STUDIES ON
HORMONAL INDUCTION OF LACTATION IN COWS

BY

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DECLARATION

I hereby declare that this thesis entitled "STUDIES ON HORMONAL INDUCTION OF LACTATION IN COWS" is a bona fide 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,
6 -8-1977.

CERTIFICATE

Certified that this thesis, entitled "STUDIES ON HORMONAL INDUCTION OF LACTATION IN COWS" is a record of research work done independently by Sri. P.M. Joseph 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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INTRODUCTION

INTRODUCTION

Adoption of scientific principles in breeding, feeding and management practices for increased milk production has resulted in animals with high milk producing potentialities. Crossbreeding with exotic breeds has increased the genetic potentiality of Indian cows for milk production severalfold. One of the problems faced by the dairy farmers is the poor breeding efficiency of the cows. This may be attributed to several factors like infection, malnutrition and management. Whatever be the etiology, the economic loss sustained by the farmer due to the poor reproductive performance of his dairy animals is considerable. Sometimes it becomes difficult for the heifers to get conceived. Some animals may attain puberty and calve only very late. Certain cows calve once or twice and then go dry without getting pregnant. In a country like India, where slaughter of cows is highly restricted, the expenditure incurred by the farmer for the maintenance of such animals is very high. The loss will be much larger if the infertile cow is of high genetic potentiality. In fact the economic viability of any dairy undertaking depends not only on milk yield but also on the breeding efficiency of the cows. Many potentially valuable dairy animals are discarded from the herds each year because of breeding failures. It has been observed by Iton (1974) that beyond a certain level, milk

yield and reproductive performance are antagonistic to each other. The result of first insemination of dairy cows was found to be satisfactory upto 4000 kg of milk, beyond which there was seemed to have a steady decline in the rate of conception to the first insemination. He observed that fertilization rates were highest when daily milk yield was 10-15 kg and it markedly declined when the yield exceeded 20 kg. This clearly indicates the possibility of reduction in breeding efficiency as the production potential is increased.

The development of a technique to initiate lactation in animals that fail to conceive is of considerable significance. Heifers that fail to conceive or attain puberty very late and calve much later can profitably be used for milk production before they calve and their milk producing ability can thus be exploited. Similarly cows of high milk producing potentialities which go dry without conception after one or two calvings can be used as a potential source of milk. Some workers have observed that the milk yield in induced lactation can be taken as a criterion to assess the future milk production of heifers and culling of heifers can be made much earlier. Another advantage of this technique is the possibility that many of the infertile animals may conceive after the induction of lactation.

Scientists have been attempting for over 30 years

to induce lactation in dairy cows. Considerable progress has been attained in this direction during recent years. The parenteral administration of reproductive hormones to induce udder development and lactation is the technique that has attained much importance. The basic principle underlying this technique is to create in the animal system, a hormonal set up similar to that in a pregnant animal. Morphogenesis and biochemical differentiation of the mammary gland is regulated by a complex of hormones required at different stages of development. Through a series of studies it is established that the anterior pituitary hormones, oestrogen and adrenal steroids cause the mammary duct growth, while lobule-alveolar growth is induced by progesterone as well as prolactin in the presence of the above hormones. By 1931 it was understood that in vivo mammary morphogenesis required the presence of oestrogen for duct development and progesterone for alveolar tissue development and that lactogenesis was initiated by the release of hormones from the anterior pituitary. The considerable amount of literature on the mammogenic potencies of ovarian steroids in various species of animals provide information on the effective dose levels of the ovarian hormones for stimulating extensive duct and lobule-alveolar growth.

In earlier works oestrogen alone was used to elicit udder growth and lactation in cattle and goats. The histological

study of the mammary gland developed by the treatment of oestrogen alone showed several abnormalities. When progesterone also was given along with oestrogen these abnormalities were not present.

In most of the earlier studies the duration of the treatment varied from a few weeks to several months as this duration was thought to be required for the optimum udder development. But Smith et al. (1971) evolved a procedure in which the hormone combination was given for seven days with very good results. Since then several workers have adopted this procedure.

