INTRA-UTERINE INFUSION OF HOMOLOGOUS PLASMA IN THE TREATMENT OF ENDOMETRITIS IN COWS

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree

Master of Veterinary Science

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KERALA AGRICULTURAL UNIVERSITY

Department of Animal Reproduction

COLLEGE OF VETERINARY AND ANIMAL SCIENCES

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1995

DECLARATION

I hereby declare that this thesis entitled "INTRA-UTERINE INFUSION OF HOMOLOGOUS PLASMA IN THE TREATMENT OF ENDOMETRITIS IN COWS" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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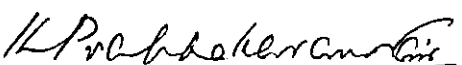
Mannuthy

24.8.1995.

CERTIFICATE

Certified that this thesis, entitled **"INTRA-UTERINE INFUSION OF HOMOLOGOUS PLASMA IN THE TREATMENT OF ENDOMETRITIS IN COWS"** is a record of research work done independently by **Sri. R. Venugopal**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.



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Dedicated to

My Parents and Teachers

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Introduction

INTRODUCTION

Livestock constitute an integral part of the rural economy of our country not only as a source of valuable animal proteins for the teeming millions but also by contributing vital motive power and organic manure for enhancing agricultural productivity. The contribution of the livestock sector to the Gross Domestic Product (GDP) is about eight to nine per cent, constituting around 25 per cent of the GDP of Rs.2018 billions from agricultural sector. Over 73 per cent of the India's rural households rear livestock to supplement their meagre income. Productivity of farm animals, on which depends the economic returns is greatly influenced by their reproductive efficiency. Infertility of cattle is multi-factorial. Among the various etiological factors, infections of the genital tract probably constitute one of the major factors responsible for bovine infertility. Genital infections result in great economic loss due to high cost of treatment and delayed conception. It may also result in permanent sterility and higher culling rate in the herd.

The standard treatment of bovine endometritis with either parentral or topical administration of antibiotics based on biosensitivity, though effective in most of the cases, has certain limitations. The prohibitive cost of treatment, the development of resistant strains of bacteria due to injudicious antibiotic therapy and the excretion of

antibiotics through milk and meat as a public health hazard impose great limitations in the extensive use of antibiotics in the treatment of bovine endometritis. This drives home the urgent need for evolving an effective non-antibiotic alternate therapy for endometritis.

Uterine infection is eliminated by the natural defence mechanism of the host which is activated during oestrum by margination of the host defence cells in the endometrium, active phagocytosis and higher concentration of immuno-globulins in the luminal secretions. For effective phagocytosis the luminal secretions should necessarily contain sufficient levels of opsonins. It has been reported that in barren mares with chronic persistent infection of the uterus, effective phagocytosis does not take place due to lack of opsonin in the uterine secretions eventhough there is sufficiently high levels in the blood (Asbury et al., 1984). It was further observed that intra-uterine infusion of homologous plasma drawn at the time of oestrum helped to eliminate uterine infection and the mares settled to service.

The objective of the present study is to evaluate the effectiveness of intra-uterine infusion of homologous plasma both in the treatment of clinical endometritis and as a post insemination intra-uterine infusion in repeat breeders with subclinical first degree endometritis.

Review of Literature

REVIEW OF LITERATURE

Bovine endometritis exists as a vexatious problem in most of the dairy herds with profound impact on the fertility of affected animals. The detrimental effect imparted by this condition on subsequent fertility can be either short term or long term depending on its severity (Arthur et al., 1989). As a short term effect it can prolong the days to conception and services per conception leading to losses from reduced milk production associated with longer calving intervals and higher insemination costs (Sandals et al., 1979; Pulfer and Riese, 1991). Extension of calving to conception interval has been shown to be an average of 12 days (Tennat and Peddicord, 1968), 20 days (Erb et al., 1981) and 10 days (Bretzlaff et al., 1982). In accordance with this, the services per conception had increased from 1.67 in normal cows to 2.0 in endometritis cases (Tennat and Peddicord, 1968).

Bacterial infections significantly increased the number of services required for conception and the number of cows culled because of infertility (Sturder and Morrow, 1978). As a long term effect endometritis can impair the fertility permanently causing higher culling rates and associated increase in replacement costs (Pulfer and Riese, 1991).

Roberts (1971) opined that dairy farmers incurred a loss of 30 dollars per day by a single day prolongation in the calving to conception interval of an animal. Bartlett et al. (1986) estimated that uterine infections diagnosed by rectal palpation, cost 106 dollars for each affected lactation by prolonging the calving interval, increasing involuntary culling rate, in medication and with milk withholding costs.

Voluminous data are available from different parts of the world about the prevalence of endometritis. Field observations on the puerperal period in 1431 dairy cows from three herds in Poland showed an average metritis incidence of 39.1 per cent with variations in individual herds from 36.6 to 44 per cent (Zezula-Szpysa et al., 1988). Borsberry and Dobson (1989) reported an incidence of 10.1 per cent endometritis from U.K. It was observed in Bulgaria that out of 112 non pregnant dairy cows between three to eight years of age 72 (50.7%) remained infertile due to chronic uterine infections based on clinico-morphological studies (Maneta et al., 1990). Gilbert (1992) reported the prevalence of bovine endometritis in U.S.A. to the extent of 7.5 to 8.9 per cent based on visible mucopurulent vaginal discharge.

Several investigations were carried out from different parts of the country in a bid to assess the prevalence of endometritis in cows. In Andhra Pradesh the condition was prevalent to the extent of 30.77 per cent in cross-breds and

19.41 per cent in indigenous cattle (Rao and Kottaya, 1976). Kaikini et al. (1981) documented an incidence of 8.76 per cent from Maharashtra while an incidence of 32.86 per cent has been reported from Karnataka (Rao et al., 1983). Rahman et al. (1990) recorded the incidence to be around 16 per cent in Kashmir, whereas it was estimated to be 29.38 and 27.09 per cent in cows and heifers of Orissa (Mohanty et al., 1992). Dhami et al. (1993) subjected 4259 animals in Gujarat for detailed clinical evaluation and 17.64 per cent of them were found to have uterine infections.

Quite a few reports are available from Kerala too.Varadarajan and Nair (1990) noted the incidence of endometritis among cross-bred cows of Kerala as 9.66 per cent which is almost in agreement with 7.95 per cent reported by Vahida (1992). However, Iyer et al. (1992) observed a higher incidence up to 20 per cent based on extensive field study and a lower incidence of 1.3 per cent based on abattoir study by Nair and Raja (1975).

Several fruitful studies on bovine endometritis were carried out to elucidate the complex nature of its etiology so as to develop suitable treatment options.

Raghavan et al. (1971) isolated bacteria such as Corynebacterium (21.42%), Diphtheroids (3.57%), Streptococcus (7.14%), Staphylococcus (10.71%), Bacillus (10.71%), E. coli (7.14%) and Proteus (3.57%) from 28 samples of uterine

discharge. Murthy et al. (1974) conducted bacteriological studies on uterine discharge of 106 repeat breeder cows which yielded non-specific bacteria in 81.33 per cent of the cases. The isolates were predominated by E. coli, Staphylococcus, Pseudomonas, Bacillus, Proteus and Corynebacterium. Similar distribution of uterine bacterial flora were obtained by Venketeswaralu et al. (1983) from 180 cows with second degree endometritis. Out of 520 samples of uterine discharge, 76.92 per cent revealed non-specific bacteria which showed maximum susceptibility to Gentamicin (Dholakia et al., 1987). Ambrose and Pattabiraman (1989) revealed nonspecific organisms in the lochial discharge of cows with puerperal infections, which were predominated by E. coli (55.9%) and Bacilli (35.3%).

There is consensus of opinion in the distribution of uterine bacterial flora of repeat breeder cows in several studies (Singh et al., 1989; Sirohi, et al., 1989 and Khan et al. 1990). Varadarajan and Nair (1990) reported that majority of uterine isolates (59%) came under the broad group of gram negative bacilli, such as Enterobacteria, Pseudomonas and Necromonas. The antibiogram studies indicated that most of the isolates were sensitive to Gentamicin. Sharda et al. (1991), isolated bacteria from 32 per cent out of fifty cervico vaginal mucus samples and obtained 26 isolates which showed maximum in vitro sensitivity to Gentamicin. A total of 127 non specific bacterial isolates were identified from

89 cows, by Sharma et al. (1993). These isolates showed maximum susceptibility to Nitrofurazone based on antibiogram studies.

Dawson (1960) reviewed the classification of endometritis according to the type and extent of discharge, as absent or intermittent in first degree cases, a continuous mucopurulent type in cases of the second degree, and a purely purulent type with some tendency to accumulation representing the third degree involvement. In an extensive study on inflammatory lesions in the uterus of over one thousand non descript cows at slaughter revealed necrotic endometritis (0.08%), acute suppurative endometritis (0.16%), acute non suppurative endometritis (0.43%), chronic non-suppurative endometritis (9.16%), perimetritis (0.16%) and hydrometra (0.24%) by Nair and Raja (1975). Mazumdar et al. (1985) detected gross lesions in the uterus of 25 cows (12.5%) out of 200 cows slaughtered. The lesions were classified as catarrhal (40%), fibrinous (36%) and suppurative (24%). The percentage of mild, moderate and severe endometritis in cows were reported to be 66.67, 22.22 and 11.11 respectively as against 62.96, 25.93 and 11.11 in heifers (Mohanty et al., 1992).

Host responses to insult may be specific or non specific. Non specific responses such as inflammation and phagocytosis are the result of initial encounter with foreign antigens. Specific responses such as the production of

antibody and cell mediated immunity, represent subsequent reaction of the host to foreign antigens (Frank et al., 1983). Neutrophils are the most important population of granulocytic phagocytes involved in host defence against invading bacteria and are most effective in extravascular tissues (Scanlan, 1988).

Non specific defence particularly phagocytosis of microorganisms by polymorphonuclear leucocytes (PMN) played a major role in the protection from and reaction to microbial invasion of the bovine uterus (Frank et al., 1983). They further added that enhanced resistance of the oestrual versus dioestral uterus to infection may be a result of enhanced phagocytic activity. Roth et al. (1983) studied the effect of oestrous cycle on functional capabilities of peripheral bovine blood PMN and found increased random migration of peripheral blood PMN at oestrus but no significant effect on other metabolic indices of PMN or ingestion of Staphylococcus aureus. Anderson et al. (1985) reported that the phagocytic activity was similar for PMN collected either from the uterine lumen or peripheral blood in case of bovines.

