, Kruif and Brand (1976) found that the conception rate of oestrus detected at PGF_2 alpha induced heat was comparable to that of controls, the values being 56 and 58 per cent for treated and control animals respectively. Roche (1977), on the other hand, reported that inseminations at 48, 60 and 72 hours of treatment with cloprostenol (500 mg) resulted in lower fertility than two insemination at 60 and 72 hours or at 48 and 72 hours after treatment. MacMillan et al. (1978) conducted trials involving 1400 lactating dairy cows and 105 Friesian heifers, which provided data for evaluation of a synthetic analogue of PGF_2 alpha (ICI 80996). Using single injection regimen in conjunction with efficient heat detection, the percentage of cows conceived during the first two weeks of seasonal breeding programme increased from 36 (in controls) to 60 in treated groups. Nair and Madhavan (1984)

induced oestrus in suboestrous animals with Estrumate and reported a conception rate of 43.4 per cent. They observed that the conception rate in the induced heat was significantly influenced by the intensity of heat. Chatterjee et al. (1989) treated suboestrous cross-bred cows with PGF_2 alpha by three different routes. It was observed that in the group treated with 25 mg Dinofertin (PGF_2 alpha THAM salt) intramuscular; 90 per cent cows manifested oestrus at an average interval of 93.80 hours after treatment with 50 per cent conception rate. In the group treated with 10 mg Dinofertin intrauterine; 90 per cent cows manifested oestrus at an average interval of 72.80 hours with 50 per cent conception rate; while in the group treated by intravulvo submucosal (IVSM) route with 10 mg, oestrus was manifested in 100 per cent animals at an average interval of 79 hours with 60 per cent conception rate. They further recommended that IVSM route was the best for induction of oestrus in suboestrous cross-bred cows.

2.7.4. Role of PGF_2 alpha in the management of Reproductive health

In an exhaustive review, Wenzel (1991) explained the use of PGF_2 alpha on reproductive health management of dairy cows and opined that the full potential of this drug has yet to be realised and some of its benefits and limitations are yet to be completely elucidated.

i. Uterine involution

After a detailed study, Etherington et al. (1984) concluded that cloprostenol treatment at day 24 postpartum was beneficial to improve the reproductive performance. Lindel and Kindahl (1983) reported that frequent injection of exogenous PGF_2 alpha would hasten uterine involution. According to Kindahl et al. (1984) and Madej et al. (1984), endogenous PGF_2 alpha concentrations were elevated during the early postpartum period. Several well controlled clinical trials supported the hypothesis that prostaglandins administered to cows 14 to 40 days post-partum would increase the first service conception rate and reduce the calving to conception interval (Young, 1984). An increased uterine motility in ovariectomised, oestrogen primed cows (Garcia-Villar et al., 1987), and in post-partum cows (Ko et al., 1985), was reported when prostaglandin was given. However, Eiler et al. (1984) and Burton et al. (1987) did not demonstrate uterine contractibility by administration of PGF_2 alpha. Young (1989) found that prostaglandins administered every 12 hours from day 3 to day 13 post calving improved uterine involution. Wichtel (1991) reported that there was evidence of endogenous prostaglandins in placental detachment and uterine involution during the first three to four weeks after calving. Research findings indicated that PGF_2 alpha used during the post-partum period

would reduce the interval to first oestrus and the number of days open through its effect on uterine involution or ovarian activity before breeding commences.

ii. Ovarian activity

Evidences indicated that prostaglandins cause the release of hypophyseal hormones in cows (Louis et al., 1974; Hafs and Manns, 1975; Oxender and Seguin, 1975; Furr et al., 1981). Scattered smooth muscle fibres in the ovary have been shown to contract rhythmically in response to prostaglandin, and demonstrated that relatively slow and strong contractions of the ovaries of non pregnant cows could be induced with PGF_2 alpha. Singh et al. (1979) compared this response with that of pregnant cow ovaries and the contractile response was found to be weak and brief. Villeneuve et al. (1987) reported that early postpartum ovarian activity, particularly in the ovary ipsilateral to the previously gravid uterine horn was hastened in cows treated with exogenous PGF_2 alpha over untreated controls. In addition PGF_2 alpha in the early post-partum period positively influenced later ovarian activity (25-35 days post-partum) (Guilbault et al., 1987; Villeneuve et al., 1987), possibly as a result of the direct action of PGF_2 alpha on follicles during the early post-partum period. It has also been hypothesised that PGF_2 alpha in the early post

partum period influence later ovarian activity as a result of its action on the hypophysis (Thatcher, 1988).

2.7.5. Prostaglandin therapy for endometritis

Prostaglandins have been recommended for the treatment of endometritis, because of its luteolytic effect and thereby reducing the period of luteal phase (Von Haam and Rosenfeld, 1942; Rowson et al., 1953). They opined that the corpus luteum would be persistent in uterine infections, luteolysis and induction of oestrus would bring about number of beneficial changes such as increased uterine leucocyte population and increased uterine contractions resulting in evacuation of uterine contents. Brand et al. (1975), listed the indications of PGF_2 alpha (Dinoprost) in bovine reproduction and opined that prostaglandin is effective in induction of oestrus through elimination of persistent corpus luteum due to purulent endometritis, pyometra and mummified foetus. Cooper et al. (1976) treated 204 cases of endometritis in cows with cloprostenol and found that 88 per cent were cured. Casagrande et al. (1977) treated 12 Nelore heifers and 53 cows for subclinical endometritis, with 4 mg synthetic prostaglandin intrauterine and found that 9 heifers and 22 cows became pregnant. Jackson (1977) obtained excellent results by treating endometritis in seven cows with 500 mic.g cloprostenol. It

was reported that all of them were in oestrus with in 2-3 days after administration of cloprostenol. In six cases the subclinical endometritis was resolved with uterine involution within seven days and uterine mucus samples showed no specific microbial growth. He also suggested on a study of chronic endometritis, in cattle that 51, out of 56 animals treated, showed oestrus within 14 days with resolution of the condition. According to Coulson (1978), treatment of chronic endometritis should be aimed to correct the abnormalities in both the ovary and uterus, and to eliminate the corpus luteum and induce a normal oestrus. This could be achieved by the luteolytic action of PGF_2 alpha (Dinoprost Tromethamine-Lutalyse) in the cow to produce regression of corpus luteum, relaxation of cervix and expulsion of uterine contents. Coulson (1978) treated 55 cows suffering from metritis with single intramuscular injection of 25 mg Dinoprost on the day of diagnosis. Oestrus and evacuation of uterine contents occurred in 13 (23.6 per cent) cows within 7 days. Twenty four (43.6 per cent), cows responded in 8-15 days post injection, and 5 (9.1 per cent) revealed no discharge. The total cases responded to treatment was 42 (76.3 per cent). The results indicated the value of Dinoprost as a first line therapy for metritis in cattle. Uwland (1978) recommended synthetic prostaglandin, cloprostenol in the treatment of endometritis in cows. Riznar et al. (1979) also reported the successful

treatment of retention of foetal membranes and endometritis with cloprostenol. All cows with retention of foetal membranes and endometritis, received 2 ml cloprostenol on days 21 and 33 postpartum and resulted in a rapid cure. Zuber (1980) obtained a conception rate of 78 per cent with a single injection of Cloprostenol in 184 cows. Ott and Gustafsson (1981) indicated that chronic or mild or moderate endometritis in cycling cows would improve with prostaglandin therapy due to increase in the number of oestrous periods, in a shorter period of time. A combination of treatment with prostaglandin and iodine was carried out by Seviek et al. (1982). Cloprostenol (0.5 mg) was given in 99 cases of endometritis, twice at an interval of 11 days, in combination with iodine therapy, and obtained a conception rate of 77 per cent. In a comprehensive trial Steffan et al. (1984) allotted 153 cows with metritis in three treatment schedule, 30 days post-partum. One group was given antibiotic intrauterine, chloramphenicol, (Framycetin, 3 doses) second group received 2 doses of PGF_2 alpha intramuscular 14 days apart and the third group formed the control. The overall clinical recovery rates, at 60 days after parturition was identical (49 per cent) in two treated groups, while it was 33 per cent in control. The mean interval from calving to conception was 147 days in the control and was shortened by 16-24 days in the treated

animals. Busch (1984) reported a definite economic advantage in using Cloprostenol for treatment of persistent corpus luteum and pyometra. According to Lusky et al. (1985) the greatest economic advantage of using cloprostenol for mucopurulent endometritis was that more cows conceived at first insemination ptimum period. Aldermir and Kilicoglu (1986) successfully treated cows with chronic endometritis using PGF_2 alpha (Dinoprost) and PGF_2 alpha analogue (Luprostiol). It was observed that all treated animals came to oestrus and became pregnant with a conception rate ranging 50 to 70 per cent. The advantage of using PGF_2 alpha for post-partum uterine infections was also explained by Paisley et al. (1986). According to them luteolysis induced by PGF_2 alpha administration decreased progesterone inhibition of the uterine defence mechanism. Oestrogen released following luteolysis, would stimulate uterine defence mechanism and also stimulate myometrial contractions, that would aid in the expulsion of uterine lochia, pus or other contents. Oestrogens might also have a stimulatory effect on phagocytosis by uterine leukocytes. Tsakalof et al. (1987) in a comprehensive trial, found that the highest fertility rate was obtained with intramuscular injection of 25 mg PGF_2 alpha supplemented with intrauterine antibiotics and sulphonamides, Ferreira et al. (1988) reported a recovery rate of 60 per cent after a single

injection of 500 mic. g of cloprostenol, and 76.7 per cent after a second treatment. Boulet (1989) treated 266 cows in three different groups. Group I received intramuscular injection of prostaglandin (Etiproston) and insertion of Chlortetra-cycline bolus in the uterus and found normal involution. Those in group II received intramuscular injection of prostaglandin and noticed that 70 per cent of the treated animals showed normal involution, while 15 per cent had endometritis at 30 days. Animals in group III received only chlortetracycline and resulted normal involution in 36 per cent of cases. In terms of first oestrus, service period and insemination index, better results were noticed in 23 cows, having chronic endometritis, which were given intramuscular injection of 25 mg Dinoprost together with the intrauterine infusion of 100 ml 4% Lotagen, compared to those treated with lotagen alone (Vukovic et al., 1989). Roy et al. (1990) reported 57 per cent pregnancy rate in 14 cases of endometritis when treated with Dinofertin (Natural PGF_2 alpha). Murray et al. (1990) did not find any significant advantage, in the efficacy by combining antibiotics and PGF_2 alpha, and reported that a single injection of Alfaprostol alone was necessary in the treatment of mild, moderate or severe endometritis. Chauhan et al. (1991) successfully treated 10 Nili-Ravi buffaloes, with mild or chronic endometritis, with 500 mic. g cloprostenol (Estrumate).

Materials and Methods

MATERIALS AND METHODS

Materials for the present study consisted of cross-bred cows (Jersey x Sindhi, Jersey x Local, Brown Swiss x Local and Holstein-Friesian x local) belonging to the Livestock Farm, Mannuthy, attached to the Kerala Agricultural University. These animals were apparently healthy and maintained under identical conditions of feed and management. All cows which did not conceive beyond 45 days post partum, were subjected to detailed clinico-gynaecological examination. Those found to have clinical endometritis as evidenced by aberrations of oestrus, abnormal discharge (Mucopurulent, white or yellow pus) and a large doughy uterus were selected. Those animals which were having corpusluteum and abnormal uterine discharge were also included in the study. The selected animals were randomly alloted to the following four treatment groups.

Group I. Ten animals in their natural oestrus were inseminated twice at 24 hour interval (control).

Group II Eleven animals were inseminated twice at 24 hour interval at natural oestrus and subjected to post insemination intrauterine antibiotic therapy 24 hour later based on the antibiotic sensitivity tests.

Group III Eleven animals were subjected to induction of oestrus by administration of PGF_2 alpha 25 mg intramuscular (Lutalyse 5 ml*) (Fig. 3.1) at 8-12 days of their cycle and inseminated twice at 24 hour interval at the induced oestrus.

Group IV Ten animals were subjected to induction of oestrus by administration of PGF_2 alpha 25 mg (Lutalyse 5ml) intramuscular at 8-12 days of cycle and inseminated twice at 24 hour interval and were given post insemination antibiotic therapy; intrauterine, based on antibiotic sensitivity, 24 hours later.

The following observations were made:

3.1. Time taken from the administration of PGF_2 alpha to the onset of oestrus in Group III and IV

Each animal after the administration of the PGF_2 alpha was closely observed at an interval of 6 hours and those found to be in oestrus were confirmed by rectal examination. The interval from treatment to the onset of oestrus was recorded as the time taken for the induction of oestrus.

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* Lutalyse (Inj): 5 ml (UpJohn)
Each ml contains Dinoprost Tromethamine equivalent to Dinoprost 5 mg

3.2. Duration of oestrus

Each cow in oestrus, in all the groups was closely observed at an interval of six hours till the symptoms of oestrus subsided. The period from the beginning to the end of clinical and behavioural signs of oestrus was considered as the duration of oestrus.

3.3. Intensity of oestrus

The intensity of oestrus was graded as intense, medium or weak from the clinical and behavioural signs (Sharma et al., 1968).

3.4. Physical changes of reproductive tract during oestrus

Physical changes of reproductive tract including congestion of vulval mucosa, oedema of vulval lips, sliminess of vulva and tonicity (low, medium and high) of uterine horns were recorded after a detailed clinico-gynaecological examination.