Eventhough both natural and synthetic oestrogens have been used for the induction of lactation, most of the workers have used natural oestrogen. Literature is scanty about the use of synthetic oestrogen for induction of lactation by the short period treatment. The present investigation has been undertaken—

1. to study the effect of combination of synthetic oestrogen and progesterone in the induction of lactation by the short term treatment as compared to the combination of natural oestrogen and progesterone;

2. to compare the responses between seven days' and fourteen days' treatments with the combination of synthetic oestrogen and progesterone;

3. to study the composition and properties of the milk produced by the induced lactation and

4. to study the effect of induction of lactation by hormones on the blood values.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Role of Hormones in the Development of Mammary Gland

Ovarian hormones.

The role of hormones in the development of mammary gland in various species have been extensively investigated. The mammary gland development that occur during the oestrus period in several species led scientists to the conclusion that mammary gland growth is stimulated by ovarian hormones. Experiments with ovarian extracts and pure oestrogen and progesterone preparations have confirmed the stimulatory action of ovarian hormones on mammary gland (Jacobson, 1961).

In cattle, goats, guinea pigs and monkeys oestrogen in physiological doses caused the growth of both duct and lobulo-alveolar system (Folley, 1956). Studies on this aspect in ruminants showed that eventhough oestrogen alone induced growth of duct and alveolar system, the response was very erratic. Mixner and Turner (1943) observed that the mammary gland developed by the action of oestrogen showed many abnormalities on histological examination. The main abnormalities observed were cystic alveoli, papillomatous outgrowths into the alveolar lumen and immature ducts and alveoli without lumen. Cowie *et al.* (1952) and Benson *et al.* (1955) have confirmed these findings. They found that these abnormalities could be avoided if progesterone also was given simultaneously with oestrogen.

In cows it was observed that oestrogen combined with progesterone made better mammary gland development and higher milk yield than by treatment with oestrogen alone (Sykes and Wrenn, 1951; Reineke et al. 1952; Folley, 1956 and Meites 1961). They found that the mammary glands developed by this procedure were free from histological abnormalities. In goats, Cowie et al. (1952) and Benson et al. (1955) administered oestrogen with progesterone and induced mammary gland development without histological abnormalities. Now it is established that both oestrogen and progesterone are required for the normal development of the mammary gland.

Pituitary hormones.

The works of Lyons et al. (1958) clearly established that an intact anterior pituitary was essential for the development of mammary gland in response to the ovarian hormones. They found that five of the six anterior pituitary hormones played an important role in mammary growth and lactogenesis. The studies of Lyons and Dixon (1966) and Norgren (1968) confirmed the role of anterior pituitary in mammary gland development.

Adrenal hormones.

The studies by Ahren and Jacobson (1957) on the effect of cortisone on the mammary glands of hypophysectomised rats, established that the general metabolic rate of the animal was important in mammary gland development. Flux (1954) tested a

number of 11-oxygenated corticoids and found that only 11-deoxycorticosterone acted synergistically with oestrogen in promoting mammary duct growth. Injection of cortisol acetate in low doses in ovariectomised mice stimulated mammary gland development (Flux and Munford, 1957). In rats with intact pituitaries cortisone stimulated secretion but not mammary growth, whereas the addition of oestrogen and progesterone promoted growth and abundant secretion (Ahren and Jacobson, 1957). Chen et al. (1955) and Lyons et al. (1958) had shown that the mammary gland growth could be induced in ovariectomised, hypophysectomised, adrenelectomised and thyroidectomised rats by giving oestrogen, progesterone, prolactin. growth hormone and cortisone but replacement of thyroid was not necessary. In cows and goats the galactopoietic effect of thyroid had been established (Meites, 1961).

Role of Hormones in Milk Secretion

For a long time it was postulated that milk secretion was a passive phenomenon taking place after the removal of the inhibitory hormones from placenta. Stricker and Grueter (1928) demonstrated lactogenesis in the pseudopregnant rabbit in response to injection of anterior pituitary extracts and they concluded that the fully developed mammary gland secreted in response to a positive hormonal stimulus. Subsequently Riddle and his colleagues (1933) identified the pituitary lactogenic principle - prolactin - with the pigeon crop-gland

stimulating factor. But it was found in hypophysectomised animals that in the absence of other anterior pituitary hormones, prolactin was not lactogenic. It was therefore suggested by Folley and Young (1941) that other pituitary hormones were also concerned in milk secretion and that it would be preferable to refer as a lactogenic hormone complex rather than a specific lactogenic hormone.