Paisley et al. (1986) attributed several reasons for the failure of uterine defence mechanism during the periods of progesterone dominance as (1) the intra-uterine pH is low, creating a more favourable environment for those bacteria regularly isolated from the bovine uterus (2) the uterine

epithelium is less permeable to bacteria and as a result leucocytic system is stimulated at a later stage (3) the leucocytes' appearance in the endometrium and uterine lumen is delayed (4) the activity of leucocytes is decreased (5) the uterine secretion has no detoxicating effect. Subandrio and Noakes (1992) found out that oyster glycogen can be used as a chemotactic agent to stimulate the migration of leucocytes in bovine uterus. The migration was influenced by the stage of cycle with the number of neutrophils recovered during oestrus being significantly less than those recovered during dioestrus.

Bouters and Vandeplassche (1977) have investigated phagocytosis in the post partum bovine uterus by counting the number and Gram staining character of bacteria ingested by isolated phagocytes. Eighty per cent of post partum phagocytosis of foreign material in the normal, involuting bovine uterus has been due to neutrophils, while the remaining 20 per cent has been due to macrophages.

The concept of local mucosal immunity has recently been extended to include the female genital tract. This implies that locally applied antigen will evoke the local production of secretory IgA antibody and possibly IgM (Woolcock, 1979). The predominant immunoglobulin in cervical mucus is IgA and within the uterus it is IgG. If bacteria such as C. foetus infect the genital tract, the vaginal IgA antibodies will immobilize the organisms and agglutinate

them. If the mucus membrane becomes inflamed, IgG antibodies derived by transudation from serum will also assist in protection (Tizard, 1977).

Mendonca et al. (1989) reported a fall in phagocytic activity of vaginal discharge between four and eight days after calving in a study covering over three hundred cows with puerperal disorders. Hussain and Daniel (1991) observed that normal and active uterine defence mechanisms have been very important for the exclusion of bacterial infection from the uterus and recovery from puerperal endometritis.

Watson et al. (1987) demonstrated a reduced migration by blood neutrophils and possible reduction in phagocytosis by uterine neutrophils in mares susceptible to endometritis. But there was no evidence of any deficiency in haemolytic complement activity.

Uterine PMN obtained from mares considered susceptible to endometritis 12 hours after uterine inoculation with Streptococcus zooepidemicus did not show chemotactic response (Cheung et al., 1985; Liu et al., 1985). This character is necessary for the neutrophils to move and phagocytise invading microbes.

Cai et al. (1994) reported that the host defence role of neutrophils in periparturient cows were impaired

principally because of a defect in killing capacity, which may increase susceptibility to infections.

Asbury et al. (1982) suggested that a defect in opsonisation of bacteria in the uterine lumen as a factor for decreased uterine resistance. Although large numbers of polymorphonuclear leucocytes are mobilised to the endometrium and migrate into the uterine lumen, as in normal mares, these polymorphonuclear leucocytes have depressed phagocytic activity. The defect appears to be at the level of bacterial opsonisation and as complement is an essential component of the opsonisation cascade reaction, a deficiency or inactivation of uterine complement may be involved (Ricketts, 1987).

The addition of serum to uterine secretion was shown to opsonise Streptococcus zooepidemicus and significantly increased the bacterial phagocytosis by equine neutrophils (Asbury et al., 1984). They measured the amount of third complement (C3) present in uterine secretions in a series of 14 mares injected with Streptococcus zooepidemicus. Ten of the 14 mares had detectable amounts of C3; however the C3 had been cleaved and rendered non-functional.

It would appear that the main common denominator in opsonic action is the ability to coat the surface of the microbe so as to decrease the surface electrical potential and promote adhesion to other such particles and to the

surface of phagocytes (Hamphrey and White, 1972). Opsonins are serum factors that render the micro organism susceptible to phagocytosis. The heat labile opsonins are inactivated by heating at 56°C for 30 minutes and are derived from complement system while serum from animals that are previously exposed to the bacteria (Immune serum) contains opsonins that are stable to this type of heat treatment (Gadebusch, 1979). Scanlan (1988) suggested that opsonins present in the serum include natural antibody, immune antibody and the C_3b fragment of complement, which bind to specific receptors on phagocytic cells. Further he added that the phagocytosis of most pathogenic bacteria require serum factors, especially antibacterial antibody and complement, whereas non-pathogenic bacteria are readily phagocytised in the absence of serum.

The addition of small amounts of serum to uterine secretions resulted in improved opsonisation and subsequent increase in the phagocytic rate in an in vitro system (Asbury et al., 1982). Further studies in this aspect revealed that the above process was complement dependent (Asbury et al., 1984). Plasma was chosen as a source of supplemental complement for its ease in preparation compared to serum (Asbury, 1984). Several experiments were carried out to provide in vivo evidence to the above said observation. A total of 22 clinical streptococcal isolates associated with endometritis in mares were tested for their ability to

withstand the natural bactericidal properties of freshly obtained blood revealed the following observations. Killing required leucocytes, but the specificity for killing appeared to reside in plasma, although plasma by itself was not bactericidal. Heat stable and heat labile components in plasma, interpreted as antibody and complement respectively, appeared necessary for killing (Causey et al., 1995).

The supernatent from the uterine flushings of mares with chronic endometritis was compared with autologous serum for its capacity as an opsonin and chemoattractant (Troedsson et al., 1990). There was a significant increase of both phagocytosis and chemotaxis when autologous serum was used compared with the supernatent from the uterine flushings.

Twenty six mares with active endometritis were treated with combinations of saline irrigations and plasma infusions. Out of which twenty four showed clinical improvement and 15 of them became pregnant after natural service. Three mares with subclinical endometritis were treated post-breeding with single infusion of plasma and all became pregnant (Asbury, 1984). Parmigiani et al. (1985) reported that when 10 mares were given two to four intra-uterine treatments with 200 ml homologous plasma, five became pregnant when served at the first oestrus after the last treatment. Three mares which did not respond to treatment had irreversible endometritis lesions. Ward (1985) in a similar study concluded that 23 out of 44 (52%) mares with active clinical endometritis were

pregnant with combinations of saline irrigation and plasma infusion. By post breeding plasma treatment nine of ten mares with subclinical infection become pregnant. In consensus with the above observations Ricketts (1987), Leblanc (1989) and Ley (1994) suggested intra-uterine infusion of homologous plasma as an effective treatment option for endometritis in mare.

However, the results obtained by Adams and Ginther (1989) did not support the hypothesis that intra-uterine infusion with plasma or saline solution is efficacious in treating endometritis. Pregnancy rate and uterine biopsy scores were not improved after either treatment and in the group treated with plasma, the degree of endometrial inflammation was greater in post treatment cycle. Experimental mares with induced endometritis were used in a controlled study by Colbern et al. (1987) to evaluate the efficacy of intra-uterine, equine plasma therapy for endometritis. No significant differences were observed between mares in the treatment or control groups intra-uterine plasma, when used alone did not alter the course of mild lymphocytic or moderate suppurative and lymphocytic endometritis. Based on histological, bacteriological and cytological uterine changes it was concluded that the plasma has a local inflammatory effect on the mare's endometrium comparable to that of normal saline (Waelchli et al., 1987).

Saini et al. (1993) studied the efficacy of different non antibiotic alternate therapies on bovine endometritis which enhanced the normal uterine defence mechanism. In a group of ten animals with endometritis three successive intra-uterine infusion of homologous plasma (50 ml) followed by insemination at the subsequent oestrus gave 20 per cent conception rate. With levamisol (s/c injection), plasma and levamisol combination, E. coli lipopolysacharide (100 ug i/u infusion) the conception rates were 50.00, 37.5 and 83.33 per cent respectively.

Numerous methods have been adopted to treat cows with endometritis, by parenteral administration or intra-uterine infusion with several antibiotic preparations (Dawson, 1960). Paisley et al. (1986) opined that the most important reason for the apparent failure of intra-uterine therapy to eliminate the uterus of bacteria is the depression of leucocyte function due to intra-uterine manipulations. This effect is magnified if the organism present in the uterus were resistant to the antibiotic used. Moreover the presence of enzymes that degrade the antibiotic, and presence of pus and debris in the uterus render intra-uterine antibiotic therapy ineffective.

Whitacre (1992) suggested that the uterus is considered as an anaerobic environment early in the post partum period which makes aminoglycoside group of antibiotics ineffective as they require oxygen for their activity.

A prospective clinical trial on intra-uterine antimicrobial treatment in reducing calving to conception interval in cows with endometritis revealed poor efficacy (Thurmond et al., 1993).

Systemic administration eliminates the risk of damage to the endometrium and gives better distribution of the drug in the tubular genital tract (Pulfer and Riese, 1991). Moreover systemic therapy claims the advantage of less chance of contamination of the genital tract when compared with repeated intra-uterine therapy (Ley, 1994). However higher drug costs and the inconvenience of repeated administrations are the main drawbacks in parenteral administration of antibiotics in the treatment of genital infections (Ley, 1994).

The need for non antibiotic alternative therapies were stressed by Gustafsson (1984) considering the increased awareness of the risk of bacterial resistance and the residual effect of these antibiotics in milk and meat, necessitating with holding time with antibiotic therapy.

Hussain and Daniel (1991) concluded that despite the widespread use of local or systemic antibiotics, antiseptics, sulphonamides and hormones, rate of recovery from endometritis and subsequent fertility of cow has not increased appreciably. They suggested alternate therapies which stimulate the natural uterine defence mechanisms such

as lipopolysacharide of E. coli, serum plasma or hyper immune serum, polymorphonuclear leucocyte extracts (PMN) and Granulocyte macrophage colony stimulating factor (G-MCSF).

Materials and Methods

MATERIALS AND METHODS

Animals for the present study consisted of sub-fertile and infertile cross-bred cows brought for treatment to the Artificial Insemination Centre attached to the Department of Animal Reproduction. The study was conducted during the period from March 1994 to January 1995. All the cows were subjected to detailed clinico-gynaecological examination based on which they were subdivided in to four groups, as described below.

Cows suffering from endometritis, with catarrhal, mucopurulent or purulent uterine discharge were selected and randomly allotted into treatment group (Group I) and control group (Group II).

Repeat breeders with subclinical first degree endometritis, which have failed to conceive with two or more inseminations and have apparently normal luminal secretions were allotted randomly into treatment group (Group III) and control group (Group IV).

Treatment modalities adopted

Group I (Endometritis)

A total of 13 cows were selected in this group and their luminal secretions were collected aseptically at the time of oestrum for standard plate count. Each cow was

given 25 to 35 ml of homologous plasma as intra-uterine infusion on the day of oestrum. These cows were subjected to detailed clinico-gynaecological examination in the succeeding heat and their luminal secretion subjected to standard plate count. Those cows which showed clinical recovery as evidenced by clear discharge were inseminated then, using semen from known fertile bulls.