3.5. Nature of oestrual mucus

Oestrual mucus of the animals belonging to all four groups were subjected to detailed examination and classified on the basis of appearance, as purulent, cloudy, with flakes of Pus and clear. Nature of oestrual mucus of animals belonging to groups III and IV was examined and classified both before and after the PGF_2 alpha treatment.

Aseptic collection of uterine discharge was carried out by using an instrument fabricated by Vahida (1992), (Fig. 3.2 & 3.3), a modification of the design by Minocha et al. (1964). The instrument used consisted of three telescoping metal tubes, the outer one having a length of 30.5 cm, with outer diameter of 0.7 cm and 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. The inner tube (sampler) was 58.5 cm long with an outer diameter of 0.3 cm. The sampler was having two holes, each 0.5 cm in length and 0.2 cm in width and one situated at 0.4 cm and 0.8 cm away from the tip, on the opposite sides and intended for aspirating the dishcarge 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 contamination during introduction. A stiletto (70 cm length and 0.20 cm diameter) was inside the sampler. The instrument was cleaned well before use and dried in an air drier. The outer tube, the inner assembly containing the second tube and sampler with the stiletto, were wraped separately in wraping 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 tube and sampler with stiletto were introduced through the outer tube, until the tip reached the site of collection in the uterus. The stiletto was removed and the sampler alone was pushed 1-3 cm further forward, 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, 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 then transferred into a collection vial, through the holes exposed by drawing back the second tube. The nature of the uterine discharge was recorded and the sample thus collected was used for isolation of the organisms, and for antibiotic sensitivity tests.

3.6. Isolation and identification of bacterial organisms

A 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 sample and incubated at 37°C for 18 to 24 hours. One half of a well

isolated single colony was subjected to Gram's staining and other half was subcultured on Mueller-Hinton Agar*/Tryptic Soy Agar** for further purification of the organism. Isolation and identification of organisms were attempted with 21 samples.

Identification of the most predominantly occurring isolates from each sample was elcidated 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).

3.7. Antibiotic sensitivity test and antibiotic treatments

3.7.1. Preparation of culture medium

The reconstituted Mueller-Hinton Agar (38g of Mueller-Hinton Agar in 1000 ml distilled water) was 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 to test whether the plates were sterile or not.

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* Mueller-Hinton Agar: Himedia Laboratory Pvt.Ltd., Bombay 400 686, India

** Tryptic Soy Agar: Span Biologicals, 174, New Industrial Estate, Udhiana, Surat, India.

3.7.2. Inoculation of plates

Few well isolated colonies of actively growing organisms were suspended into sterile peptone water so as to obtain a turbidity usually comparable to half the density of a No.1 MacFarland's 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 Mueller-Hinton Agar plate by 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).

3.7.3. Application of Antibiotic Discs

The antibiotic discs used are given below.

Zone size interpretative chart

No.	Discs used	Strength mic.g	Sensitive (mm or more)	Inter-mediate	Resistant (mm or less)
*1.	Penicillin	10 IU	29	-	28
2.	Streptomycin	10	15	12-14	11
3.	Oxytetracycline	30	19	15-18	14
4.	Nitrofurantoin	300	17	15-16	14
5.	Chloramphenicol	30	18	13-17	12
6.	Gentamicin	10	15	13-14	12
7.	Suphadiazine	300	17		12

* Diameter of zone of inhibition of staphylococci

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 inibition, the discs were kept at a minimum distance of 15 mm from ech other and also from the edges of the plate.

3.7.4. Incubation

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

3.7.5. Reading of zones of inhibition

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

3.7.6. Artificial insemination

All cows in oestrus were inseminated with fresh chilled semen twice at 24 hour interval. Cows which failed to settle with first insemination were reinseminated on subsequent oestrus.

3.7.7. Antibiotic treatment

Uterine discharge of animals in group II and IV were subjected to antibiotic sensitivity tests, and based on the results they were subjected to post insemination intrauterine antibiotic therapy at the recommended systemic dose level. Animals which showed sensitivity to gentamicin were given single intrauterine administration of 1200 mg gentamicin (Gentamicin inj*) in aqueous vehicle. Animals showed sensitivity to penicillin and streptomycin were given single intrauterine administration of 2.5 g of streptomycin and penicillin 20 lakhs units. Bistrepen** (LDV) dissolved in 20 ml distilled water. Animals which showed sensitivity to oxytetracycline were treated by single intrauterine administration of 1.5 g oxytetra-cycline (Alcyclin-0 inj***), 1.5 g of oxytetracycline dis-solved in 30 ml aqueous vehicle. Chloramphenicol sensitive cases were

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* Gentamicin inj (30 ml) (Alembic). Each ml contains Gentamicin Sulphate I.P. equivalent to 40 mg Gentamicin base.

** Bistrepen LDV (2.5 g) (Alembic). Each vial contains procaine Penicillin G, B.P. (Vet) 1,500,000 units,penicillin G sodium B.P (vet) 500,000 units. Streptomycin sulphate B.P. (Vet) equivalent to 2.5 g of streptomycin base.

*** Alcyclin 0 inj (30 ml) (Alembic). Each ml contains oxytetracycline dihydrate I.P. equivalent to Anhydrous oxytetracycline 50 mg.

treated by single intrauterine administration of 1500 mg chloramphenicol (Enteromycetin)**** mixed in 30 ml sterile distilled water. Similarly, animals which were sensitive to Nitrofurantoin were treated by single intrauterine administration of nitrofurazone 0.2 per cent W/W 30ml (Furacin Vet 30ml*****). All the antibiotic treatments were done 24 hours after the second insemination.

3.8. Number of inseminations per conception

Those cows which failed to conceive were reinseminated on subsequent oestrus and pregnancy diagnosis was done by rectal examination, between days 45 and 60. Number of inseminations required per conception was calculated in each group.

3.9. Conception rate

First service conception rate and overall conception rate of each group was calculated.

**** Enteromycetin (8 ml inj, Days Pharma).Each ml contains Chloramphenicol I.P. 125 mg.

***** Furacin Vet 30 ml (Alembic). Nutrofurazone B.P. 0.2 per cent W/W in a water soluble base.

'se (Inj) 5 ml (UpJohn)

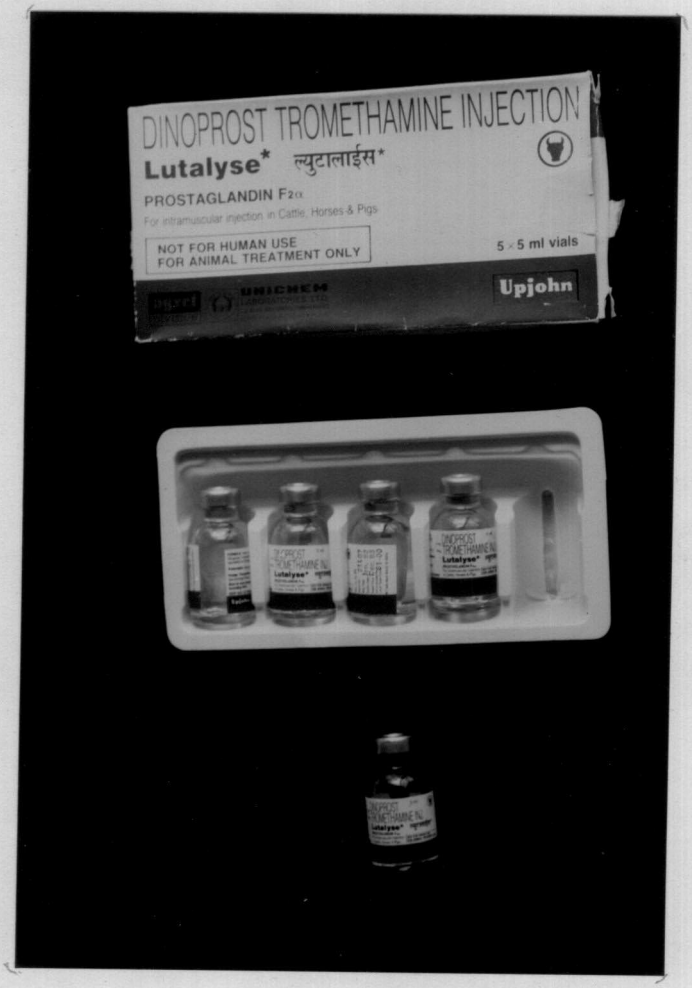
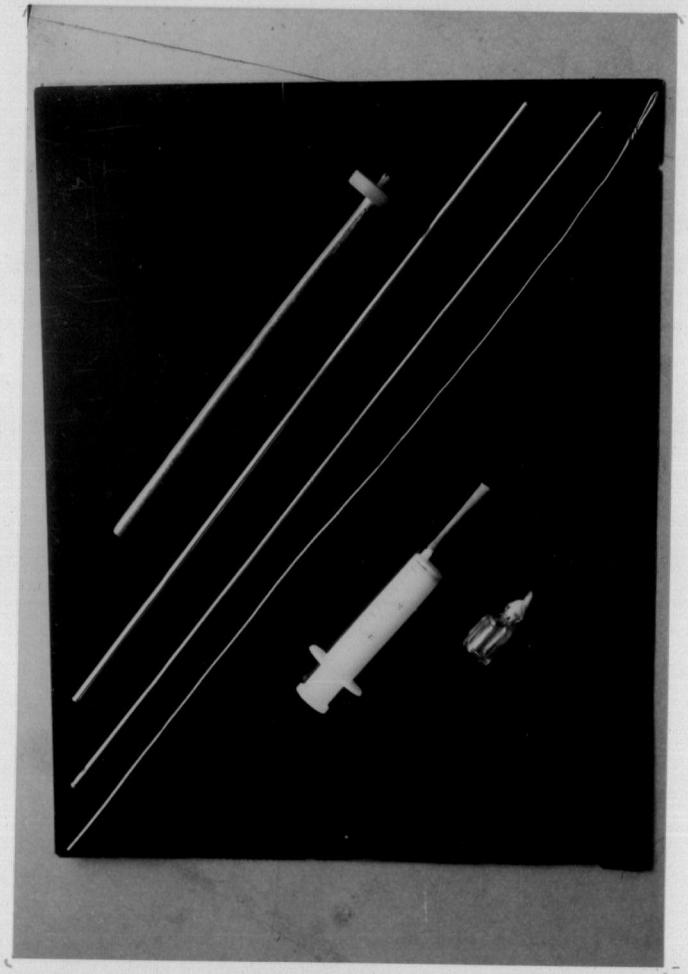
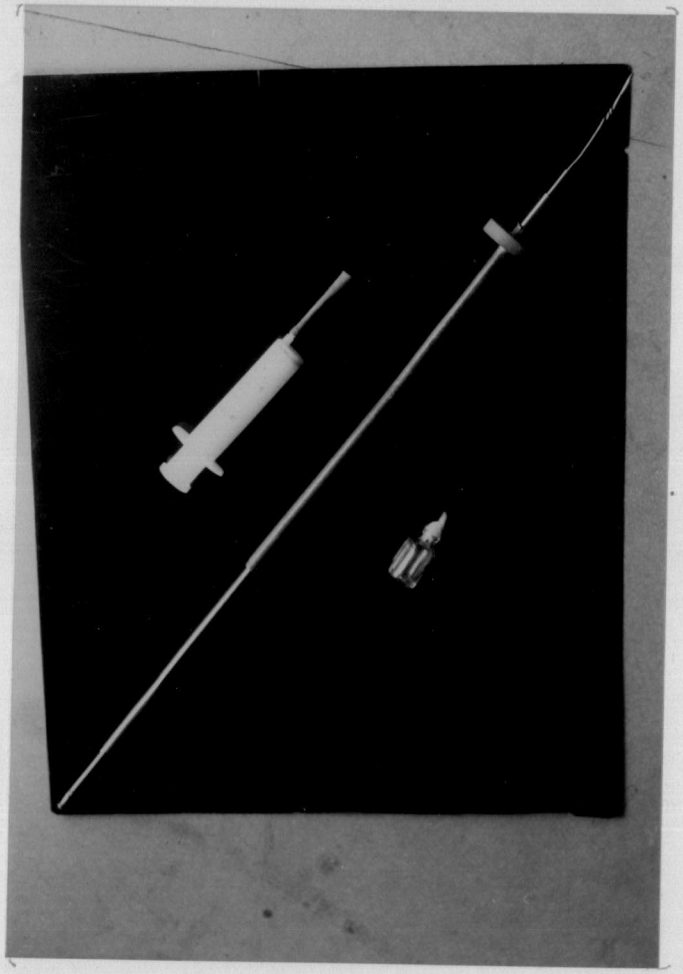

DINOPROST TROMETHAMINE INJECTION
Lutalyse*
ल्युटालाईस*
PROSTAGLANDIN F2α
For intramuscular injection in Cattle, Horses & Pigs
NOT FOR HUMAN USE
FOR ANIMAL TREATMENT ONLY
5 x 5 ml vials
UNICHEM
Upjohn

Fig. 3.2. Instrument used for the aseptic collection of uterine discharge

Fig. 3.3. Instrument for the aseptic collection of uterine discharge: Different parts

Outertube, middle tube, sampler tube, stiletto, syringe with adaptor and collection vial





RESULTS

Results of the investigation of the therapy for endometritis using PGF_2 alpha are presented in table 1 to 12 and figures 1 to 9.

4.1. Time taken for the induction of oestrus

Time taken for the induction of oestrus in animals in group III and IV is presented in table 1. All the animals in group III and IV evinced oestrus after administration of Lutalyse. It could also be seen that the interval from the administration of the drug to the expression of oestrus ranged from 48 to 120 hours with an average of 61.81 hours in group III. The time taken for induction of oestrus after the administration of drug in group IV was found to be within a range of 36 to 72 hours and averaged 54 hours.