Studies of Lyons et al. (1958) showed the minimal hormonal requirement for milk secretion in rats. In rats lobulo-alveolar proliferation was induced by treatment with a combination of oestrogen, progesterone, prolactin, growth hormone and adrenal corticoids; then milk secretion could be initiated by continuing with the injection of prolactin and corticoids. In vitro organ culture studies with lobulo-alveolar tissue maintained in a synthetic medium, confirmed the importance of the above hormone combination (Rivera, 1964).

Studies on hypophysectomised lactating goats by Cowie and Tindal (1961) and Cowie (1970) had shown that ovine prolactin, bovine growth hormone and adrenal corticoid gave substantial restoration of milk yield and further addition of thyroid hormone to these three hormones fully restored the milk yield to the pre-operation level.

A widely held concept related to milk secretion is that steroids from the ovaries and placenta can inhibit milk secretion by exerting a direct inhibitory effect on the mammary epithelium.

rendering it unresponsive to the circulating pituitary and placental lactogenic hormones. In addition to this, the high level of oestrogen in the blood during pregnancy inhibit the release of prolactin from the pituitary and at parturition as the oestrogen concentration is reduced, the low level of oestrogen permits increased prolactin output and permit the lactogenic hormone to act on the alveolar cells. Another view is that the high level of adrenal steroids at the time of parturition helps in the initiation of lactation (Cowie, 1969). Smith et al. (1973) have shown that the level of corticosteroids reached a high level at the time of parturition. In some species suckling stimulus itself plays a major role in stimulating lactogenesis (Cowie, 1970).

It is now well established that the secretory activity is regulated by hormones from the anterior pituitary and its target organs like adrenal and thyroid (Cowie and Tindal, 1971). In ruminants it has been shown that unlike in non-ruminants prolactin is concerned with the onset of mammary secretion rather than with its maintenance (Cowie, 1976).

Hormonal Induction of Lactation

The knowledge of the hormonal set up in the animal body during pregnancy (Erb et al. 1968; Wetteman and Hafs, 1973; and Smith et al. 1973) and the hormones responsible for the mammary gland development and milk secretion, prompted the scientists

to conduct experiments on the hormonal induction of lactation. The knowledge that oestrogen alone caused both duct and alveolar growth in cows and goats and the discovery of synthetic oestrogen and progesterone contributed a lot in this regard. The experimental procedure was to administer oestrogen alone or oestrogen and progesterone combination to induce udder development. Oestrogen also acted as a stimulant for the secretion of prolactin, to initiate lactation. So it was customary to give a boosting dose of oestrogen, after the administration of oestrogen and progesterone, to stimulate the secretion of prolactin (Meites and Turner, 1942). Meites (1961) reported that induction was more complete in heifers than in cows.

Oestrogen.

Growth of mammary gland and induction of lactation in cows and goats by the administration of oestrogen alone had been reported by various authors. Williams and Turner (1962) and Turner et al. (1963) injected oestradiol benzoate at the rate of 0.3 mg/100 lb body weight in heifers daily for 14 days and induced lactation. Similarly Hindery and Turner (1964, 1967) injected oestradiol benzoate 0.3 mg/100 lb body weight daily for 14 days in one group of animals and 0.5 mg/100 lb body weight for seven days in another group of heifers and cows. In both the cases lactation was initiated. This procedure was repeated at 63 days interval three times. They showed that upto four re-initiations could be successfully conducted. Hindery and

Turner (1968) injected oestradiol benzoate 1.1 mg/100 kg body weight in five cows and six heifers for seven days and started milking them from the third day. Three additional seven days treatments were given at 63 days interval. The cows reached a maximum daily yield of 14.2 kg after the first series of injections and 19.3 kg after the second series of injections. These were 50 per cent of the corresponding yields in the previous normal lactation. Similarly the heifers reached a maximum daily yield of 9.1 kg and 11.5 kg. Naito et al. (1968) induced lactation in 30 Holstein heifers using oestrogen alone. They gave subcutaneous injection of 5 mg diethylstilboestrol on every third day for 30 days with good results. They concluded that this procedure could be used to predict the future production of the heifers.

In addition to the histological abnormalities of the mammary gland developed by the treatment with oestrogen alone, this method had other disadvantages. The milk yield was less than normal lactation. Another disadvantage was that the cows might show side effects like nymphomania and fracture of pelvis; thus rendering the animal useless for future production (Sykes and Wrenn, 1951; Benson et al. 1955 and Turner et al. 1956).