Group II (Control)

The luminal secretions of 10 cows allotted to this group were subjected to standard plate count. They were administered intra-uterine infusion of 25 to 35 ml of sterile normal saline. In the succeeding heat the bacterial load in the uterine discharge was estimated by standard plate count and those animals which had either clear and cloudy discharge were inseminated with semen from known fertile bulls.

Group III (Repeat breeders with subclinical first degree endometritis)

Twenty two cows allotted to this group were inseminated with semen from known fertile bulls followed by intra-uterine infusion of 25 to 35 ml of homologous plasma, 24 h after insemination.

Group IV (Control)

Twenty cows under this group were inseminated with semen from known fertile bulls without post insemination plasma treatment.

Aseptic collection of uterine discharge

The instrument used for aseptic collection of mucus samples from the uterus was a simple one designed for this study in modification to that designed by Vahida (1992). The equipment comprised of an inner glass pipette of 40 cm length and 0.6 cm outer diameter which snugly fits into an outer metal tube 30 cm length and 0.6 cm inner diameter (Fig.3.1). The equipment was assembled by introducing the sterile glass pipette into the outer metal vaginal tube, until the tip of the pipette reaches the cranial end of the metal tube. A rubber adaptor for the syringe was fixed at the caudal end of the glass pipette.

After cleaning the external genitalia of the cow, assembled equipment was introduced into the vagina until the tip of the metal tube reaches the external os of the cervix. Keeping it firmly fixed to the cervix the inner glass pipette was introduced into the uterus and the discharge was aspirated. The inner glass pipette was then withdrawn into the outer tube and the equipment was taken out. The uterine discharge was then transferred into a sterile graduated test

tube after assessing the physical properties of the discharge and was subjected to standard plate count (Benson, 1990).

The procedure for the plate count was modified as given below since the uterine discharge was viscous and stringy in nature.

Plate count technique

A known quantity of uterine aspirate was taken in a sterile test tube and diluted ten fold using sterile normal saline. This was thoroughly mixed using a cyclomixer. Ten fold dilutions were made with sterile normal saline until a dilution of 1/10000 was obtained and at each dilution thorough mixing was carried out with the help of cyclomixer. The diluted uterine discharge in 0.5 ml quantities were mixed with autoclaved plate count agar* at 50°C in sterile petri plates and then incubated at 37°C for 24 to 48 h. Bacterial colonies in the plates were counted using Qubec colony counter (Fig.3.2). For each sample two plates were counted and the average was taken.

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* Plate Count Agar (Himedia lab. Bombay)
Ingredients: Grams/litre
Tryptone - 5.0
Yeast extract - 2.5
Dextrose - 1.0
Agar - 15.0
Suspended 23.5 grams in 1000 ml distilled water.
Boiled to dissolve the medium completely

Preparation and handling of plasma

Jugular blood in quantities of 50 to 60 ml was drawn aseptically into test tubes containing 3.8 per cent sodium citrate solution in the ratio of one part of citrate to nine parts of blood (Schalm et al., 1975). The blood was thoroughly mixed with citrate solution both during drawing of blood and afterwards in order to prevent clotting. Plasma was separated by centrifuging the sample at 3000 rpm for fifteen minutes (Fig.3.3) and drawn into a 50 ml glass syringe for intra-uterine infusion.

In vitro test to study the antibacterial effect of plasma

1. Principle

Turbidity of a bacterial suspension is directly related to its bacterial load. A reduction in turbidity of the suspension on addition of plasma is considered as a decrease in bacterial load.

2. Requirement

1. Plasma collected aseptically from cows during oestrum.

2. Bacterial suspension : Uterine secretions drawn aseptically from cows suffering from endometritis were seeded in nutrient broth and incubated at 37°C for 18 h

in order to obtain the bacterial culture. This bacterial culture was used undiluted for the in vitro study.

3. MacFarland Nephalometer Standards:

This is generally used to assess the turbidity of a bacterial suspension. The standard tubes (Fig.3.4) were prepared by mixing one per cent sulphuric acid and one per cent barium chloride in sterile nutrient broth as per the details given below (Sonnenwirth and Jarrett, 1982).

MacFarland Nephalometer Standards

Tube number (turbidity)	Sulphuric acid 1% solution ml	Barium chloride 1% solution ml	Density of bacteria (millions/ml)
1	9.9	0.1	300
2	9.8	0.2	600
3	9.7	0.3	900
4	9.6	0.4	1200
5	9.5	0.5	1500
6	9.4	0.6	1800
7	9.3	0.7	2100
8	9.2	0.8	2400
9	9.1	0.9	2700
10	9.0	1.0	3000

d. Sterile normal saline

The protocol for the in vitro experiment using plasma and saline (control) is furnished below. The test was carried out in six sterile test tubes.

Tube	Plasma trial		Tube	Saline trial	
	Plasma	Bacterial suspension		Saline	Bacterial suspension
P_1	0.1 ml	0.9 ml	C_1	0.1 ml	0.9 ml
P_2	0.3 ml	0.7 ml	C_2	0.3 ml	0.7 ml
P_3	0.5 ml	0.5 ml	C_3	0.5 ml	0.5 ml

The tubes were then incubated at 37°C and the turbidity was assessed at varying intervals of time upto seven hours using Mac Farland Nephalometer standards by visual comparison. The experiment was replicated six times for accuracy in results.

Observations made

1. The interval from intra-uterine treatment to onset of next heat and clinical recovery.

2. The texture of the uterus, the nature of discharge, and the intensity of oestrum before and after treatment.

3. Uterine bacterial load prior to and post treatment in group I and II.

4. The antibacterial effect of plasma based on <u>in vitro</u> studies.

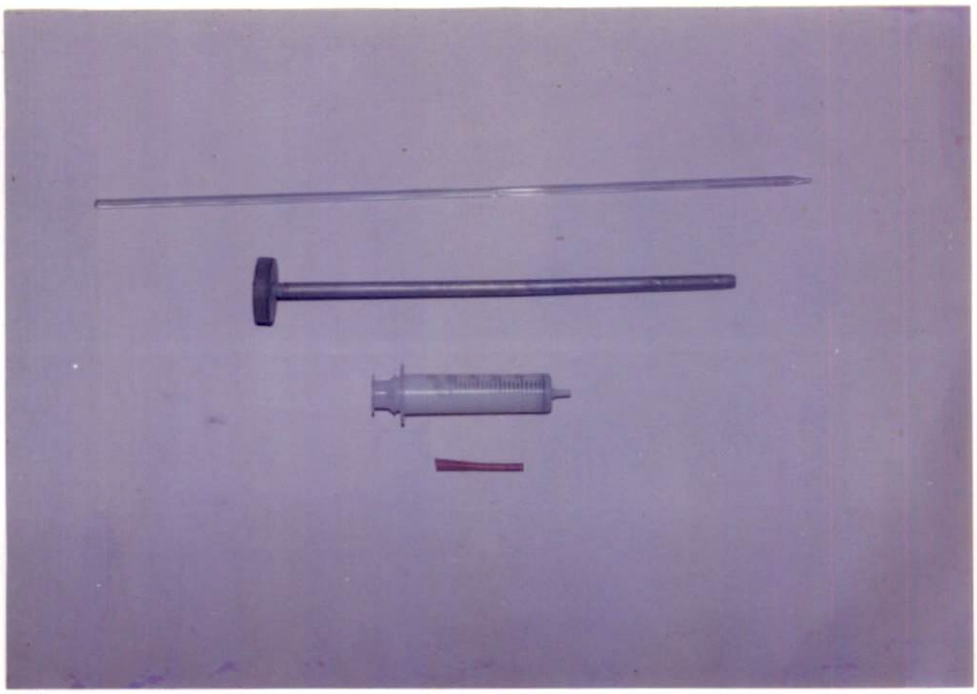
5. The conception rate - The first insemination conception rate was recorded on the basis of actual pregnancy diagnosis at 45 to 60 days of gestation in all the groups.

Assembly and analysis of the data

The data obtained were subjected to statistical analysis for proper interpretation of the results (Snedecor and Cochran, 1967).

Fig.3.1 Instrument used for aseptic collection of uterine discharge

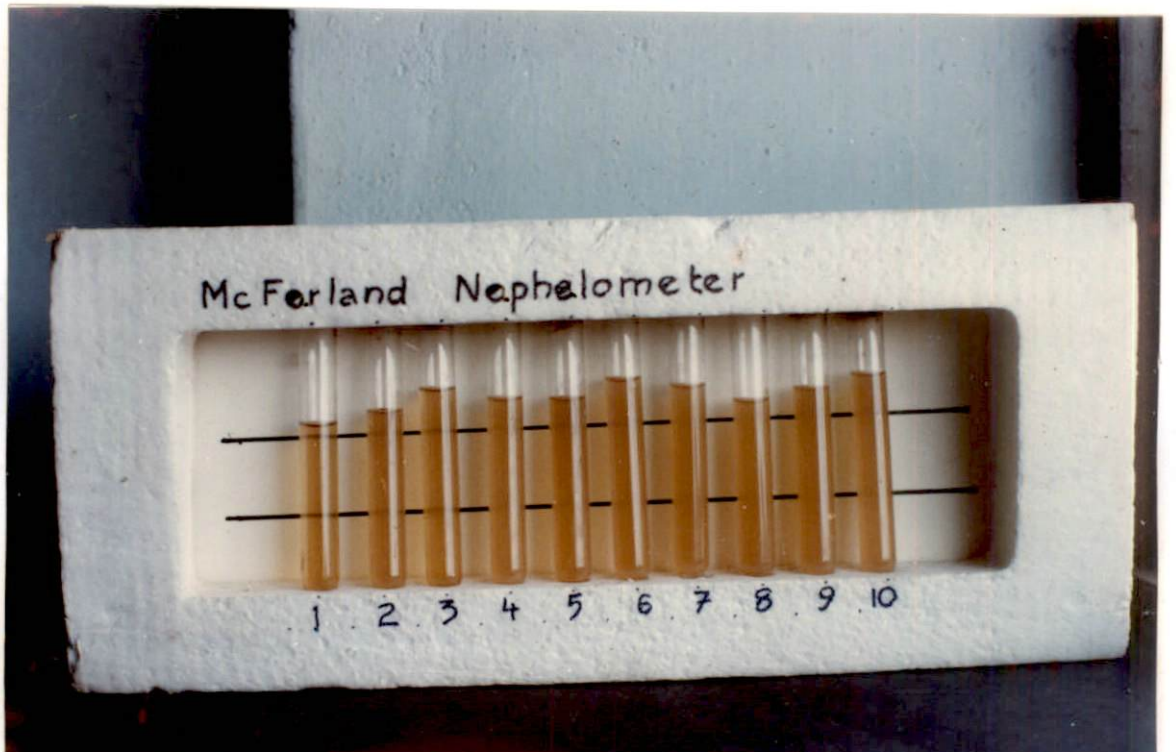
Fig.3.2 Colony counter




DALAL DIGITAL COLONY COUNTER
MAIN
INDICATOR
POWER
ON
PRESS TO
RESET ZERO
MANUAL
COUNT
SWITCH
FUSE

Fig.3.3 Plasma separated from blood

Fig.3.4 MacFarland Nephalometer tubes


Mc Farland Nephelometer
1 2 3 4 5 6 7 8 9 10

Results

RESULTS

Results of the study on the effect of intra-uterine infusion of homologous plasma in the treatment of endometritis and repeat breeding due to subclinical first degree endometritis in cows are presented in tables 1 to 9 and figures 1 to 11.