4.2. Duration of oestrus

The duration of oestrus is presented in table 2. It varied from 12-24 hours (mean 21.6 hours) 18-24 hours (mean 23.36 hours), 24-48 hours (mean 28.36 hours) and 24-48 hours (mean 31.6 hours) in group I, II, III and IV respectively. Analysis of data revealed a significant difference in the duration of oestrus between groups. It was further revealed that duration of oestrus was significantly different between groups I and IV ($t' = 2.8910$) and II and IV ($t' = 2.6445$).

Tables

4.3. Intensity of oestrus

The data presented in table 3 and figure 1 revealed that in group I, out of 10 animals 6 (60 per cent) showed intense oestrus, 3 (30 per cent) medium oestrus and 1 (10 per cent) weak signs of oestrus. Similarly, in group II, out of 11 animals, 8 animals (72.72 per cent) showed intense oestrus, 2 animals (18.18 per cent) with medium oestrus and one animal (9.09 per cent) showed weak oestrus. It could also be noted that, the percentage of intense, medium and weak oestrus was 66.66, 23.80 and 9.52 per cent respectively in natural oestrus. It may also be seen from the table 4 and figure 2 that the occurrence of intense, medium and weak oestrus was 8 (72.72 per cent), 2 (18.18 per cent) and 1 (9.09 per cent) respectively in group III and the same in group IV was 6 (60 per cent) 2 (20 per cent) and 2 (20 per cent) respectively when oestrus was induced with Lutalyse. The percentage of intense, medium and weak oestrus in both induced groups put together was however, 66.66, 19.04 and 14.28 per cent respectively.

4.4. Physical changes of reproductive tract

Physical changes of reproductive tract during natural oestrus (Group I and II) and induced oestrus (group III and IV) are presented in table 5 and 6 and figures 3 and 4 respectively. In group I, the percentage of animals showing

oedema of vulva, congestion of vulval mucosa, vulval discharge and sliminess was in 80, 100 and 100 per cent while the values in group II were 81.81, 90.90 and 90.90 per cent respectively. Similarly, the percentage of animals which showed high, medium and low uterine tone was 70,30 and 0 per cent respectively in group I, while it was 63.63, 36.36 and 0 per cent in group II respectively. The overall value when the two groups put together (natural oestrus) were 80.95, 95.25 and 95.25 per cent in respect of vulval oedema, congestion of vulval mucosa and vulval discharge and sliminess, where as the tonicity of uterus was high, medium and low in 66.66, 33.33 and 0 per cent. All the animals in group III showed oedema of vulva, congestion of vulval mucosa and vulval discharge and sliminess. Similarly, tonicity of uterus was high in all experimental animals in group III. In group IV also all the animals showed vulval oedema, congestion of vulval mucosa and sliminess and vulval discharge, while tonicity of uterus was high in 60 per cent animals and medium in 40 per cent animals. Thus it could be seen from the table that all the animals in which oestrus was induced showed vulval oedema, congestion discharge and sliminess, and the maximum percentage of these animals showed high tonicity of uterus.

4.5. Nature of oestrual discharge

Perusal of data presented in table 7 and figure 5 revealed that out of 10 animals in group I, 3 (30 per cent) had purulent discharge, 2 (20 per cent) discharge with flakes and in 5 (50 per cent) it was cloudy. In group II out of 11 animals 4 (36.36 per cent) had purulent discharge, 2(18.18 per cent) had discharge with flakes of pus and in 5 (45.45 per cent) it was cloudy. Data presented in table 8 and figures 6 and 7 showed that in groups III and IV the number of animals having pruulent vaginal discharge 3(27.27 per cent) and 5 (50 per cent) respectively before treatment. After treatment, however, the number of animals with purulent discharge was reduced to 2 (18.18 per cent) and 1 (10 per cent) in the respective groups. Similarly in groups III and IV the number of animals with flakes was 2 (18.18 per cent) and 3 (30 per cent) before treatment which were reduced to 1 (9.09 per cent) and 1 (10 per cent) respectively after treatment. So also, out of 6 animals (54.54 per cent) in group III having cloudy discharge before treatment, the number of reduced to 1 (9.09 per cent) after treatment with Lutalyse. Similarly in group IV out of 2 animals (20 per cent) which had cloudy discharge, 50 per cent were cured after treatment. It could also be seen from the table 8 and figures 6 and 7 that out of 11 animals in group III and also out of 10 animals in group IV in which

discharge was not clear before treatment, 63.63 per cent in group III and 70 per cent in group IV showed clear discharge after the treatment.

4.6. Isolation, identification and antibiogram of bacterial organisms

Uterine discharge from 21 animals were subjected to isolation and identification of bacteria and the results are given in table 9 and 10. The organisms were S. aureus (14.28 per cent), (Fig 4.1), coagulase negative staphylococci spp. (9.52 per cent), Bacillus spp. (23.80 per cent), Corynebacterium spp (9.52 per cent), Pseudomonas (14.28 per cent) (Fig. 4.2) and Citrobacter (23.84 per cent) and the yeast Candida guilliermondii (4.76 per cent), (Fig. 4.3 & 4.4). Among the S. aureus species 33.33 per cent were sensitive to gentamicin and chloramphenicol and resistant to Penicillin, Streptomycin, oxytetracycline, sulphadiazine and nitrofurantoin. All the coagulase negative staphylococci were sensitive to sulphadiazine, nitrofurantoin, chloramphenicol and gentamicin, but were resistant to Penicillin. While 50 per cent of the isolates were sensitive to Oxytetracycline, the remaining were resistant to oxytetracycline. All the Bacillus spp. isolated were sensitive to Streptomycin, chloramphenicol, nitrofurantoin and gentamicin. Eighty per cent of the

Bacillus isolates were sensitive to penicillin, oxytetracycline and sulphadiazine. Corynebacterium spp. isolated were sensitive to oxytetracycline, sulphadiazine, chloramphenicol and gentamicin. Fifty per cent of them were sensitive to penicillin and Streptomycin. All the Pseudomonas isolates were resistant to all the seven antibiotics tested. All the citrobacter isolates were resistant to penicillin, streptomycin and Furazolidone. Sixty per cent of them were sensitive to oxytetracycline and gentamicin, 80 per cent to sulphadiazine and 20 per cent to chloramphenicol.

It could be seen from the table 11 and figure 8 that, out of 20 samples 16 (80 per cent) were sensitive to gentamicin 14 (70 per cent) sensitive to chloramphenicol, 11 (55 per cent) to sulphadiazine. 11 (55 per cent) to oxytetracycline, 10 (50 per cent) to nitrofurantoin 5 (25 per cent) to streptomycin and 4 (20 per cent) to penicillin. (Fig. 4.5, 4.6 and 4.7)

4.7. Number of inseminations per conception

The number of inseminations required per conception and conception rates are presented in table 12 and figure 9. Perusal of data revealed that, the number of animals conceived was 3 out of 10, 6 out of 11, 9 out of 11 and 10

out of 10 in group I, II, III and IV respectively. The number of inseminations required per conception in the respective groups was 5.66, 3.83, 1.77 and 2.20.

4.8. Conception rate

The conception rate for first insemination in group I to IV were 30, 30, 54.53 and 50 per cent respectively, while the overall conception rates were found to be 30, 54.54, 81.80 and 100 per cent respectively. Analysis of data revealed that the overall conception rate was significantly different between groups. It was further observed that the overall conception rates between groups I and III ($t' = 5.5886$); I and IV ($t' = 4.8341$) and II and IV ($t' = 2.9186$) were significantly different.

Tables

Table 1. Time taken for induction of oestrus

Groups	No. of animals	No. of animals evinced oestrus	Time taken for induction of oestrus (hours)	
			Range	Mean
III	11	11	48-120	61.81
IV	10	10	36-72	54.00

Inference: Time taken for the induction of oestrus between group III and IV is not significantly different ($t' = 0.9759$)

Table 2. Duration of oestrus in experimental animals.

Groups	No. of animals in each group	Duration of oestrus (hours)	
		Range	Mean
I	10	12-24	21.60
II	11	18-24	23.36
III	11	24-48	28.36
IV	10	24-48	31.60

Inference: Duration of oestrus between groups I and IV ($t' = 2.8910$) and II and IV ($t' = 2.6445$) were significantly different.

Table 3. Intensity of oestrus in Group I and II

Groups	No. of animals	Intensity of natural oestrus					
		Intense		Medium		Weak	
		Number	Percent	Number	Percent	Number	Percent
I	10	6	60.00	3	30.00	1	10.00
II	11	8	72.72	2	18.18	1	9.09
Total	21	14	66.66	5	23.80	2	9.52

Table 4. Intensity of oestrus in Group III and IV

Groups	No. of animals	Intensity of induced oestrus					
		Intense		Medium		Weak	
		Number	Percent	Number	Percent	Number	Percent
III	11	8	72.72	2	18.18	1	9.09
IV	10	6	60.00	2	20.00	2	20.00
Total	21	14	66.66	4	19.04	3	14.28

Table 5. Physical changes of the reproductive tract during natural oestrus (Group I and II)

Groups	No. of animals	Oedema of vulva		Congestion of vulval mucosa		Sliminess and vulval discharge		Tone of the uterine horns					
								High		Medium		Low	
		No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent
I	10	8	80.00	10	100.00	10	100.00	7	70.00	3	30.00	-	-
II	11	9	81.81	10	90.90	10	90.90	7	63.63	4	36.36	-	-
Total	21	17	80.95	20	95.25	20	95.25	14	66.66	7	33.33	-	-

Table 6. Physical changes of the reproductive tract during induced oestrus (Group III and IV)

Groups	No. of animals	Oedema of vulva		Congestion of vulval mucosa		Sliminess and vulval discharge		Tone of the uterine horns					
								High		Medium		Low	
		No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent
III	11	11	100.00	11	100.00	11	100.00	11	100.00	-	-	-	-
IV	10	10	100.00	10	100.00	10	100.00	6	60.00	4	40.00	-	-
Total	21	21	100.00	21	100.00	21	100.00	17	80.95	4	19.04	-	-

Table 7. Nature of discharge in Group I and II

Groups	No. of animals in each group	Nature of Discharge							
		Purulent		With flakes		Cloudy		Clear	
		No.	Percent	No.	Percent	No.	Percent	No.	Percent
I	10	3	30.00	2	20.00	5	50.00	-	-
II	11	4	36.36	2	18.18	5	45.45	-	-

Table 8. Nature of Discharge in Group III and IV

Groups	No. of animals in each group	Nature of Discharge							
		Purulent		With flakes		Cloudy		Clear	
		BT	AT	BT	AT	BT	AT	BT	AT
III	11	3 (27.27)	2 (18.18)	2 (18.18)	1 (9.09)	6 (54.54)	1 (9.09)	-	7 (63.63)
IV	10	5 (50.00)	1 (10.00)	3 (30.00)	1 (10.00)	2 (20.00)	1 (10.00)	-	7 (70.00)

BT - Before treatment ; AT - After treatment
Figures in parenthesis represent the percentage

Table 9. Identification of organisms

Sl.No.	Organisms identified	Number	Per cent
1.	Staphylococcus aureus	3	14.28
2.	Coagulase negative staphylococci spp.	2	9.52
3.	Bacillus spp.	5	23.80
4.	Corynebacterium spp.	2	9.52
5.	Pseudomonas spp.	3	14.28
6.	Citrobacter spp.	5	23.84
7.	Candida guilliermondii	1	4.76
	Total	21	100.00

Table 10. Bacterial isolates and their sensitivity pattern

Isolate No.	Name of organisms	Antibiotics used						
		P	St	O	Z	C	F	J
1.	S. aureus	R	R	R	R	R	R	R
2.	S. aureus	R	R	R	R	R	R	R
3.	S. aureus	R	R	R	R	S	R	S
4.	Coagulase negative staphylococci	R	R	S	S	S	S	S
5.	Coagulase negative staphylococci	R	R	R	S	S	S	S
6.	Bacillus spp.	S	S	S	S	S	S	S
7.	Bacillus spp.	S	S	S	R	S	S	S
8.	Bacillus spp.	S	S	S	S	S	S	S
9.	Bacillus spp.	S	S	S	S	S	S	S
10	Bacillus spp.	R	S	R	S	S	S	S
11	Corynebacterium spp.	S	S	S	S	S	S	S
12	Corynebacterium spp.	R	R	S	S	S	S	S
13	Pseudomonas spp.	R	R	R	R	R	R	R
14	Pseudomonas spp.	R	R	R	R	R	R	R
15	Pseudomonas spp.	R	R	R	R	R	R	R
16	Citrobacter amalonaticus	R	R	S	S	R	R	S
17	Citrobacter amalonaticus	R	R	S	S	R	R	S
18	Citrobacter amolonaticus	R	R	S	S	R	R	S
19	Citrobacter spp.	R	R	R	S	R	R	R
20	Citrobacter spp.	R	R	R	R	S	R	R

P=Penicillin, St=Streptomycin, O=Oxytetracycline, C=Chloramphenicol, F=Nitrofurantoin J=Gentamicin, S=Sensitive, R=Resistant.
Z = Sulphadiazine

Table 11. Response to sensitivity tests

Sl. No.	Drugs	Sensitive		Resistant		Total
		Number	Percent	Number	Percent	
1.	Penicillin	4	20	16	80	20
2.	Streptomycin	5	25	15	75	20
3.	Oxytetracycline	11	55	9	45	20
4.	Chloramphenicol	14	70	6	30	20
5.	Sulphadiazine	11	55	9	45	20
6.	Nitrofurantoin	10	50	10	50	20
7.	Gentamicin	16	80	4	20	20