Combination of oestrogen and progesterone.

As early as 1943, Mixner and Turner suggested that the histological abnormalities in the udder of goats, developed by

treatment with oestrogen alone could be avoided by the combined administration of oestrogen and progesterone. In goats they injected 100 /μg of diethylstilboestrol and 20-30 mg progesterone per day for 60 days and found that the mammary gland had tightly packed alveolar cells similar to those of udders at mid-pregnancy.

Sykes and Wrenn (1951) injected 6 mg of diethylstilboestrol and 240 mg progesterone in calves daily for five months which resulted in the development of almost histologically normal udder tissue. Similarly, Turner et al. (1956) induced mammary gland growth in cows by injecting 100 /μg of oestradiol benzoate and 100 mg progesterone daily for 180 days. By injecting a triggering dose of oestrogen at the rate of 0.3 mg per day for a further period of 14 days they induced lactation in those animals. There was marked increase in milk yield after 3-6 days and the peak yield was reached by about 14 weeks.

In another experiment Turner et al. (1963) compared the results of treatments in heifers with oestrogen alone and treatment with oestrogen and progesterone combination. They found that the treatment with the hormone combination was superior to the treatment with oestrogen alone.

Benson et al. (1955) studied the histology of the udder developed by the treatment with the hormone combination in goat and found normal alveolar growth. Surgical removal and histological examination of the mammary gland at the end of injections with the hormone combination revealed that the gland consisted of

much stroma with relatively small amount of paranchymal tissue consisting of immature alveoli(Cowie et al. 1965). This led them to the conclusion that most of the lobulo-alveolar growth occurred during lactation and that milking stimulus was responsible for most of the lobulo-alveolar growth. Using ovariectomised and hypophysectomised goats they tested this possibility and confirmed that the development was pituitary mediated. This enabled them to suggest that regular milking should be commenced along with oestrogen treatment in artificial induction of lactation.

Sud et al. (1968, 1971) induced mammary gland growth in normal and hypophysectomised heifers by injecting 400 /ug of oestradiol 17 beta-and 100 mg of progesterone three times weekly for 20 weeks. Histologically normal udder tissue was observed. In heifers Sud (1972) developed the mammary gland by this method and initiated lactation using 9-Fluroprednesolone.

Tripathi (1973) induced lactation in nine heifers and nine cows by injecting 3625 mg progesterone and 525 mg stilboestrol within a period of 4-5 months. In the last 10 days the amount of progesterone was reduced. Milking was started from the second month of injection. There was milk secretion in four heifers and five cows.

All the above mentioned works involved the treatment with the hormone combination for prolonged periods ranging from

60-180 days as this time was thought to be required for full mammary gland development (Turner et al. 1956).

Investigation by Smith et al. (1971) showed that 17 beta-oestradiol and progesterone for seven days induced colostrum formation in non-lactating and non-pregnant heifers and cows. Eventhough the responses were varying, this procedure showed promise for commercial application.

Injection of 17 beta oestradiol and progesterone at the rate of 0.1 mg and 0.25 mg/kg body weight respectively for seven days induced the formation of a fluid similar in composition to normal bovine colostrum, in cows (Smith et al. 1973). They concluded that 0.1 mg/kg body weight for seven days may be the lower pharmacological dose of oestrogen required to initiate lactation and that the low dose levels used in previous works might be the reason for unsatisfactory results.

Smith and Schanbacher (1973, 1974) injected 17 beta oestradiol and progesterone at the rate of 0.1 mg and 0.25 mg/kg body weight respectively for seven days into nine cows and one heifer. Half the daily dose was given subcutaneously at 12 hour intervals. In the heifer the injections were given for 10 days. There was induction of lactation in seven out of the 10 animals. Lactation was commenced 11-21 days after the first injection and the peak yield was attained in 30-50 days. They observed that the animals in fifth to seventh day post-oestrus responded better than the animals in mid cycle and with functioning corpus-luteum.

Erb et al. (1973) and Moss et al. (1975) injected 17 beta-oestradiol and progesterone subcutaneously to three cows and five heifers. The daily dose was divided equally into two and was given at 12 hour intervals. Milk yield increased slowly from the 10th to the 21st day and reached the maximum by about 60 days after the last injection.