1. Intra-uterine homologous plasma treatment for endometritis

Characteristics of oestrual mucus prior to and after intra-uterine treatment in group I and II are presented in table 1, figure 1 and 2. The oestrual mucus became clear following plasma therapy in 100.00 per cent of the cases in group I as against 70 per cent in group II. The oestrual mucus in the ensuing cycle was cloudy in 30 per cent cases in control group.

To evaluate the effect of treatment on the consistency and texture of the uterus, the tone was graded as high, medium, low and doughy. It was observed that in endometritis cases the uterine tone was consistently low or doughy which on intra-uterine plasma therapy improved to medium and high tone in 38.46 and 23.08 per cent cases respectively. In contrast to this in control animals which received intra-uterine saline therapy there was no definite improvement in the uterine tone (Table 2, Fig.3 and 4).

The intensity of oestrum based on both clinical and behavioural signs of heat was graded as intense, intermediate and weak. All the cows suffering from endometritis which were brought under treatment showed either intermediate (38.46%) or weak (61.54%) heat which showed considerable improvement on plasma therapy in that, nearly 76.92 per cent showed intense heat as against only 23.08 per cent with intermediate heat. Similar improvement in the intensity of oestrum was also noticed in control cows receiving saline therapy (Table 3,Fig.5 and 6).

Time interval from intra-uterine treatment to succeeding oestrus in animals belonging to group I and II are presented in table 4 and figure 7. It could be seen that in group I 53.85 per cent of the cows came to oestrus after a normal interval of 18 to 21 days, whereas 38.46 per cent had prolonged and 7.69 had shortened oestrous cycle. Similarly in group II 70 per cent showed normal cycle length as against 30 per cent with prolonged inter oestrus interval and none showing short cycle. The mean time interval from intra-uterine treatment to succeeding oestrus was found to be 28.23 $\pm$ 4.67 days in group I as against 20.3 $\pm$ 0.4 days in group II.

The mean uterine bacterial load before and after intra-uterine treatment in group I and II were estimated and analysed (Students' T test). The data are presented in

table 5 and figure 8. Eventhough the mean uterine bacterial load showed reduction after intra-uterine treatment in both the groups, it was not statistically significant.

Perusal of the data presented in table 6 and figure 9 on first insemination conception rate revealed 53.85 per cent conception rate in group I as against 40 per cent in group II.

Post insemination plasma treatment

The repeat breeder cows belonging to group III and IV were subdivided further into those animals which had received third insemination and those with more than three inseminations. First insemination conception rate of the animals after post insemination intra-uterine plasma treatment are presented in table 7 and figure 10. Cows with three inseminations in group III and IV showed conception rates of 50 and 30 per cent respectively, whereas cows with more than three inseminations recorded conception rates of 28.57 and 10 per cent respectively. The mean conception rate for group III cows were 36.36 as against 20 per cent for group IV.

2. <u>In vitro</u> test on antibacterial effect of plasma

The data on the reduction in turbidity based on MacFarland Nephalometer readings in both plasma and saline

tubes, recorded at different time intervals upto seven hours of incubation are presented in table 8 and figure 11. The statistical analysis of the data are presented in table 9. There was progressive reduction in turbidity at different time intervals of incubation in plasma tubes with significant drop at 0 to 1 h ($P<0.05$) whereas in saline tubes no significant reduction in turbidity was recorded.

Table 1. Characteristics of oestrual mucus in endometritis cases before and after intra-uterine treatment

Nature of oestrual mucus	Group I (n=13)				Group II (n=10)			
	BT		AT		BT		AT	
	No.	%	No.	%	No.	%	No.	%
Purulent	1	7.69	-	-	-	-	-	-
Muco-purulent	7	53.85	-	-	6	60	-	-
Cloudy	5	38.46	-	-	4	40	3	30
Clear	-	-	13	100.00	-	-	7	70

BT - Before treatment AT - After treatment

Table 2. Changes in uterine tone in endometritis cases before and after intra-uterine treatment

Tone of uterus	Group I (n=13)				Group II (n=10)			
	BT		AT		BT		AT	
	No.	%	No.	%	No.	%	No.	%
High	-	-	3	23.08	-	-	-	-
Medium	-	-	5	38.46	-	-	-	-
Low	3	23.08	5	38.46	2	20	5	50
Doughy	10	76.92	-	-	8	80	5	50

BT - Before treatment AT - After treatment

Table 3. Intensity of oestrum prior to and after intra-uterine therapy in group I and II

Intensity of oestrum	Group I (n=13)				Group II (n=10)			
	BT		AT		BT		AT	
	No.	%	No.	%	No.	%	No.	%
Intense	-	-	10	76.92	-	-	7	70
Inter-mediate	5	38.46	3	23.08	4	40	3	30
Weak	8	61.54	-	-	6	60	-	-

BT - Before treatment AT - After treatment

Table 4. Time interval from intra-uterine therapy to succeeding oestrus in endometritis cases

Groups	Number of animals	Long cycle (>21 d)		Normal cycle (18 to 21 d)		Short cycle (<18 d)		Mean ± SE
		No.	%	No.	%	No.	%	
Group I	13	5	34.46	7	53.85	1	7.69	28.23 ± 4.67
Group II	10	3	30.00	7	70.00	-	-	20.30 ± 0.40

Table 5. Uterine bacterial load before and after intra-uterine therapy in endometritis

Groups	Bacterial count (millions/ml)		T value
	Before treatment	After treatment	
Group I (n=13)	0.3417 ± 0.3368	0.2367 ± 0.3034	0.7325 NS
Group II (n=10)	0.0920 ± 0.0679	0.0470 ± 0.0292	1.5111 NS

NS - T values are statistically non-significant

Table 6. First insemination conception rate in endometritis cases after treatment

Groups	Number of animals	Animals conceived	Percentage of conception
Group I	13	7	53.85
Group II	10	4	40.00

Table 7. First insemination conception rate in repeat breeders with first degree endometritis after treatment

Groups classification		Number of animals	Animals conceived		Mean conception rate (%)
			No.	%	
Group III (n=22)	3rd AI	8	4	50	36.36
	More than 3AI	14	4	28.57	
Group IV (Control) (n=20)	3rd AI	10	3	30.00	20.00
	More than 3AI	10	1	10.00	

Table 8. Turbidity readings in different trials (Mean ± SE)

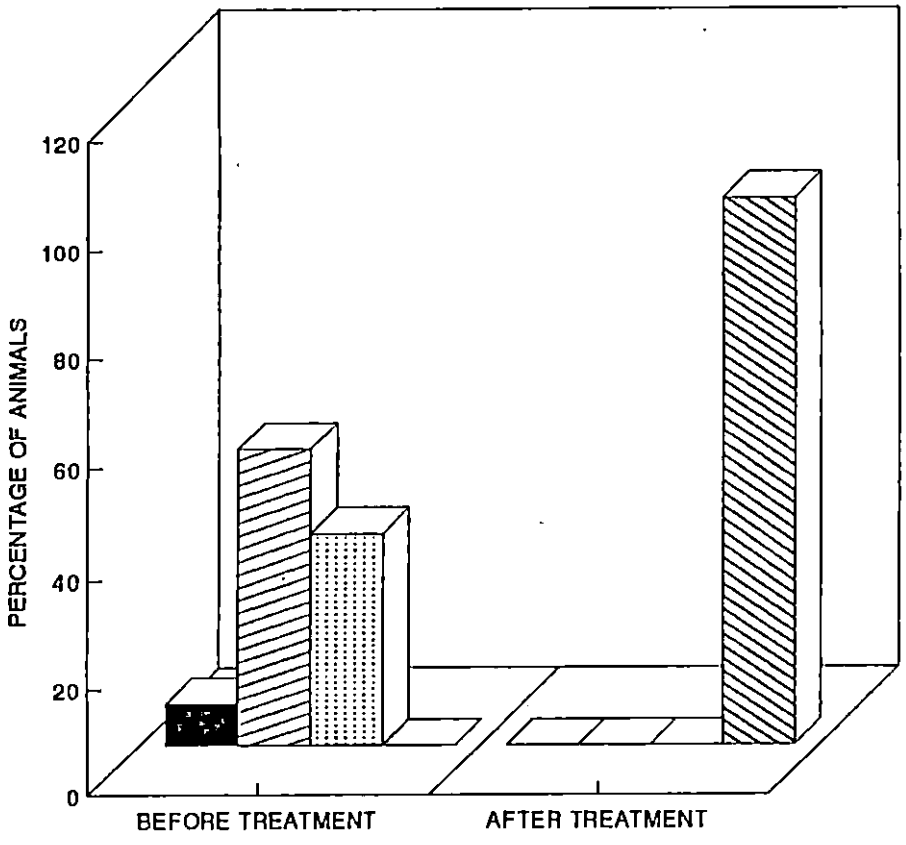
Time interval	Plasma trial			Saline trial		
	P_1	P_2	P_3	C_1	C_2	C_3
0 hr	4.00± 0.26	3.17± 0.40	2.50± 0.34	3.50± 0.34	2.67± 0.42	2.00± 0.26
1 hr	3.17± 0.40	2.67± 0.33	2.00± 0.26	3.33± 0.33	2.67± 0.42	2.00± 0.26
3 hr	2.50± 0.34	2.00± 0.26	1.50± 0.34	2.67± 0.21	2.17± 0.31	1.83± 0.31
5 hr	2.16± 0.31	1.50± 0.34	1.17± 0.17	2.50± 0.34	1.83± 0.31	1.25± 0.25
7 hr	1.83± 0.31	1.50± 0.34	1.00± 0.00	2.17± 0.31	1.83± 0.31	1.25± 0.25

Table 9.Mean reduction in turbidity (Nephalometer readings) with respect to different time intervals (Mean ± SE)

Trials	0-1 h	0-3 h	0-5 h	0-7 h
		a	b b	b
Plasma tubes	0.611 ± 0.1182	0.407 ± 0.0575	0.333 ± 0.0323	0.253± 0.0217
Saline tubes	0.056 ± 0.0555	0.167 ± 0.0485	0.172 ± 0.0406	0.238± 0.0203

Interpretation : Means bearing different superscripts differ significantly ($P<0.05$). Significant reduction in turbidity occurred within 0 to 1 h in plasma tubes.