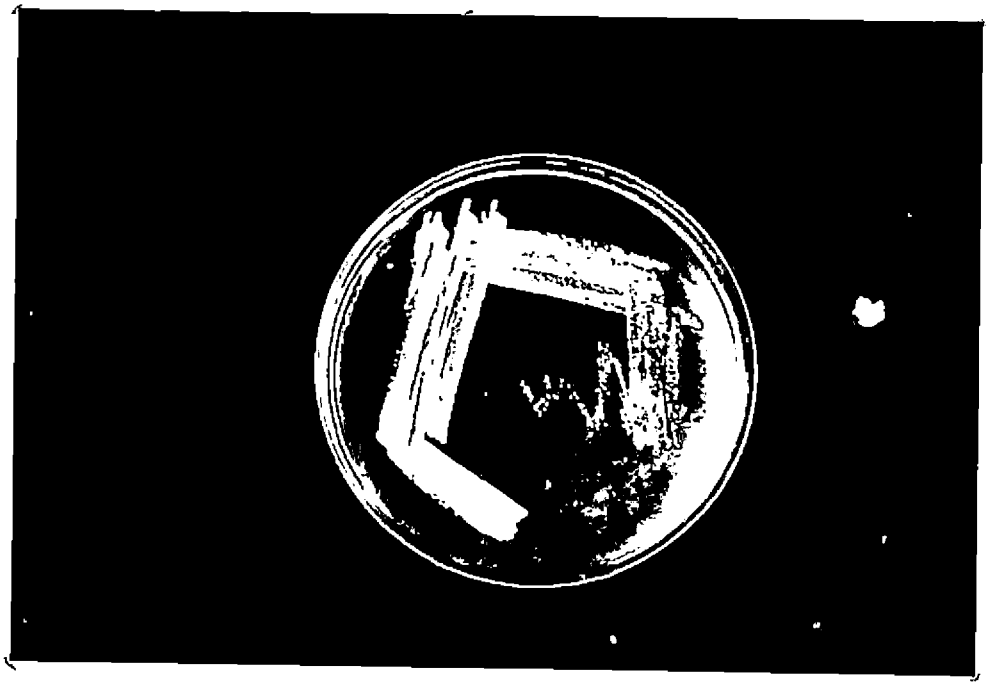
Table 12. Number of inseminations required per conception and conception rate

Groups	No. of animals in each group	No. of animals conceived	No. of inseminations required per conception	Conception rate for first insemination	Overall conception rate
I	10	3	5.66	30.00	30.00
II	11	6	3.83	30.00	54.54
III	11	9	1.77	54.53	81.80
IV	10	10	2.20	50.00	100.00

Inference: Significant difference in the overall conception rate between group I and IV ($t' = 4.8341$)
Significant difference in the overall conception rate between group II and IV ($t' = 2.9186$)
Significant difference in the overall conception rate between group I and III ($t' = 3.5886$)

Fig. 4.1. Staphylococcus aureus culture on Mueller Hinton Agar

Fig. 4.2.



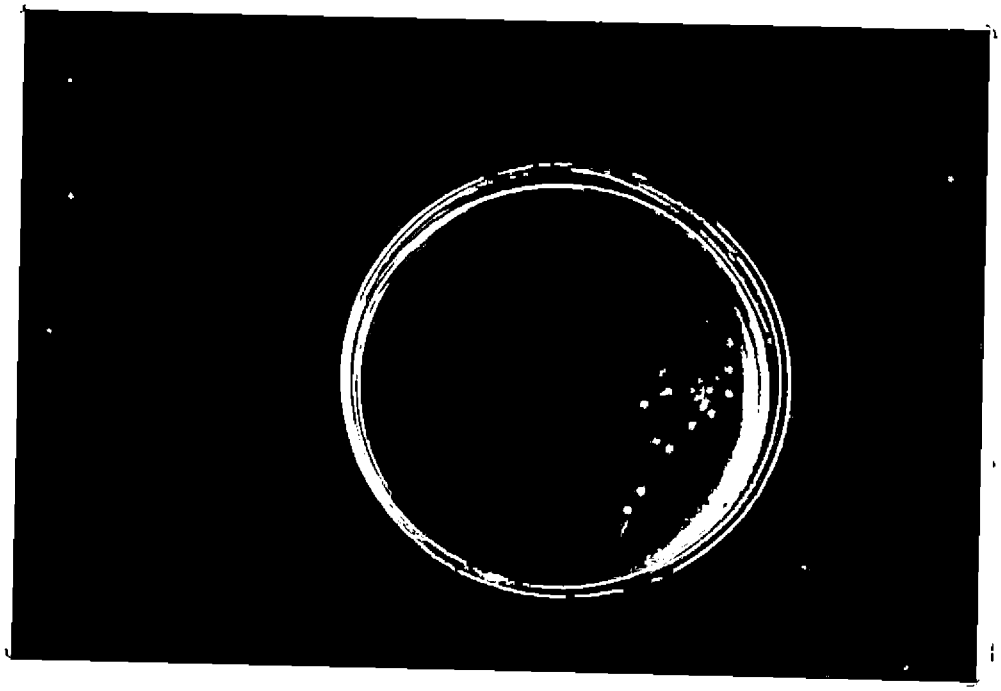
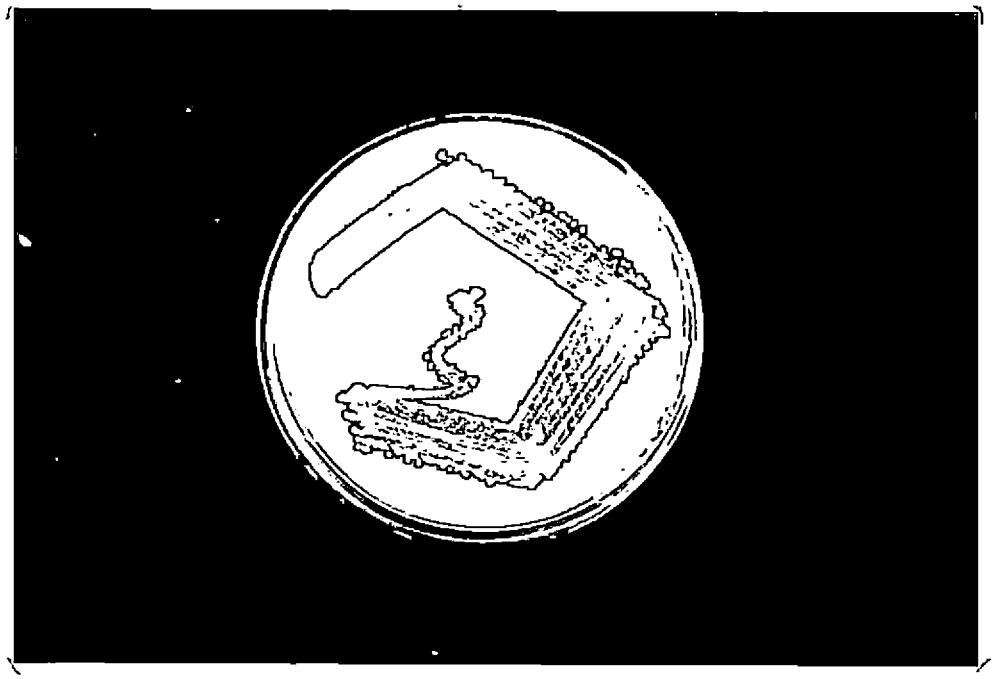


Fig. 4.3. Candida guilliermondii culture on Potato Dextrose Agar

Fig. 4.4. Candida guilliermondii stained smear



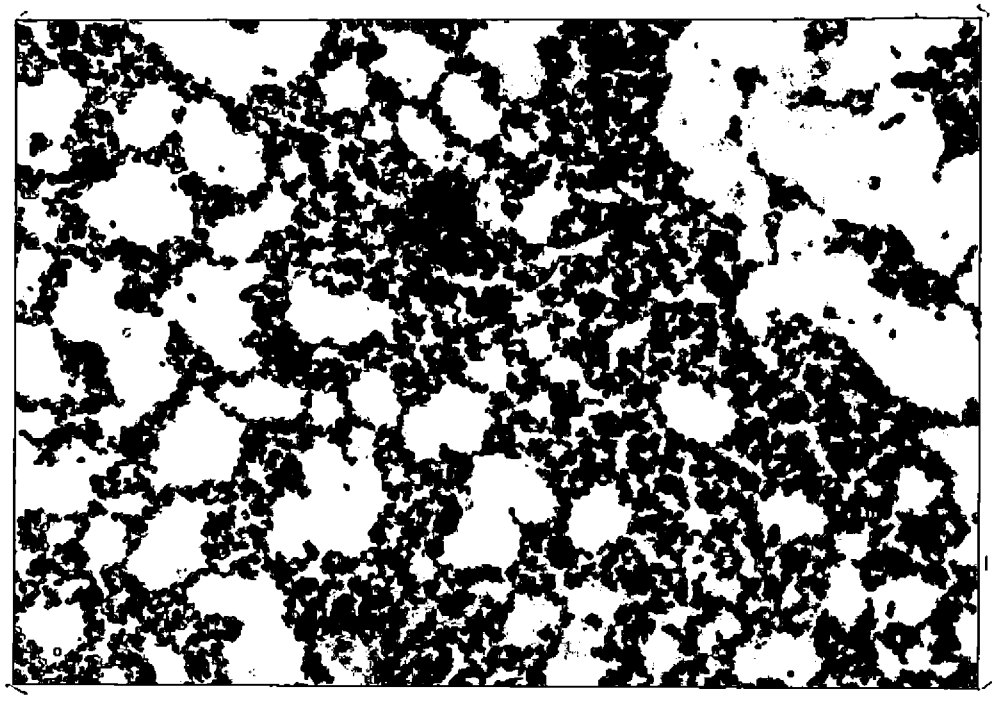


Fig. 4.5. Bacterial isolate sensitive to Penicillin, Streptomycin, Chloramphenicol, Tetracycline, Nitrofurantoin and Sulphadiazine

Fig. 4.6. Bacterial isolate sensitive to Tetracycline, Chloramphenicol, Streptomycin, Nitrofurantoin and resistant to Penicillin and Sulpha diazine

Fig. 4.7. Bacterial isolate resistant to Streptomycin, Nitrofurantoin, Chloramphenicol, Penicillin, Gentamicin and Tetracycline