Collier et al. (1975b) injected 2 mg dexamethasone per day on the 17th to 21st day in addition to the normal course of oestrogen and progesterone. Of the 18 cows and five heifers, all except two came into lactation. Howe et al. (1975) injected a dose of 0.028 mg dexamethasone per kg body weight on days 18, 19 and 20 following the oestrogen and progesterone treatment for the induction of lactation. They found that much of the mammary gland development was noticed after the commencement of lactation. This was contrary to the prepartum enlargement in normal lactation.

Fulkerson and McDowell (1974) induced udder development in non-pregnant ewes by injecting 240 µg of oestradiol benzoate and 60 mg of progesterone every third day for 60 days. Subsequently either 10 mg dexamethasone trimethyl acetate or 5 mg oestradiol benzoate and 12.5 mg progesterone was injected for six days. Both these treatments produced further udder development and initiated lactation.

Sukumaran and Pavithran (1975) induced lactation in non-pregnant non-lactating goats using stilboestrol dipropionate and

progesterone at the rate of 0.1 mg and 0.25 mg/kg body weight respectively for seven days.

Monk et al. (1973) estimated the blood plasma progesterone and oestrogen in non-lactating and non-pregnant heifers induced to lactate, on the first and seventh day of injection and also 3rd, 8th, 11th and 22nd day after the last injection. They found that during injection the amount of progesterone approximated that of the luteal phase of oestrus cycle and the level of free and urinary oestrogen approximated those one day before parturition. Based on the estimated values of oestrogen, progesterone and prolactin in the blood plasma of cows induced to lactate, Erb et al. (1976) suggested that for better performance in induced lactation, the treatment should be started during three to eight days after oestrus. The daily dose of oestradiol should be decreased with progesterone unchanged for the first seven days and oestrogen alone should be continued for seven more days.

Narendran et al. (1974) found that in hormone induced lactation the mammary gland showed a number of immature and developing alveoli and ducts with no other abnormality. The time lapse to reach the peak yield was explained as the time taken for the maturation of these alveoli. Histological and biochemical studies of the mammary gland in induced lactation revealed lactogenesis on the 8th day while it was absent in the glands of unsuccessful cows (Collier et al. 1976).

Compounds other than oestrogen and progesterone.

For artificial induction of lactation, in addition to oestrogen and progesterone, several other compounds, alone or in combination with oestrogen and progesterone were tried. Bryant et al. (1968) experimented with sheep and found that tranquilizer acepromazine caused increase in plasma prolactine level and induced lactation.

Sud et al. (1970) fed melengestrol acetate (17-acetoxy-6-methyl-16-methylenepregna-4,6-diene-3,20-dione) a new progestational compound, to two groups of five cows each at the rate of 0.5 mg and 1.0 mg per day respectively for 20 weeks. Another group of five ovariectomised cows were injected with 400 /µg of oestradiol 17 beta-and 100 mg progesterone intramuscularly three times in a week for 20 weeks. Histological examination of the udder tissue developed by these methods showed that all these methods were equally effective in bringing about udder development. Sud and Meites (1971) fed ovariectomised and normal rats with melengestrol acetate alone and in combination with oestradiol and found that for effective induction of lactation intact ovary must be present and melengestrol acetate feeding was more effective for induction of lactation if given along with oestradiol.

Injection of 9-Fluroprednesolone acetate (Predef) a glucocorticoid was used for induction of lactation in 24 Holstein heifers in which the mammary gland was developed by the injection of either 100 mg progesterone and 400 /µg of oestradiol or 200 mg

progesterone and 800 /µg of oestradiol thrice weekly for 20 weeks (Sud, 1972). The milk yield was higher in the Predef treated group showing its efficiency to initiate lactation.

Fulkerson and McDowell (1975) studied the development of mammary gland and induction of lactation in ergocryptin treated ewes using syntocinon, dexamethasone trimethyl acetate and oestrogen and progesterone. The study revealed that syntocinon and dexamethasone trimethyl acetate were either lactogenic per se or effected the release of a lactogenic hormone complex other than prolactin whereas oestradiol and progesterone were lactogenic by virtue of the influence of oestrogen on secretion of prolactin.