FIG.1 CHARACTERISTICS OF OESTRUAL MUCUS BEFORE AND AFTER INTRA-UTERINE TREATMENT IN GROUP I



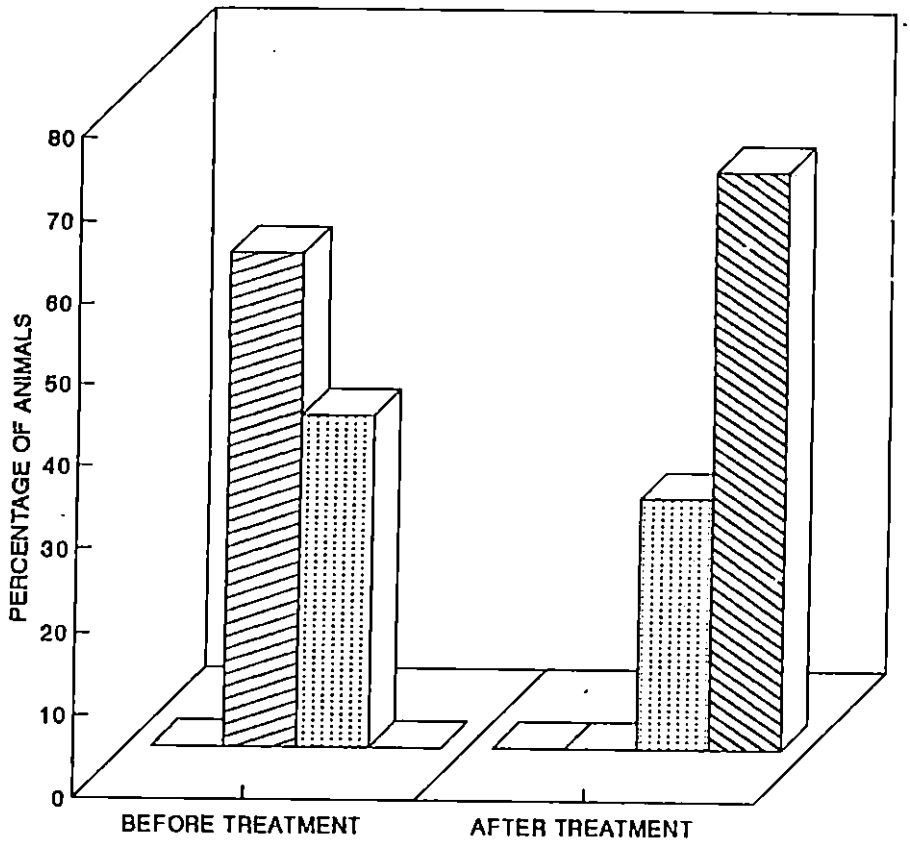
Purulent	7.69	0
Muco purulent	53.84	0
Cloudy	38.46	0
Clear	0	100

NATURE OF OESTRUAL MUCUS

TREATMENT GROUP

Purulent Muco purulent Cloudy Clear

FIG.2 CHARACTERISTICS OF OESTRUAL MUCUS BEFORE AND AFTER INTRA-UTERINE TREATMENT IN GROUP II



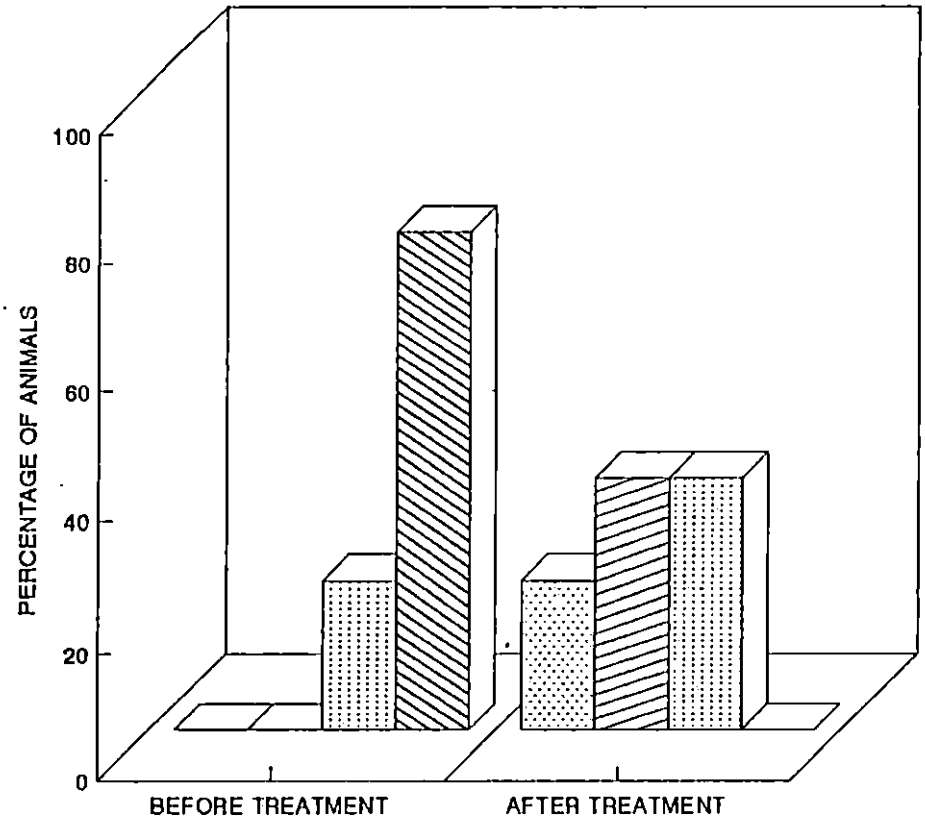
Purulent	0	0
Muco purulent	60	0
Cloudy	40	30
Clear	0	70