FIG. 1. INTENSITY OF NATURAL OESTRUS IN GROUP I AND II

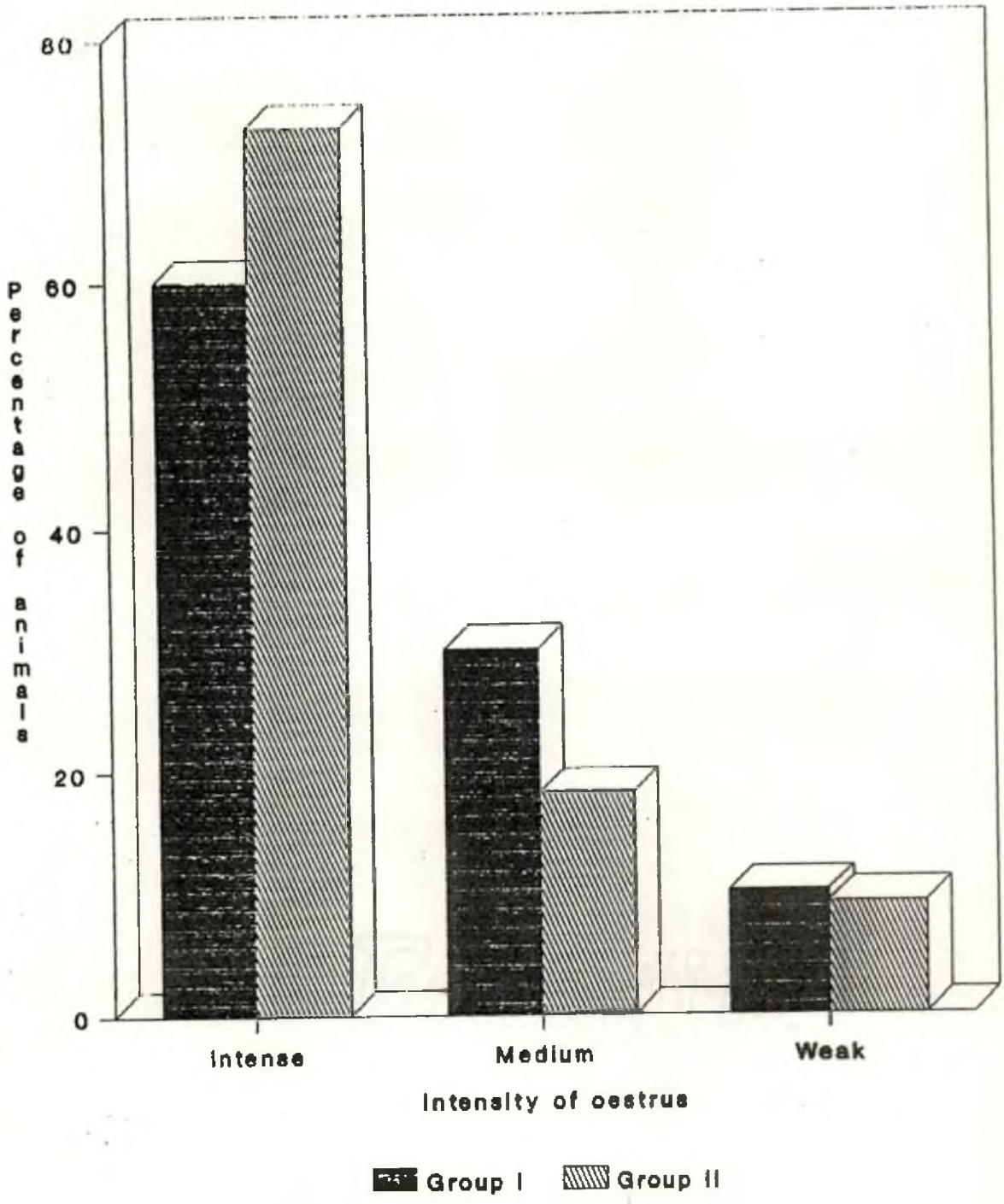


FIG. 2. INTENSITY OF INDUCED OESTRUS IN GROUP III AND IV

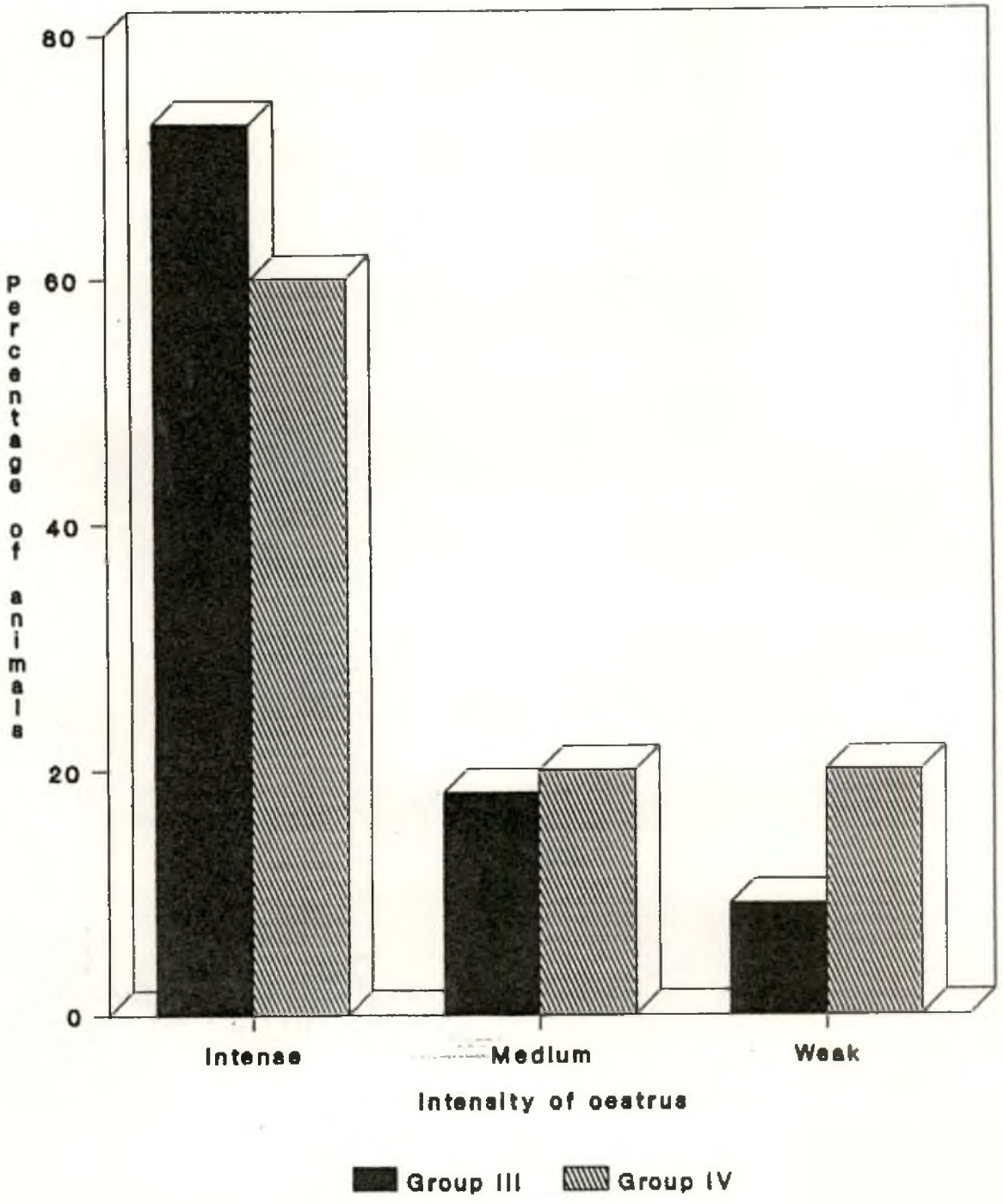
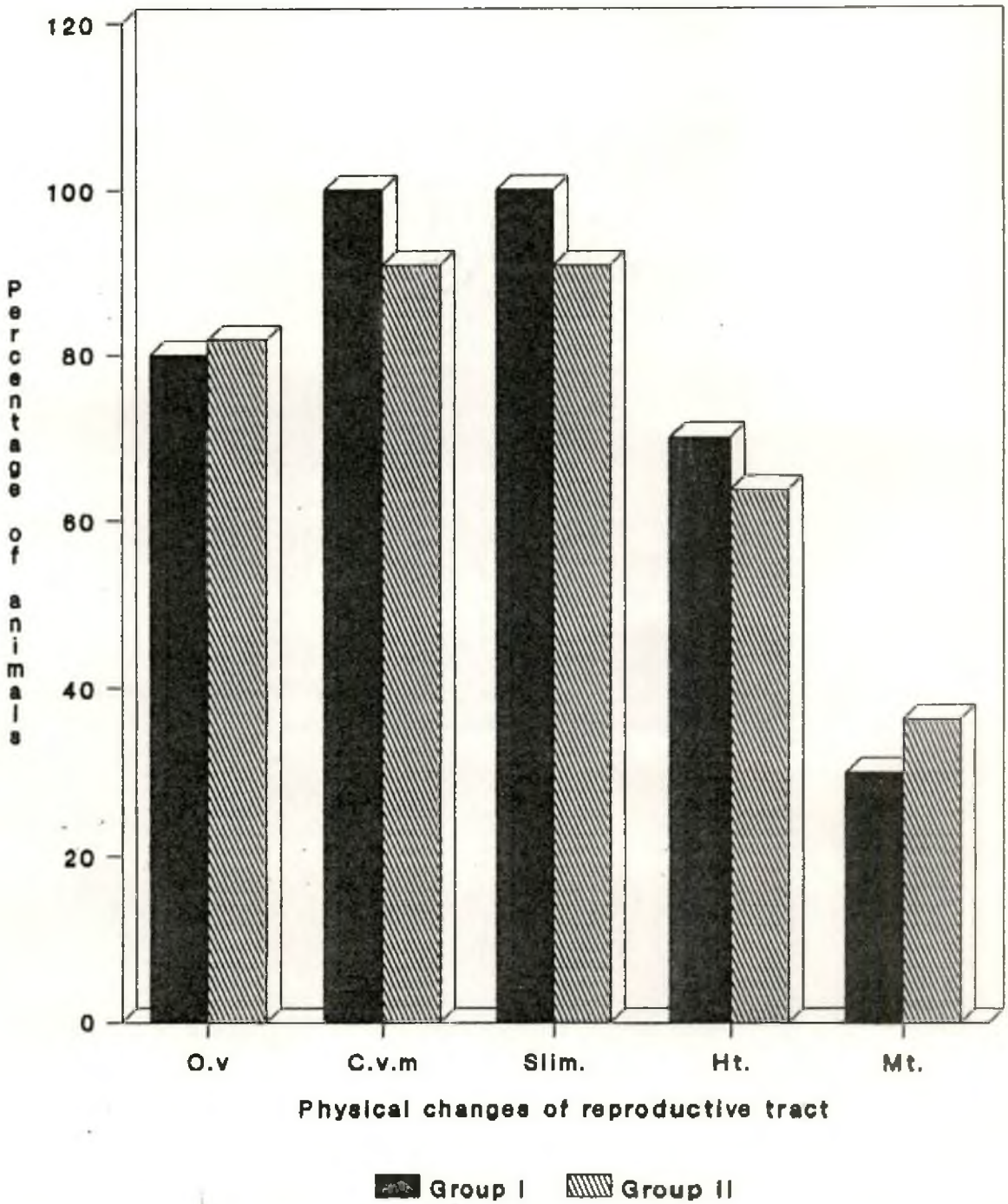
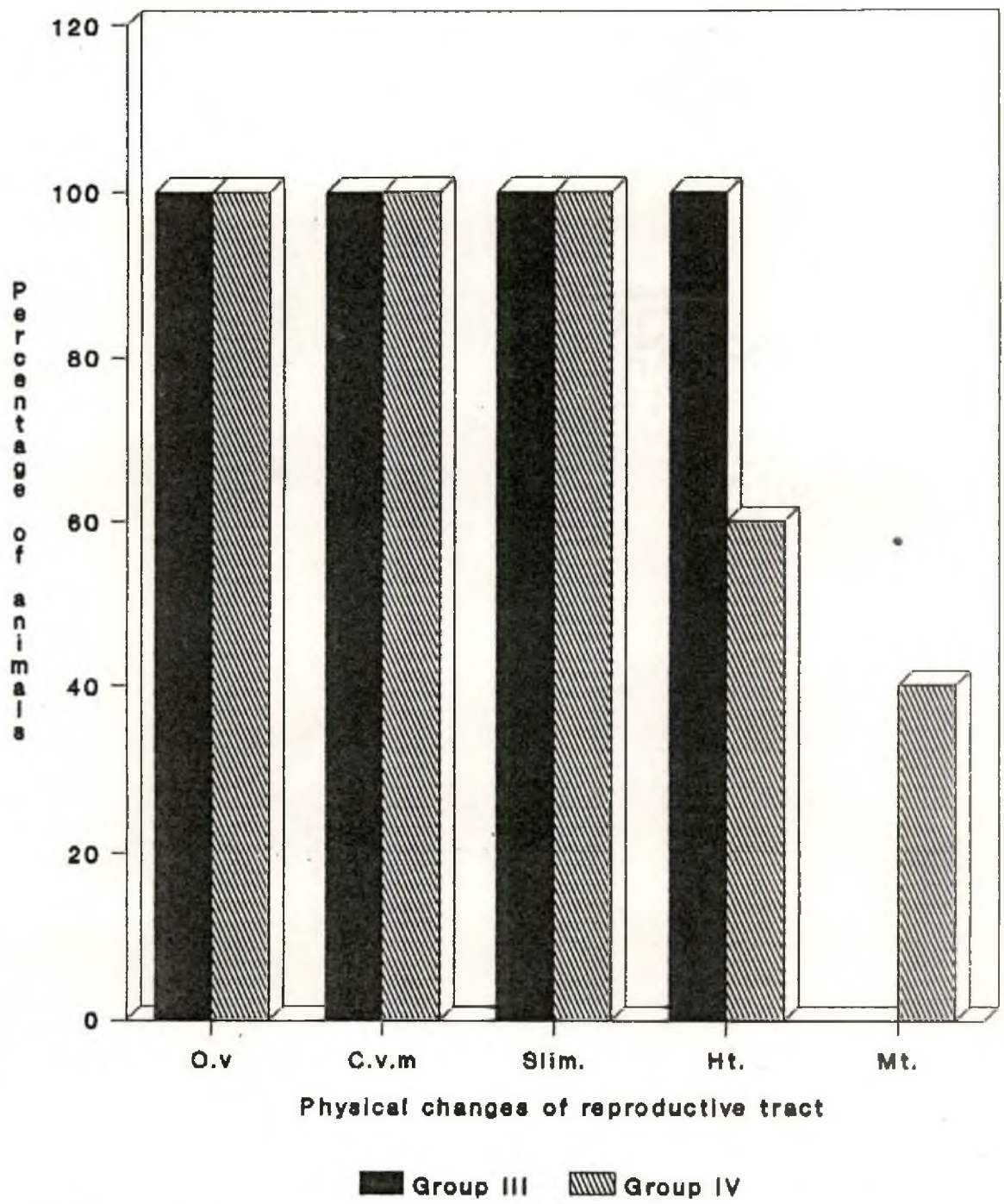


FIG. 3. PHYSICAL CHANGES OF REPRODUCTIVE TRACT DURING NATURAL OESTRUS



O.V. = Oedema vulva; C.v.m. = Congestion of vulval mucosa; Slim = Sliminess and discharge; Ht. = Hightone; Mt = Mediun tone

FIG. 4. PHYSICAL CHANGES OF REPRODUCTIVE TRACT DURING INDUCED OESTRUS



O.v. = Oedema vulva; C.v.m. = Congestion of vulval mucosa;
Slim = Sliminess and discharge; Ht. = High tone; Mt = Medium tone

FIG.5. NATURE OF DISCHARGE IN GROUP I AND II

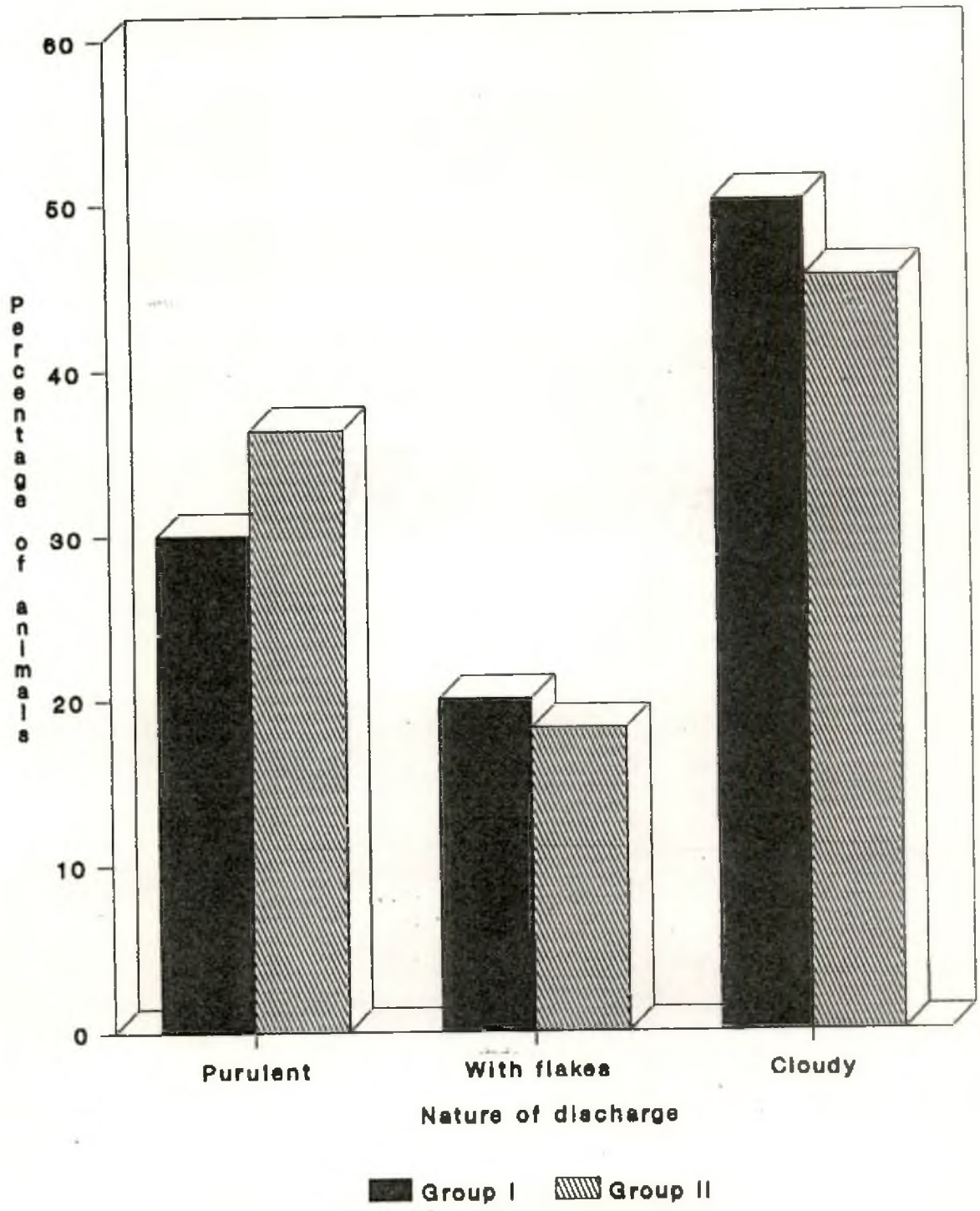


FIG.6. NATURE OF DISCHARGE IN GROUP III AND IV BEFORE TREATMENT

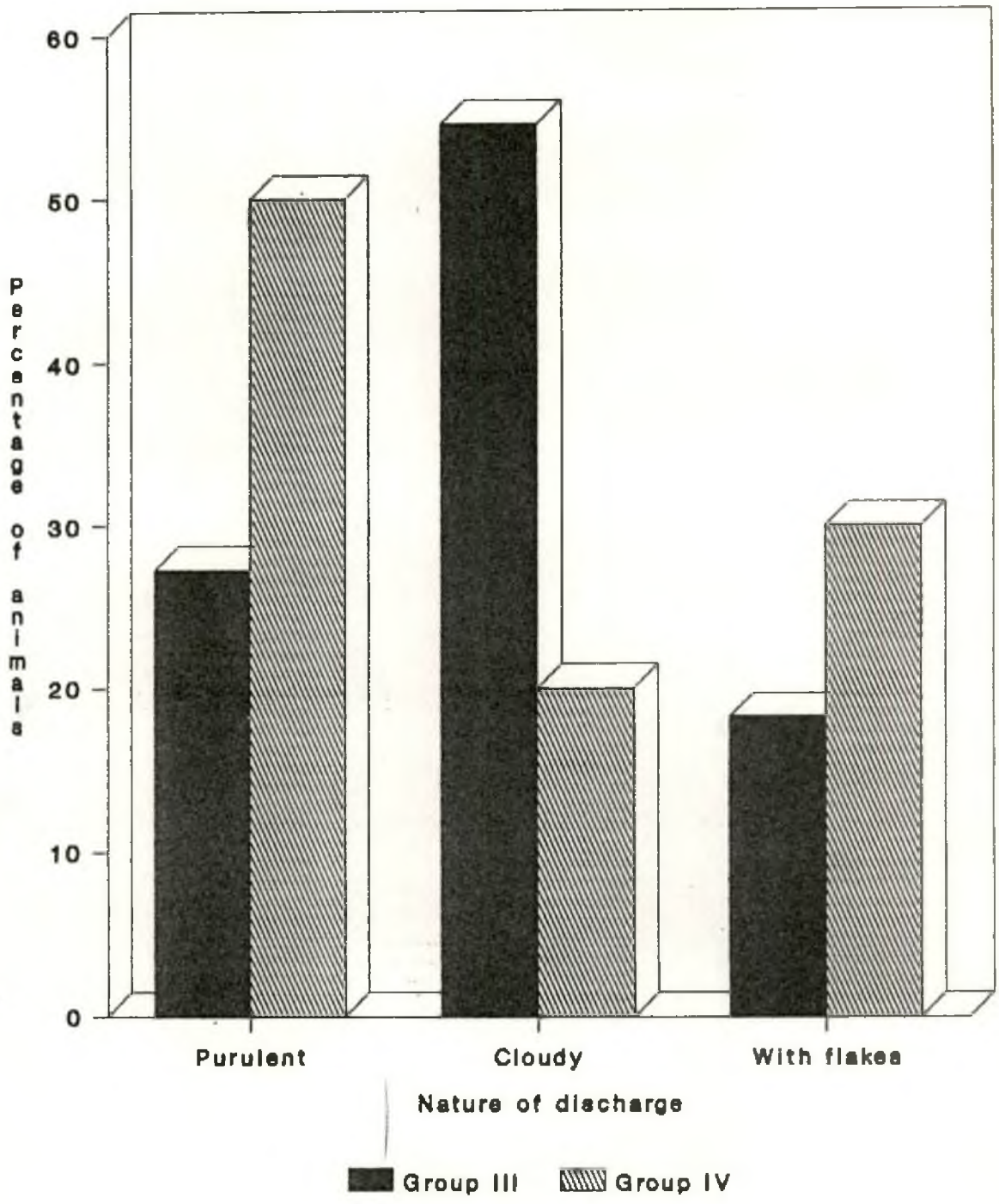


FIG.7. NATURE OF DISCHARGE IN GROUP III AND IV AFTER TREATMENT

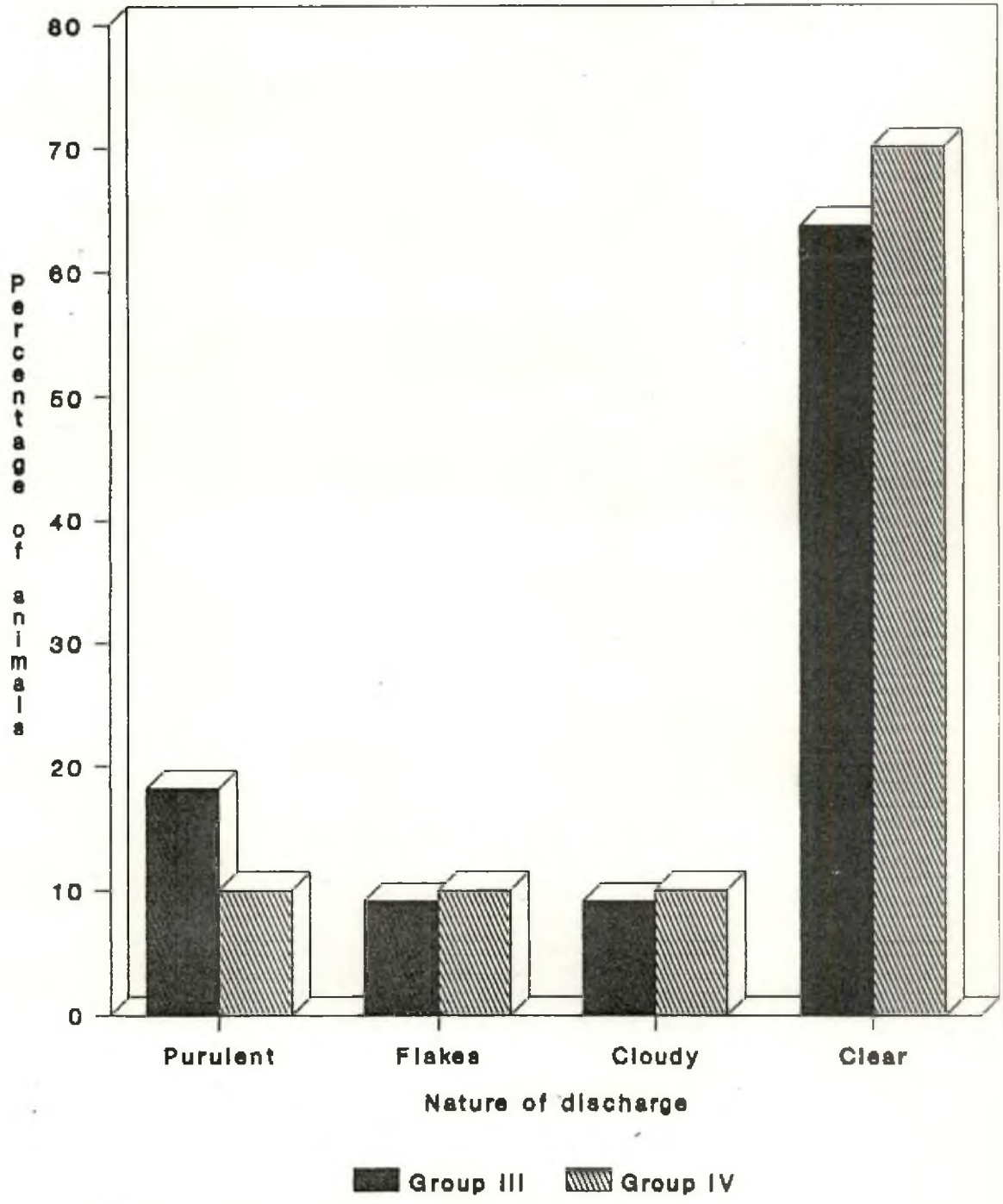
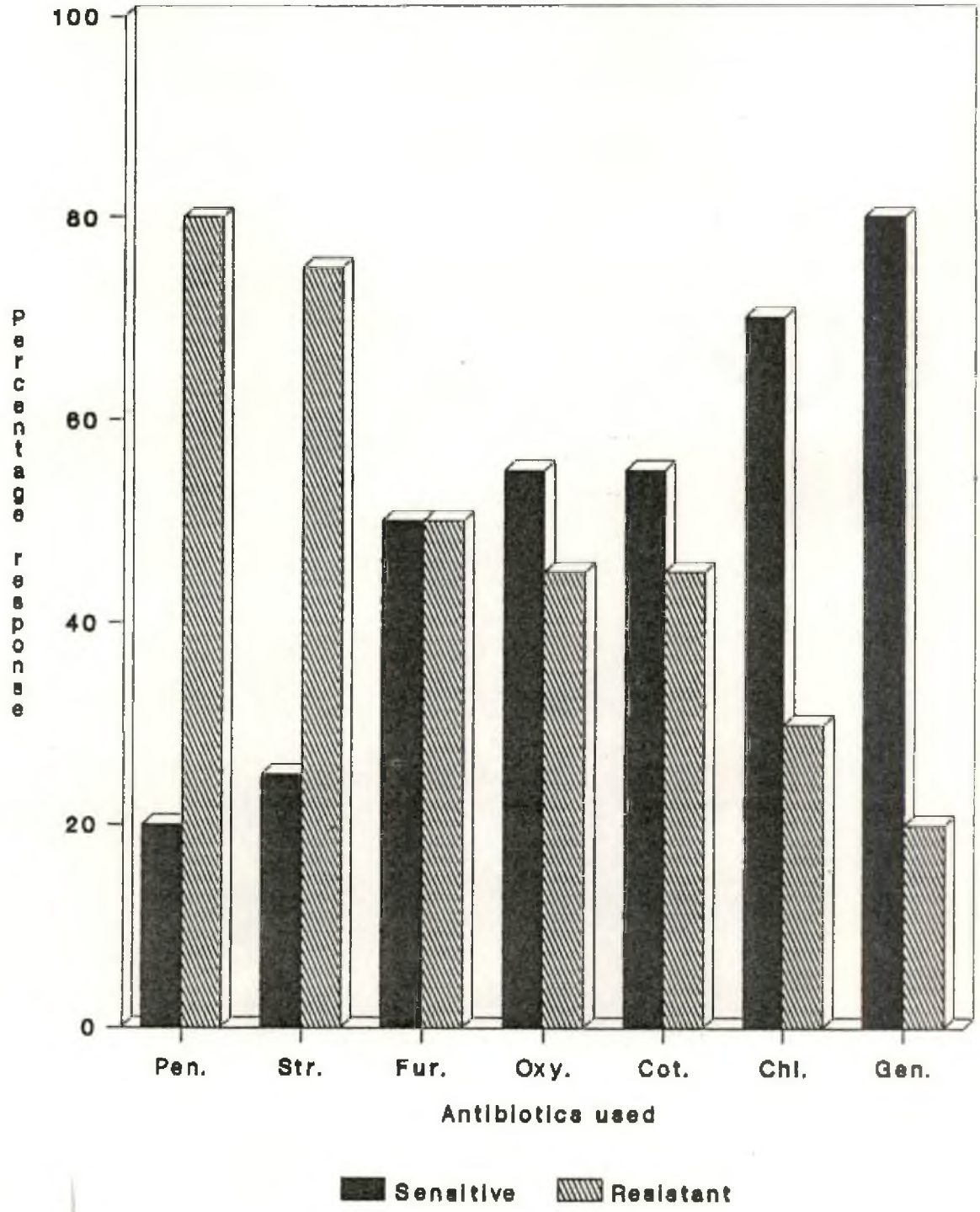
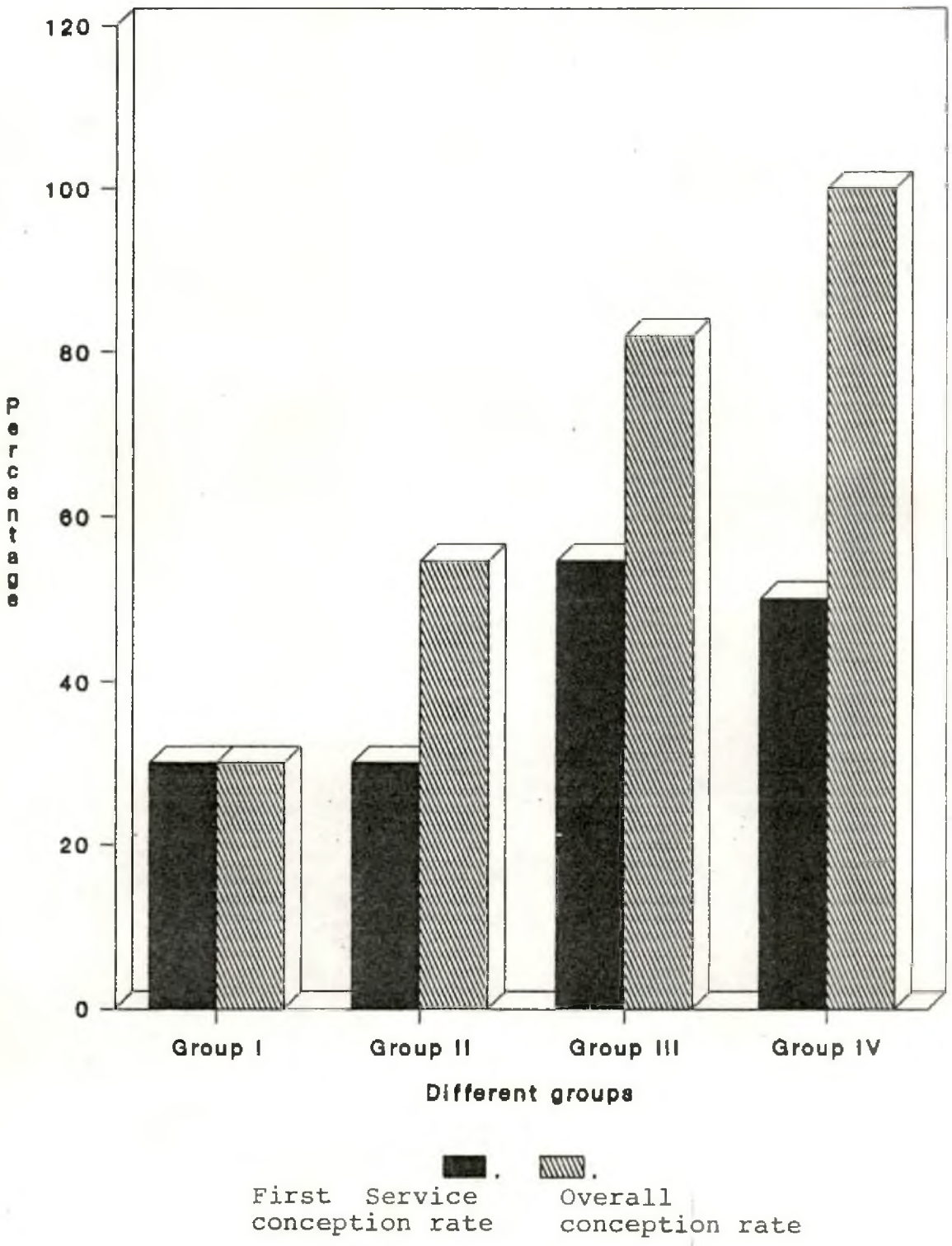