NATURE OF OESTRUAL MUCUS

CONTROL GROUP

Purulent Muco purulent Cloudy Clear

FIG.3 CHANGES IN UTERINE TONE BEFORE AND AFTER INTRA-UTERINE TREATMENT IN GROUP I

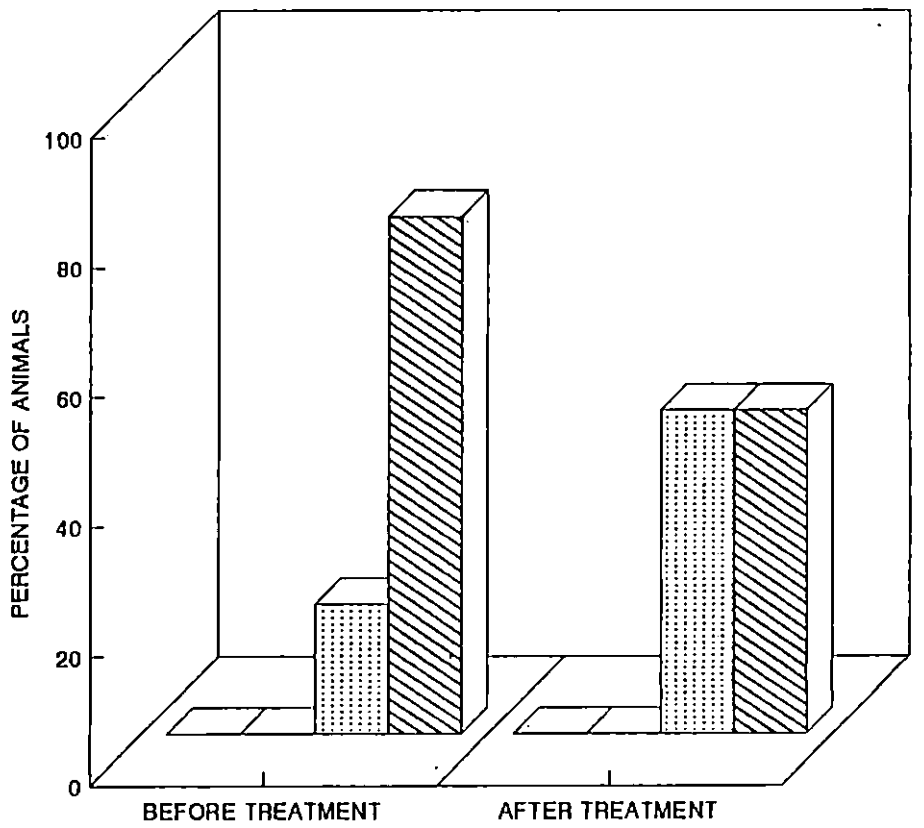


High	0	23.08
Medium	0	38.46
Low	23.08	38.46
Doughy	76.92	0

TONE OF UTERUS

TREATMENT GROUP
High Medium Low Doughy

FIG. 4 CHANGES IN UTERINE TONE BEFORE AND AFTER INTRA-UTERINE TREATMENT IN GROUP II



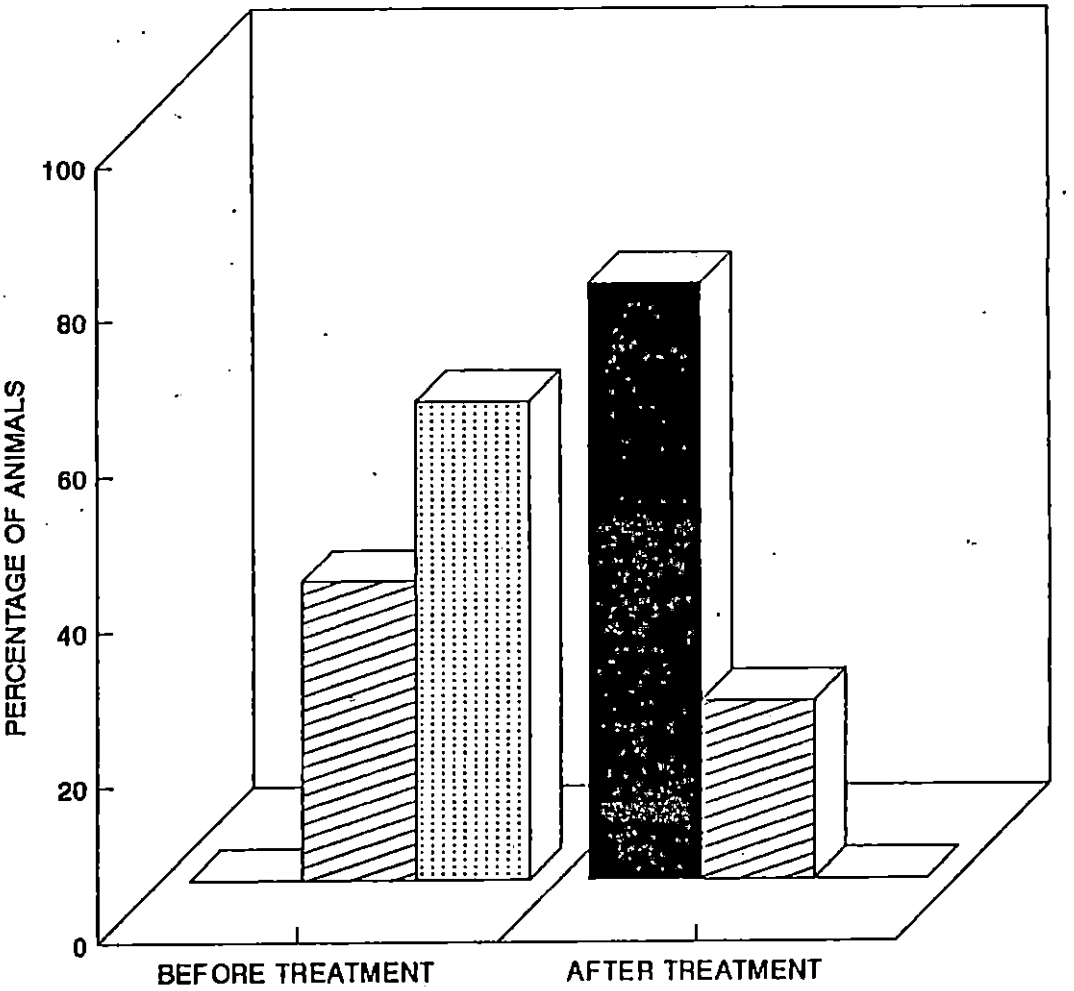
High	0	0
Medium	0	0
Low	20	50
Doughy	80	50