FIG. 8. **RESPONSE TO SENSITIVITY TESTS**



Pen. = Penicillin; Str. = Streptomycine; Fur. = Nitrofurantoin;
Oxy. = Oxytetracycline; Cot. = Sulphadiazine; Chl. = Chloramphenicc
Gen. = Gentamicin

FIG. 9. CONCEPTION RATES IN DIFFERENT GROUPS



Discussion

DISCUSSION

Bovine endometritis has been considered as the most prevalent of all the common periparturient diseases of dairy cattle with greatest impact on fertility (Jackson, 1977; Seguin, 1980; Murray et al., 1990; Gilbert, 1992). The magnitude of this condition is so great that it increases the service period and lengthen the intercalving period with resultant economic loss. Several therapeutic measures have been tried in the past for the treatment of this important condition with varied results (Moberg, 1952; Cooper, 1974; Pant et al., 1991). Because of the complex nature of the etiology of this melody, a rational approach for its treatment is a necessity.

Several lines of treatment have been tried in the past for bovine endometritis like antibiotics, antiseptics, oestrogens (Ziv, 1980; Bretzlaff et al., 1983; Jayappa and Loken, 1983; Gustafsson, 1984; Ball et al., 1984), but the use of PGF_2 alpha is of a recent origin. Although very little information is available on the beneficial effect of PGF_2 alpha, in treating clinical endometritis in cycling cows, favourable results have been reported by Coulson (1978) and Ott and Gustafsson (1981). Since then several trials have been carried out to study the utility of PGF_2 alpha and its structural variants for endometritis (Chauhan et al., 1984; Paisley et al., 1986; Youngquist and Braun,

1986; Studer and Holtan, 1986; Pant et al., 1991). The present investigation was taken upto compare the efficacy of PGF_2 alpha and antibiotics with the ultimate object of evolving a suitable non antibiotic therapy for post partum clinical endometritis

The material used for the present study consisted of 42 crossbred cows belonging to Livestock Farm attached to the Kerala Agricultural University, which were maintained under identical conditions of feed and management, but did not conceive beyond 45 days post partum and were diagnosed to have clinical endometritis.

5.1. Time taken for the induction of oestrus

Perusal of data presented in table 3 and 4 revealed that out of 21, all the animals evinced oestrus after administration of lutalyse. This is in accordance with the findings of earlier workers that majority of the animals treated with PGF_2 alpha would show oestrus within a period of 3-4 days (Lauderdale, 1972; Liehr and Marion, 1972; Louis et al., 1972; Rowson et al., 1972; Oxender et al., 1974; Lauderdale, 1975; Seguin, 1979; Schams and Karg, 1982; Martinez and Thibier, 1984; Stotts et al., 1987; Kelton, 1989; Pant et al. 1991). Interval from the administration

of the drug to the expression of oestrus ranged from 48 to 120 hours with an average of 61.81 hours in Group III. The mean time taken for induction of oestrus after the administration of drug in group IV was found to be 54 hours and ranged from 36 to 72 hours. Thus it could be seen that, on an average, 58.95 hours were required to induce oestrus in experimental animals. Similar observations were also made by Roche (1974), Cooper and Furr (1974), Philipsen and Rasbech (1974), Ganeswaran and Patil (1975) Barnabe (1975), Becze et al. (1976), Gupta et al. (1978), Drew and Gould (1980), Seguin et al. (1985), Kelton (1989) and Pant et al. (1991), who reported that oestrus could be induced by administration of PGF_2 alpha in cycling cows within 2-4 days of administration. On the contrary, poor response of oestrus induction by PGF_2 alpha was reported by Eddy (1977), Leidl (1978), Singh et al. (1979), Khurana (1979), Chauhan et al. (1980), Momont and Seguin (1984) and Seguin et al. (1985). This might be due to treatment of cows during the non responsive stage of oestrus cycle as reported by Chauhan et al. (1980).

5.2. Duration of oestrus

The average duration of oestrus was 21.6 hours, 23.36 hours, 28.36 hours and 31.60 hours in Group I, II, III and IV respectively. Analysis of data revealed that the duration

of oestrus in group IV was significantly longer than the other groups which had natural oestrus. Although, the duration of oestrus in group III was not significantly longer than group I and II, it appeared that the oestrus of animals administered with PGF_2 alpha was of longer duration than that of natural oestrus. Thus it could be assumed that the duration of induced oestrus did show slight to marked variation than normal oestrus in cross-bred cows. This is in contrast to the earlier reports of Elving et al. (1975) and Nair and Madhavan (1984) who reported that duration of induced oestrus using PGF_2 alpha did not show marked variation from the normal oestrus in cross-bred cows. Although, there is paucity of information on the nature and duration of prostaglandin induced oestrus in cross-bred animals, Louis et al. (1973) and Louis et al. (1974) reported that the physiological events which follow treatment with PGF_2 alpha were not distinguishable from those which followed naturally occurring luteolysis. However, it was further reported that the response to PGF_2 alpha in terms of the duration of oestrus and onset of oestrus was reported to be variable especially in lactating dairy cattle. This was attributed to the ovarian status at the time of PGF_2 alpha administration. In this study all the experimental animals were in early lactation and the variation in duration of oestrus between natural and induced

oestrus might be attributed to this as reported by Macmillan (1983) and Fortin et al. (1988).

5.3. Intensity of oestrus

Data presented in table 3 and 4 and figure 1 and 2 revealed that the percentage of intense medium and weak oestrus was 66.66 per cent, 23.80 per cent and 9.52 per cent respectively in natural oestrus while the respective values in induced group were 66.66 per cent, 19.04 per cent and 14.28 per cent respectively. Although comparable data on the intensity of induced oestrus are not available, Ginther (1968), and Nair and Madhavan (1984) reported a high incidence of weak signs of oestrus when oestrus was induced with PGF_2 alpha, and attributed this to a partial luteolysis resulting in weak expression of oestrus in animals affected with subclinical uterine infections. The present study also concurs with the above observation since the percentage of weak oestrus (14.28 per cent) in the experimental animals was higher than that of natural oestrus (9.52 per cent).

5.4. Physical changes in reproductive tract

Perusal of data presented in table 5 and 6 and figures 3 and 4 revealed that the physical changes of reproductive

tract did not show any variations between animals showing natural oestrus and those in which oestrus was induced. All the animals in which oestrus was induced showed vulval oedema, congestion of vulval mucosa and sliminess, and maximum percentage of these animals showed high tonicity of uterus, similar to natural oestrus. This is in full agreement with that of Schultz (1980), Seguin (1980) and Wenzel (1991), who also reported that the cyclical changes of reproductive tract were not affected by induction of oestrus with PGF_2 alpha, and remarked that luteolysis and changes of reproductive tract either occurring naturally or by induction with exogenous PGF_2 alpha are similar in nature.

5.5. Nature of oestrual discharge

Data presented in table 7 and 8 and figures 5, 6 and 7 show the percentage of animals having purulent discharge, discharge with flakes, cloudy and clear discharge in all the four groups. Perusal of data in table 8 reveal that in group III, when treated with PGF_2 alpha alone, there was a marked reduction in the percentage of animals with purulent discharge, discharge with flakes and cloudy discharge. It could also be seen that none of these animals had clear discharge before treatment. The percentage of animals with clear discharge after treatment was 63.63 per cent.

Similarly in group IV when the animals were given antibiotics and PGF_2 alpha, the percentage of animals showing purulent discharge, discharge with flakes and cloudy discharge also reduced considerably, although, it was not markedly different from those in group III. Similarly, the number of animals showing clear discharge in the group after treatment was 70 per cent, which clearly indicates the beneficial effect of PGF_2 alpha in evacuating the purulent exudate from the uterus. Several workers have reported the beneficial effect of PGF_2 alpha in uterine infections by effecting evacuation of the uterine contents and thereby enhancing the healing of endometrium (Paisley et al., 1986; Arthur et al., 1989; Murray et al., 1990; Wenzel, 1991). The ability of PGF_2 alpha for stimulating the phagocytosis by uterine leucocytes and making the uterine discharge clear has also been well documented (Vandeplassche, 1984; Paisley et al., 1986).

5.6. Isolation, identification and antibiogram of bacterial organisms

The present study isolated coagulase negative staphylococcus spp. (9.52 per cent), S. aureus (14.28 per cent), Bacillus spp. (23.80 per cent), Corynibacterium spp. (9.52 per cent), Pseudomonas spp. (14.28 percent), citrobacter spp. (23.84 per cent), Candida guilliermondii

(4.76 per cent) from the uterine exudate of experimental animals (Table 9). Isolation of various microbial agents have been reported earlier also. Hinze (1959) opined that nonspecific opportunist pathogens are the most important causes of endometritis and that these organisms might have reached the uterus from vagina at oestrus or at parturition. He also remarked that most of these organisms would have been normal inhabitants of the dairy cattle. Elliot et al. (1968) identified 36 species of bacteria. Hartigan et al. (1974) isolated C. pyogenes as the most common pathogen in post partum uterine infections. According to Benjamin et al. (1982) S. aureus, E. coli and corynebacteria are the most common pathogens infecting the uterus during the puerperal period. However, Martine and Thibier (1984) remarked that the epidemiological pattern of endometritis need not be generalised in all cases. David and Bonnier (1984), Singh et al. (1989), Khan et al. (1990), Biolatti (1991), Kudryavtsev (1991) and Vahida (1992) also isolated the common microorganisms from the uterine exudate as reported in the present study. However, perusal of literature did not reveal isolation of Candida guilliermondii as reported in the present study. This organism was isolated from uterine exudate of one cow in which discharge did not become clear even after treatment with PGF_2 alpha. Probably further studies on this organism and its treatment are warranted.

A critical study on the sensitivity test of the uterine exudate revealed (table 10, 11 and figure 8) that maximum per cent of animals were sensitive to gentamicin followed by chloramphenicol, oxytetracycline co-trimoxazole and Furazolidone. On the other hand, maximum resistance was shown to penicillin and streptomycin. This might be on account of the indiscriminate use of penicillin and streptomycin in treating infections making them resistant to these antibiotics. Koleff et al. (1973) by conducting sensitivity tests of bacterial flora of uterine contents of cows having endometritis found that the most effective antibiotics was ampicillin, followed by chloramphenicol and tetracycline. Sinha et al. (1977) recommended chloramphenicol as the most effective drug for treatment of endometritis. Kharade and Kulharni (1983) found that most of the uterine isolates were sensitive to gentamicin and Kanamycin. Varadarajan et al. (1985), Sudhaker (1986), Varadarajan and Nair (1989), Vahida (1992) also concurred that gentamicin was the most effective drug for clinical endometritis. This is in agreement with present findings. The finding that the majority of animals were resistant to penicillin and Streptomycin was reported earlier also (Iyer, 1992).

In the present study, in all the experimental animals, the dose of antibiotics given intrauterine was the same as

recommended for systemic use. Arthur (1989) also recommended that the dose for intrauterine use should be similar to that of systemic administration. It could also be seen that better conception rate was obtained by administration of oxytetracycline, chloramphenicol, co-trimoxazole and Furazolidone. Similar findings have also been reported earlier (Guedawy et al., 1983; Moore et al., 1984; Varadarajan, 1985; Sudhaker, 1986). Vahide (1992) stressed the efficacy of gentamicin for treatment of uterine infections and remarked that systemic administration was found to be more effective than uterine administration. In the present study, however, only intrauterine administration was done and data for systemic administration is lacking for comparison.

5.7. Number of inseminations per conception

The number of inseminations required per conception, the conception rate for first insemination and overall conception rate are presented in table 12 and figure 9. It may be seen that the number of insemination required per conception was 5.56, 3.83, 1.77 and 2.20 in groups I, II, III and IV respectively.

5.8. Conception rate

The conception rate for first insemination was 30,30, 54.53 and 50 per cent in the groups I, II, III, and IV respectively, while the respective values for overall conception rate were 30, 54.54, 81.80 and 100 per cent. It could be assumed from the above data that the number of insemination required per conception was significantly less in group III and IV than the other two groups. It may also be noted that the conception rate in the first insemination were also higher in group III and IV, compared to that in group I and II. Significant difference in overall conception rate was also observed between group I and IV, group II and IV and group I and III. However, no significant difference was observed in the overall conception rate between group III and IV. This clearly indicates that the overall conception rate of the animals treated with prostaglandin alone and in combination with antibiotics were higher than those of other groups of animals. Thus it could be inferred that administration of PGF_2 alpha alone or in combination with antibiotics would be beneficial in the treatment of endometritis. However, since no beneficial effect in conception rate was noticed between the animals in group III and IV, it could be inferred that administration of PGF_2 alpha alone would be beneficial to improve the overall fertility of the animals. Moreover, it may be

noted that the number of insemination per conception was less in group III than group IV. Vandeplassche (1984) and Paisley et al. (1986) opined that by intrauterine therapy there was risk of introducing new infection into the uterus, injuring the endometrium and depressing uterine phagocytic activity and drug resistance. They also warned that the economic losses resulting from unnecessary therapy and milk withdrawal after antibiotic therapy have to be weighed against any gains resulting from this therapy. The preponderence of literature also supports the view that there is only occasional beneficial effect with more of economic loss from antibiotics and antibacterial therapy for uterine disorders especially in post partum period. Because PGF_2 alpha therapy has the advantage of no requirement for milk withdrawal and no drug resistance, it can be recommended as a treatment regime for endometritis. It may also be pointed out that administration of PGF_2 alpha in endometritis has not only the therapeutic value for uterine infections, but also shortens the interval from parturition to oestrus, thus reducing the calving to conception interval.

Summary

SUMMARY

The aim of the investigation was to assess the therapeutic value of PGF_2 alpha for evolving a non antibiotic alternative in the treatment of post partum clinical endometritis.

The materials used for the investigation consisted of 42 crossbred cows belonging to the livestock farm attached to the Kerala Agricultural University, Mannuthy. These animals were maintained under identical conditions of feed and management. All animals which have not conceived beyond 45 days post partum were subjected to detailed clinico gynaecological examination and those found to have clinical endometritis were selected and randomly allotted into four treatment groups. Group I consisted of ten animals which were watched for their natural oestrus and inseminated twice at 24 hours interval (control). Group II consisting of 11 animals were inseminated twice at 24 hours interval at their natural oestrus, and subjected to post insemination intrauterine antibiotic therapy 24 hours later, based on antibiotic sensitivity tests. Eleven animals in group III were subjected to induction of oestrus by administration of PGF_2 alpha (Lutalyse) 25 mg intramuscular, 8-12 days of their cycle and inseminated twice at 24 hour interval at the induced oestrus. Ten animals in group IV, were subjected to

induction of oestrus as in Group III and inseminated twice at 24 hours interval and were given post insemination intrauterine antibiotic therapy based on sensitivity tests, 24 hours later.

It was noticed that the time taken for induction of oestrus, after administration of PGF_2 alpha varied from 48-120 hours with an average of 61.81 hours in group III and 36-72 hours with a mean of 54 hours in group IV. The duration of oestrus showed significant difference between groups. It averaged 21.6 hours in group I, 23.36 hours in group II, 28.36 hours in group III and 31.60 hours in group IV. Among the experimental animals in group I, 60 per cent showed intense oestrus, 30 per cent showed medium and 10 per cent weak oestrus. In group II, the percentage of animals showing intense, medium and weak oestrus was 72.72, 18.18 and 9.09 per cent respectively. The percentage of animals showing intense, medium and weak oestrus in natural oestrus was 66.66, 23.80 and 9.52 respectively while the values in animals with induced oestrus was 66.66 per cent, 19.04 per cent and 14.28 per cent respectively. The percentage animals showing oedema of vulva was 80, 81.81 in group I and II respectively. Congestion of vulval mucosa was noticed in 100 and 90.90 per cent of animals, sliminess of vagina in 100 and 90.90 per cent in group I and II respectively, while

the percentage of animals showing high, medium and low uterine tone was 70, 30, and 0 per cent in group I, 63.63, 36.36 and 0 per cent in group II respectively. It could also be observed that all the animals in which oestrus was induced showed vulval oedema, congestion and sliminess and maximum percentage of these animals showed high tonicity of uterus.

In group I, 30 per cent of animals had purulent discharge, 20 per cent discharge with flakes and in 50 per cent the discharge was cloudy. In group II, the percentage of animals with purulent discharge, discharge with flakes and cloudy vaginal discharge was 36.36, 18.18 and 45.45 per cent respectively. It may also be noted that in group III, the percentage of animals with purulent discharge was reduced from 27.27 per cent to 18.18 per cent, the percentage of animals with flakes was reduced from 18.18 per cent to 9.09 per cent, and cloudy discharge from 54.54 to 9.09 per cent after treatment. It may also be noted that in group IV, the percentage of animals with purulent discharge was reduced from 50 to 10 per cent after treatment, 30 to 10 per cent in respect of discharge with flakes and 20 per cent and 10 per cent cloudy discharge. The percentage of clear cervical mucous after treatment was, however, 63.63 and 70 per cent in group III and IV respectively.

The common organisms identified were S. aureus (14.28 per cent), coagulase negative staphylococci (9.52 per cent), Bacillus spp. (23.80 per cent), corynebecterium spp (9.52 per cent), pseudomonas (14.28 per cent) and citrobacter (23.84 per cent). It is also interesting to note that in one animal (4.76 per cent) Candida guilliermondii was isolated.

Antibiogram of uterine exudate revealed that 80 per cent was sensitive to gentamicin, followed by chloramphenicol (70 per cent), sulphadiazine and oxytetracycline 55 per cent each. The majority of organisms (80 per cent) were resistant to penicillin.

The number of insemination required for conception in all the four groups varied from 1.77 (group III), 2.2 (group IV), 3.83 (group II) and 5.66 (group I).

The conception rate for first insemination was 30 per cent in group I and II, 50 per cent in group IV and 54.53 per cent in group III. The overall conception rate was found to be 100 per cent in group IV, 81.80 per cent in group III, 54.54 per cent in group II and 30 per cent in group I.

From the foregoing paragraph it could be inferred that the conception rate for first insemination was higher in group III and IV than group I and II. But the overall conception rate in group III and IV did not differ significantly. It would therefore appear that there is no added advantage in improving the fertility by administration of a combination of antibiotic and PGF_2 alpha, and that the administration of PGF_2 alpha alone would be beneficial to improve the overall fertility of the animals. It may further be noted that the number of insemination required for conception was less in group III than that of group IV.

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PROSTAGLANDIN THERAPY FOR POST - PARTUM CLINICAL ENDOMETRITIS

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ABSTRACT

The object of the investigation was to evaluate the therapeutic values of PGF_2 alpha, for evolving a non antibiotic alternative for the treatment of post partum clinical endometritis.

For this, 42 cross-bred cows, belonging to University Livestock Farm, Mannuthy, having post partum clinical endometritis were divided into four groups. Group I consisted of 10 animals which were watched for their natural oestrus and inseminated twice at 24 hours interval. In group II, 11 animals were observed for their natural oestrus and inseminated twice at 24 hours interval and were given post insemination intrauterine antibiotic treatment 24 hours later based on antibiotic sensitivity test. Eleven animals in group III were subjected to induction of oestrus by administration of PGF_2 alpha (Lutalyse) 25 mg intramuscular 8-12 days of their cycle and inseminated twice at 24 hours, at the induced oestrus. Group IV consisted of 10 animals subjected to induction of oestrus as in group III and inseminated twice at 24 hours interval and were given post insemination intrauterine antibiotic therapy based on sensitivity tests, 24 hours later.

The observations made and inferences drawn are given below. The interval from the administration of PGF_2 alpha to the onset of oestrus ranged from 48-120 hours (mean 61.81 hours) and 36 to 72 hours (mean 54.0 hours) in group III and IV, respectively. The mean duration of oestrus was 21.6 hours, 23.36 hours, 28.36 hours and 31.60 hours in the four groups respectively. The duration of oestrus showed significant variation between groups I and IV (t' = 28910) and between groups II and IV (t' = 2.6445). The percentage of intense, medium and weak oestrus was 66.66, 23.80 and 9.52 per cent respectively in natural oestrus and 66.66, 19.04 and 14.28 in induced oestrus respectively. The difference in the intensity of oestrus between natural and induced oestrus was not significantly different, although, a slightly high incidence of weak oestrus was observed, when oestrus was induced with Lutalyse. Physical changes of the reproductive tract like oedema of the vulva, congestion of vulval mucosa and sliminess did not show any variation between the natural oestrus and induced oestrus. The percentage of animals showing purulent discharge, discharge with flakes and cloudy discharge showed a marked reduction when treated with PGF_2 alpha alone and a combination of PGF_2 alpha and antibiotics. Similarly the percentage of animals showing clear discharge increased enormously by above

treatments. The bacterial organisms isolated from the uterine discharges were citrobacter spp. 23.84 per cent, Bacillus spp. 23.80 per cent, S. aureus 14.28 per cent, Pseudomonas 14.28 per cent, Corynebacterium spp. 9.52 per cent, Coagulase negative staphylococci, 9.52 per cent and the yeast Candida guilliermondii 4.76 per cent. Gentamicin was the most sensitive antibiotic for most of the organisms isolated, followed by chloramphenicol, oxytetracycline and sulphadiazine. Penicillin was the most resistant followed by streptomycin and nitrofurantoin. Significant difference in the overall conception rate was observed between different groups; the overall conception rate was significantly higher in group IV than in group I and II (t' = 4.8341 between groups I & IV and t' = 2.9186 between groups II & IV). Significantly higher conception rate was observed in group III than group I also (t' = 5.5886). The number of inseminations required per conception was lowest in group III and highest in group I.

Thus, it appeared that PGF_2 alpha in combination with antibiotic was beneficial in the treatment of clinical endometritis. But since the number of inseminations required for conception was lower in group III than group IV and because, there is no significant difference in the overall conception rate, between these two groups, it could

be inferred that administration of antibiotics along with PGF_2 alpha did not have any added advantage. Furthermore, considering the harmful effects of administration of antibiotics, it may be stated that PGF_2 alpha alone would be beneficial in the treatment of post partum clinical endometritis and can be recommended as the drug of choice.