TONE OF UTERUS

CONTROL GROUP

High Medium Low Doughy

FIG.5 INTENSITY OF OESTRUM BEFORE AND AFTER INTRA-UTERINE TREATMENT IN GROUP I

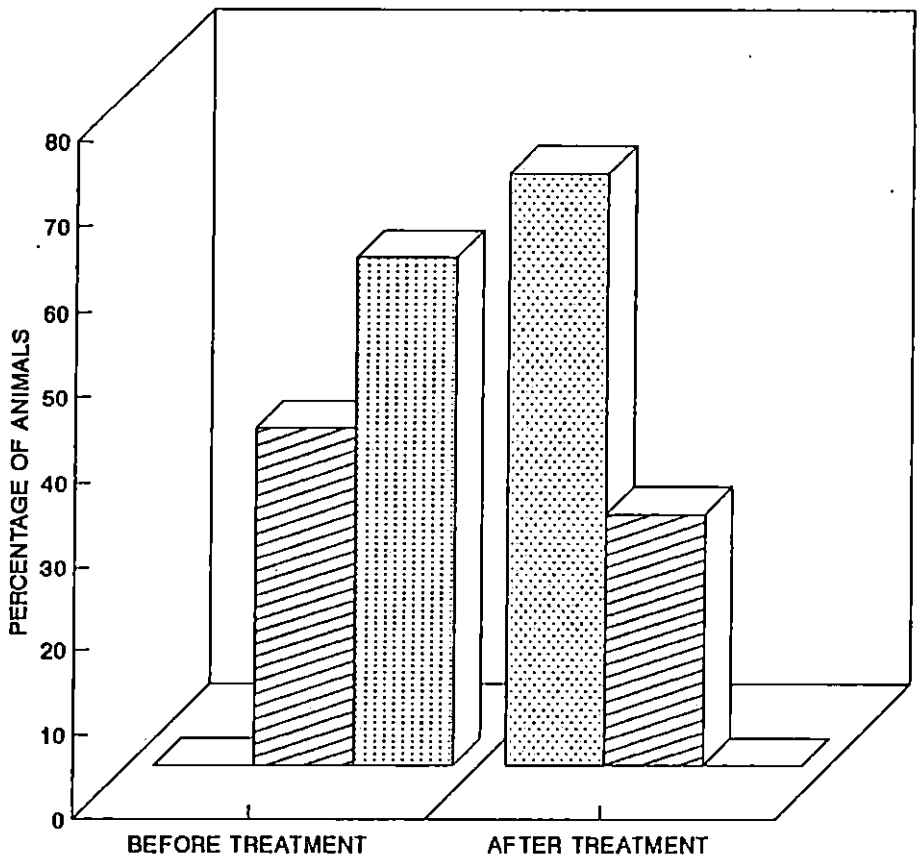


Intense	0	76.92
Indermediate	38.46	23.08
Weak	61.54	0

INTENSITY OF OESTRUM

TREATMENT GROUP
Intense Indermediate Weak

FIG.6 INTENSITY OF OESTRUM BEFORE AND AFTER INTRA-UTERINE TREATMENT IN GROUP II



Intense	0	70
Intermediate	40	30
Weak	60	0

INTENSITY OF OESTRUM

CONTROL GROUP

Intense Intermediate Weak

FIG.7 TIME INTERVAL FROM INTRA-UTERINE TREATMENT TO SUCCEEDING OESTRUS

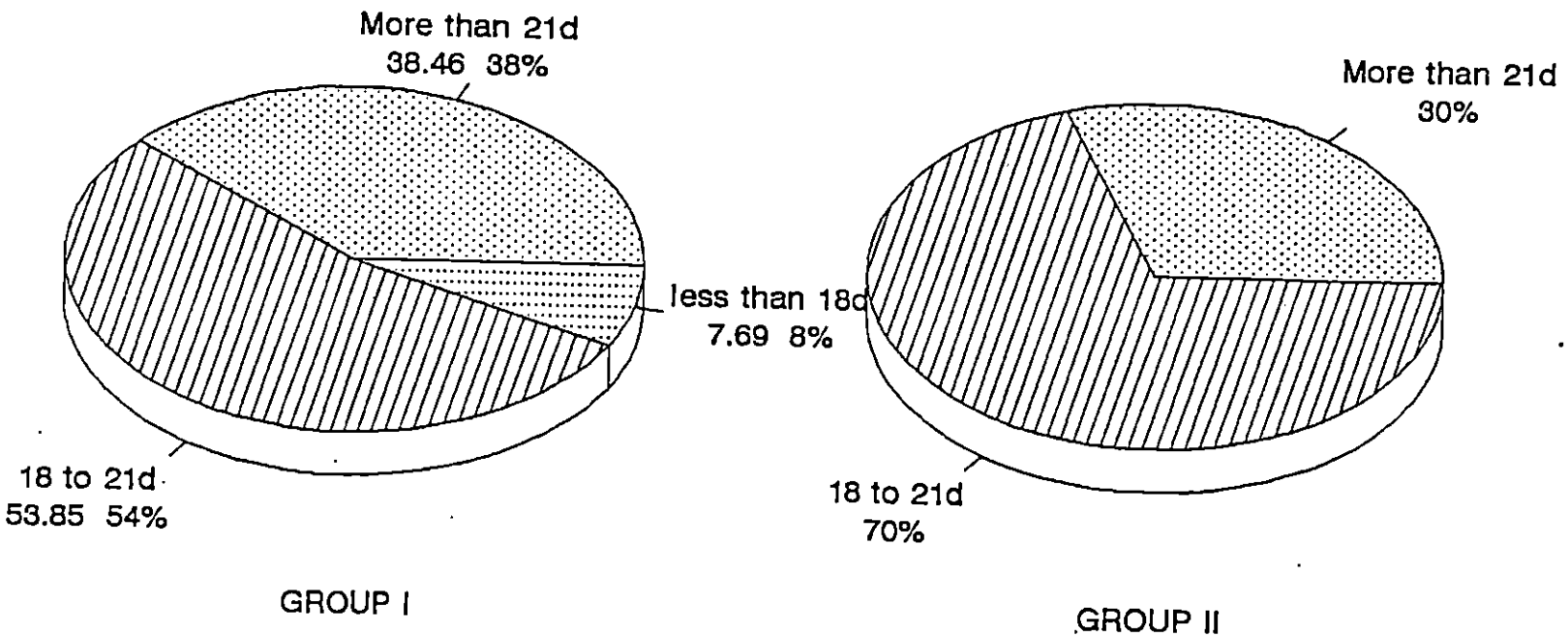


FIG.8 UTERINE BACTERIAL LOAD BEFORE AND AFTER INTRA-UTERINE TREATMENT

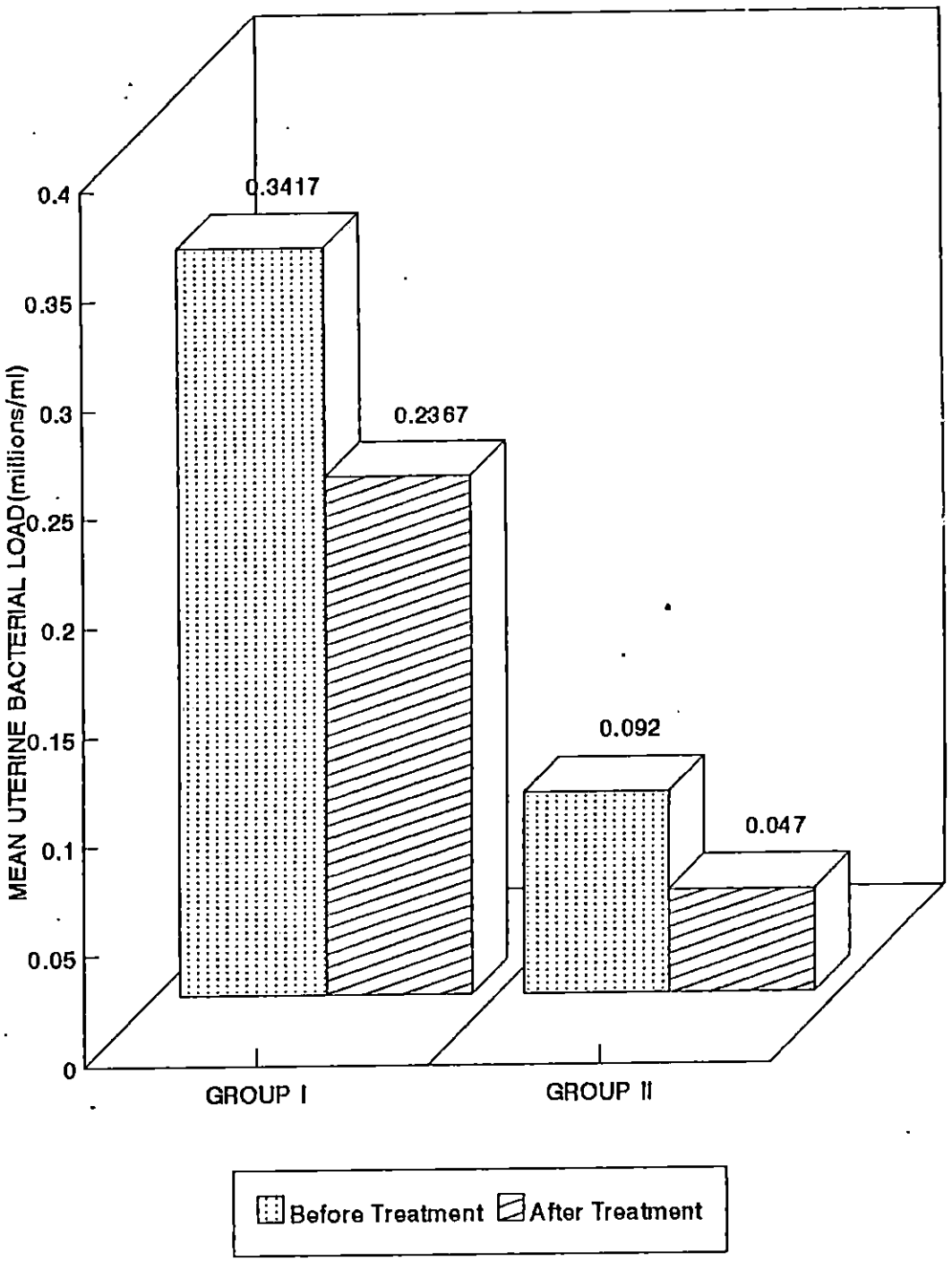
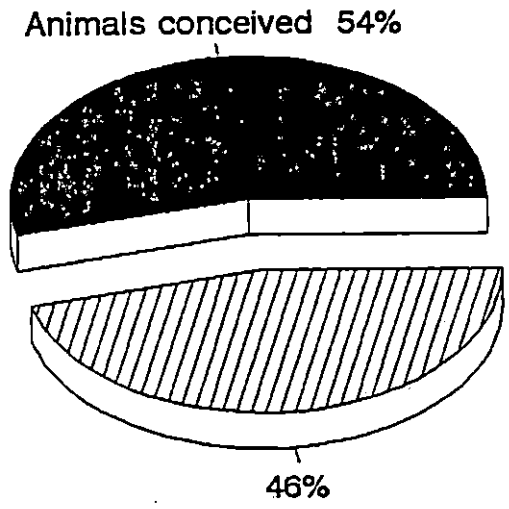
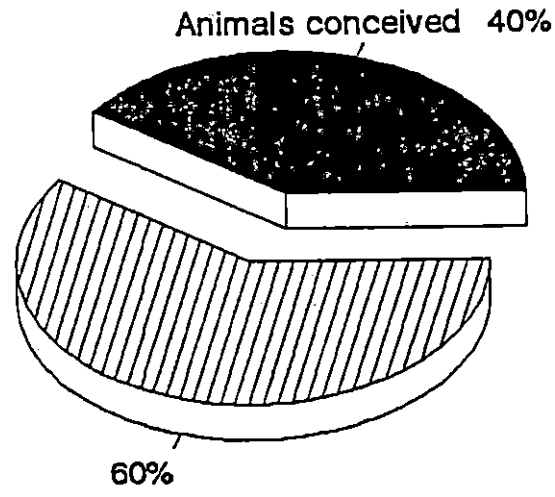


FIG.9 FIRST INSEMINATION CONCEPTION RATE IN GROUP I AND II

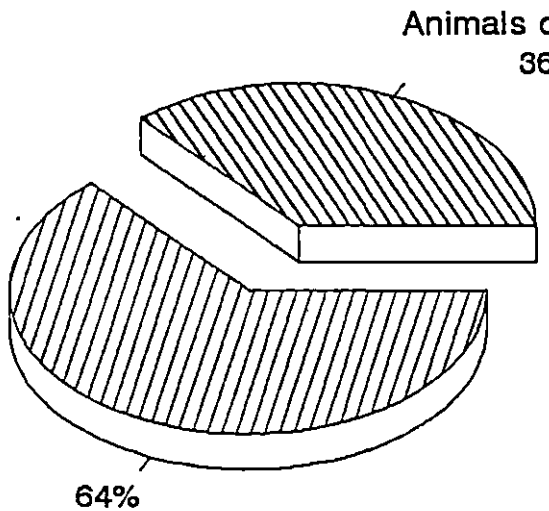


GROUP I

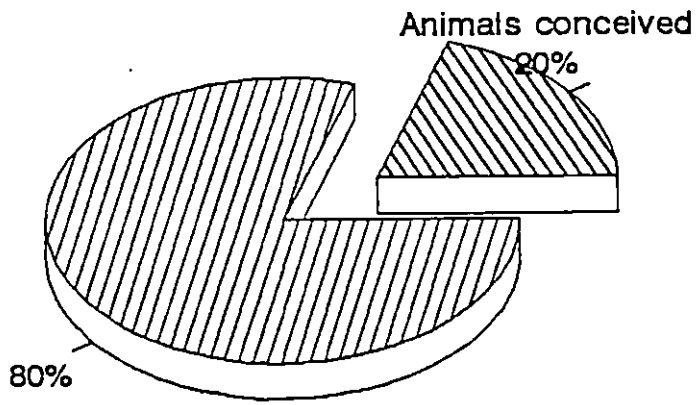


GROUP II

FIG.10 FIRST INSEMINATION CONCEPTION RATE IN GROUP III AND IV

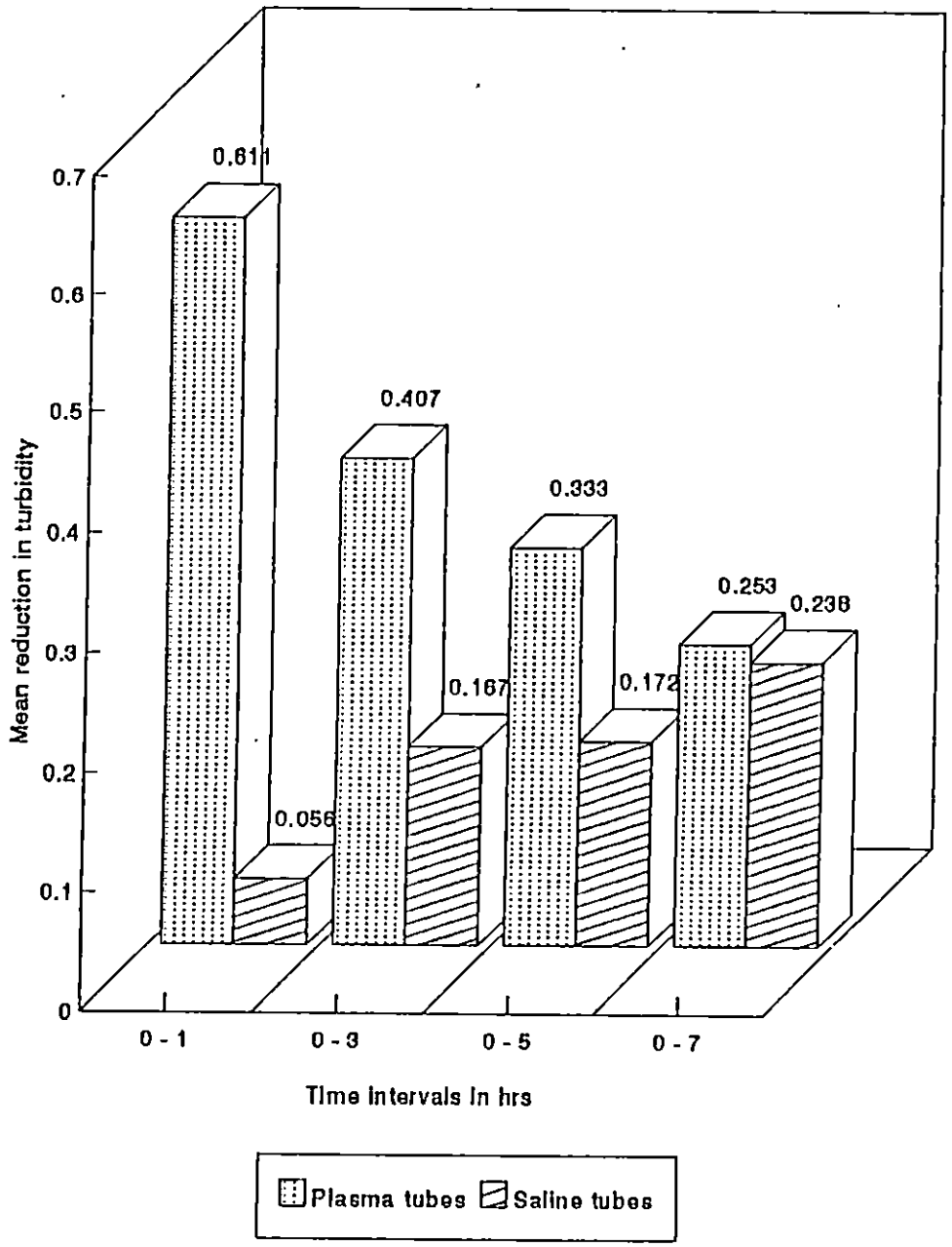


GROUP III



GROUP IV

FIG.11 REDUCTION IN TURBIDITY (NEPHALOMETER READING) WITH RESPECT TO DIFFERENT TIME INTERVALS



Discussion

DISCUSSION

Bovine endometritis is a serious infertility problem affecting reproductive efficiency thereby hampering economic livestock production (Bretzlaff et al., 1982; Gilbert, 1992). Based on the results of controlled studies many treatment lines which include antibiotics (Koleff et al., 1973; Namboodiripad et al., 1976; Ziv, 1980; Sharma et al., 1988; Dholakia et al., 1987; Varadarajan and Nair, 1990; Vahida, 1992) antiseptics (Grunnert et al. 1973; Sequin et al., 1974; Gupta et al. 1990) hormones (Roberts, 1956; Gustafsson, 1984) and prostaglandins (Jackson, 1977; Coulson, 1978; Ott and Gustafsson, 1981; Jacob, 1993) are in vogue. Eventhough most of these treatments are effective in relieving infection and improving fertility of the affected animals to a great extent, they have their own limitations. Since livestock rearing in the state is an avocation of economically backward sections of the society, the prohibitive cost of antibiotics and hormones acts as a great deterrent in the treatment of endometritis. This drives home the need for evolving a viable less expensive and non antibiotic therapy for effective treatment of endometritis and hence this work.

Perusal of the data presented in Tables 1 to 3 reveals that there is definite change in the nature of oestrual mucus, tone of the uterus and intensity of oestrum with plasma treatment. All cases of endometritis with

purulent or mucopurulent or cloudy discharge became clear after plasma treatment. However the uterine discharge remained cloudy in 30 per cent of the cases in control group. In most of the cases of endometritis the uterine tone was either low or doughy which improved to medium or high following plasma treatment. Similarly the intensity of oestrum in the affected animals which was in the range of intermediate to weak heat showed definite improvement to intense heat signs.

Definite improvement in the uterine tonicity, intensity of oestrum and clearing of uterine mucus can be attributed to increased phagocytosis aided by the complement in the plasma. Moreover the immunoglobulins present in the plasma might have also helped to eliminate infection by their direct bacteriostatic and bacteriolytic effect.

In vitro test on the antibacterial effect of plasma based on reduction in turbidity on MacFarland Nephalometer readings also support the above contention. The reduction in turbidity was significant ($P<0.05$) in plasma tubes compared to saline tubes. Maximum reduction had occurred within 0 to 1 h interval in plasma tubes which indicates an immediate antibacterial effect of plasma.

The interval from intra-uterine infusion to succeeding oestrum was found to vary following plasma treatment when compared to saline infusion. The mean duration

was found to be 28.23 ± 4.67 days in the group which received plasma as against 20.3 ± 0.4 days in saline group. This is on account of the fact that a sizable number of cows in the plasma treated group had prolonged cycle. Infusion of substances that irritate the uterus may cause the oestrous cycle to be either prolonged or shortened depending on the stage of oestrous cycle when the infusion was administered (Oxender and Seguin, 1976 and Morrow, 1980). Since saline is no irritant the cycle length was not prolonged in that group. On the other hand plasma containing many factors might have acted as a mild irritant resulting in the prolongation of the cycle. Adams and Ginther (1989) recorded mild endometrial inflammation based on biopsy studies in animals treated with plasma.

The mean uterine bacterial load before and after intra-uterine treatment with plasma did not vary significantly. Eventhough clinical recovery occurred following plasma treatment, the uterine discharge showed a bacterial load of 0.2367 ± 0.3034 millions/ml. This may be explained on the basis of an earlier report that the normal puerperium of a cow could harbour bacteria (Ambrose and Pattabiraman, 1989).

There was definite improvement in the conception rate following a single intra-uterine infusion of homologous plasma. This is in agreement with the similar reports on

plasma treatment of endometritis in mares with chronic endometritis (Asbury 1984; Parmigiani, 1985; Ward, 1985).

The fact that there was still fairly heavy bacterial load in the uterus after single intra-uterine infusion clearly points out that the infection is not completely eliminated eventhough the uterine discharge was clear in all the cases. It is therefore felt that conception have been further improved if plasma infusion were repeated in two or three succeeding cycles.

Cows with three inseminations in group III and IV showed conception rates of 50 and 30 per cent respectively, whereas cows with more than three inseminations recorded conception rates of 28.57 and 10 per cent respectively. First insemination conception rate in repeat breeder cows which had failed to settle with three or more inseminations showed definite improvement following post insemination plasma therapy. Moreover 42.85 per cent of the cases in more than three AI group receiving plasma infusion had a history of unsuccessful antibiotic treatment. Eventhough comparable figures are lacking in cows, significantly high conception rate following post insemination plasma therapy has been reported in mares with subclinical endometritis (Asbury, 1984; Ward, 1985).

It could be concluded that homologous plasma infusion into the uterus could be adopted as a therapy for endometritis and as a post insemination therapy for repeat breeding.

Summary

SUMMARY

Bovine endometritis is an economically significant problem in most of the dairy herds with immense bearing on the fertility of affected animals. Several treatment regimes with varying degree of success have been evolved based on extensive field trials. The objective of the present investigation was to formulate an effective non-antibiotic alternate therapy for bovine endometritis by enhancing the normal uterine defence mechanism. With this aim in view intra-uterine infusion of homologous plasma rich in complement and immunoglobulins was adopted as a treatment for endometritis. Post insemination infusion of homologous plasma was also adopted in repeat breeders with subclinical first degree endometritis.

The luminal secretions of cows with endometritis randomly allotted to group I (n=13) and II (n=10) were collected aseptically at the time of oestrum and subjected to standard plate count to assess the bacterial load. Animals in group I were given 25 to 35 ml of homologous plasma as intra-uterine infusion on the day of oestrum, whereas animals in the control group (group II) were given 25 to 35 ml of sterile normal saline. Bacterial load in the luminal secretions of cows belonging to both the groups in succeeding oestrus following treatment were estimated by standard plate count. Cows showing recovery based on clinicogynaecological

examination following treatment were inseminated with semen from known fertile bulls.

Repeat breeder cows with first degree endometritis were randomly allotted to group III (n=22) and IV (n=20). Cows in group III were inseminated with semen from known fertile bulls followed by intra-uterine infusion of 25 to 35 ml of homologous plasma, 24 h after insemination. The control group (group IV) consisting of twenty repeat breeder cows with subclinical endometritis were inseminated with semen from known fertile bulls and left without any treatment. First insemination conception rate of cows belonging to all the four groups was estimated by actual pregnancy verification.

An in vitro test to study the antibacterial effect of plasma was carried out by measuring the reduction in turbidity of a bacterial suspension on addition of plasma. The turbidity reduction was estimated at varying intervals of time by visual comparison with standard MacFarland Nephalometer tubes.

Results obtained and inferences drawn are summarised below. The oestrual secretions became clear following plasma infusion in 100.00 per cent of the cases in group I as against 70 per cent in group II. However the luminal secretion was cloudy in 30 per cent cases in group II following saline treatment. In group I cows with

consistently low or doughy uterine tone, intra-uterine plasma treatment had improved uterine consistency to medium and high tone in 38.46 and 23.08 per cent of the cases respectively. However no definite improvement was recorded in control group (group II). The intensity of oestrum showed comparable results in that it improved from intermediate/weak heat to intermediate/intense heat after intra-uterine infusion in group I and II.

The mean duration from intrautrine infusion to succeeding oestrum was found to be 28.23 ± 4.67 days in group I as against 20.3 ± 0.4 days in group II.

The mean uterine bacterial load before and after intra-uterine treatment did not vary significantly in group I and II, eventhough there was reduction in the bacterial load of uterus.

The data on first insemination conception rate revealed 53.85 per cent conception rate in group I as against 40 per cent in group II.

The repeat breeder cows with subclinical first degree endometritis belonging to group III and IV were subdivided further into those animals which had received three inseminations and those with more than three inseminations. Cows with three inseminations in group III and IV showed conception rates of 50 and 30 per cent respectively, whereas

cows with more than three inseminations recorded conception rates of 28.57 and 10 per cent respectively.

The in vitro test on antibacterial effect of plasma revealed that maximum reduction in turbidity occurred within 0 to 1 h ($P<0.05$) interval in plasma tubes, whereas in saline tubes, no significant reduction in turbidity with respect to time was recorded.

In general, it can be inferred from the present study that endometritis can be effectively treated with intra-uterine infusion of homologous plasma drawn from the cow on the day of oestrum. Both clinical recovery and conception could be achieved in a shorter period of time. Similarly repeat breeder cows can be successfully made to conceive with a single intra-uterine infusion of plasma 24 h after insemination.

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INTRA-UTERINE INFUSION OF HOMOLOGOUS PLASMA IN THE TREATMENT OF ENDOMETRITIS IN COWS

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ABSTRACT OF A THESIS

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ABSTRACT

The present study was undertaken on infertile cows with uterine infections, with the objective to evaluate the beneficial effect of intra-uterine infusion of homologous plasma as a non antibiotic alternate therapy for bovine endometritis. The luminal secretions of cows with clinical endometritis allotted to group I (n=13) and II (n=10) were aseptically collected at the time of oestrum and subjected to plate count to assess the uterine bacterial load prior to treatment. Cows belonging to group I were given 25 to 35 ml of homologous plasma as intra-uterine infusion on the day of oestrum whereas in control group (group II) the cows were given 25 to 35 ml of sterile normal saline as intra-uterine infusion.

Luminal secretions of all the cows were collected aseptically at the succeeding oestrus following treatment and the uterine bacterial load was estimated. Cows which showed recovery in terms of clinico-gynaecological findings were bred artificially.

Following plasma therapy definite improvement as evidenced by clear uterine discharge, higher uterine tone and intensity of oestrum was noticed in the treatment group. These changes were not pronounced in the control group. The mean uterine bacterial load showed reduction after

intra-uterine treatment in both group I and II, however the reduction was statistically insignificant. The mean duration from intra-uterine treatment to succeeding oestrus was prolonged in group I (28.23 ± 4.67 d) as against normal in group II (20.3 ± 0.4 d). The first insemination conception rate recorded in group I was 53.85 per cent as against 40 per cent in group II showing that there was definite improvement in conception rate following plasma treatment.

Repeat breeder cows with subclinical first degree endometritis allotted to group III were administered 25 to 35ml homologous plasma 24 h after insemination whereas repeat breeders (group IV) were left untreated following insemination. The mean conception rate for group III was 36.36 as against 20 per cent for group IV. This result also signifies the beneficial effect of plasma treatment in improving conception rate of repeat breeders.

An <u>in vitro</u> test to study the antibacterial effect of plasma was carried out by measuring the reduction in turbidity of a bacterial suspension on addition of plasma. It was found that the reduction in turbidity was significant ($P<0.05$) in plasma tubes compared to saline tubes. Maximum reduction had occurred within 0 to 1 h interval in plasma tubes. This indicated an immediate antibacterial effect of plasma

It could be inferred from the above findings that

homologous plasma drawn from the cow at the time of oestrum has significant bactericidal and bacteriostatic properties and when infused into the uterus of cows affected with endometritis helps in quicker clinical recovery and better conception rate. Post insemination plasma infusion into the uterus also improved conception rate in repeat breeders. Hence plasma treatment is recommended as an effective less expensive and non antibiotic alternate therapy for endometritis and repeat breeding in cows.