The effect of reserpin on plasma prolactin level and milk yield in induction of lactation was studied by Collier et al. (1975a) and Bauman et al. (1976). Reserpin caused increased prolactin level and increased milk yield in artificial induction of lactation.

Route, dose and ratio of hormones for induction of lactation.

Different routes of administration of the hormones for induction of lactation have been adopted. The dynamics of hormone absorption for successful induction of lactation showed the importance of selecting the appropriate route for the administration of hormones (Smith and Schanbacher, 1974).

The major routes of administration are parenteral, oral and percutaneous, out of which the parenteral is the mostly used one. Among the parenteral routes subcutaneous injection seems to be the most popular one. Hindery and Turner (1968), Tripathi (1973), Fulkerson et al. (1975) and most of the workers in the seven day treatment method used this route for the administration of the hormones.

Eventhough the intramuscular route of administration of hormone was not successful for the induction of lactation (Smith and Schanbacher, 1974) many others tried this route and found successful (Sud et al. 1971; Pinheiro et al. 1974 and Sukumaran and Pavithran, 1975).

Garg and Nangia (1972) obtained mammary gland development and initiation of lactation in Haryana cows by subcutaneous implantation of tablets containing oestrogen and progesterone. But the milk yield was not much. The main handicap in this method was the difficulty to control the rate of absorption of the hormones.

Folley et al. (1941) applied oestrogen locally on the mammary gland of goats and induced udder growth. But in cows it was found that only very little yield could be obtained by local application of diethylstilboestrol.

Attempts to find out the optimum dose and ratio of oestrogen and progesterone to be given for the induction of lactation

were made from the very outset of the knowledge of the synchronising effects of oestrogen and progesterone. Studies with goats by Mixner and Turner (1943) showed that daily injection of 20-30 mg progesterone and 100 to 150 µg diethylstilboestrol (1: 200) for 60 days gave good results. The absolute amounts of the hormones employed appear to be as important as the ratio of the two hormones for producing maximum udder growth. Cowie et al. (1952) injected a combination of 1 mg hexoestrol and 40 mg progesterone (1: 40) daily for 70 days in goats and reported that the quantitative histological measurements were not significantly different from those treated with hexoestrol alone. But Benson et al. (1955) used 0.5 mg hexoestrol and 70 mg progesterone (1: 140) and found normal udder development.

Sud et al. (1968) reported that in heifers 800 µg oestrogen and 200 mg progesterone or 400 µg oestrogen and 100 mg progesterone (1: 250) was the optimum combination for udder development. The hormones were injected three times in a week for 20 weeks. Smith and Schanbacher (1974) induced lactation in heifers by injecting 0.1 mg 17 beta oestradiol and 0.25 mg progesterone per kilogram body weight for seven days (1: 2.5). Similarly the ratios 1: 2 and 1: 1 also were effective in inducing lactation by this method.

After extensive studies Benson et al. (1957) concluded that the ratio of hormones was of no significance as altering the dose level but maintaining the ratio gave different growth responses

and the absolute quantities of the two hormones were the factor of real importance. But Smith and Schanbacher (1974) observed that the total amount of hormones injected had little effect on the ability of the treatment to initiate lactation and neither amount nor ratio of 17-beta-oestradiol and progesterone was critical in induction of lactation.

Composition of milk in Induced lactation

The studies on the composition of milk from hormone induced lactation had been made by several scientists. The general observation was that after the hormone treatment, a secretion similar in composition to colostrum filled the udder. Folley and Malpress (1944) reported slightly higher protein and a low lactose content. The fat content was variable. Depending on the rate of increase in yield the colostrum changed to normal milk. Perrin (1955) induced lactation in pairs of identical twins and studied the milk composition. They found that the fat and protein were slightly higher eventhough the values were within the range of normal chemical composition. In buffaloes with induced lactation the colostrum changed to normal milk from the fourth day of milking (Shalash et al. 1964). The observation made by Fulkerson and McDowell (1974) was that the composition of milk in ewes with induced lactation was similar to normal ovine milk.

Narendran et al. (1974) induced lactation in Holstein cows and studied the composition of the milk. They reported that with

regard to fat, protein and lactose the composition of milk from induced lactation was not different from that of the milk from normal lactation during the first 21 days following parturition. Erb et al. (1976a) conducted detailed study of the composition induced lactation milk. Their observation was that lactose and fat increased more slowly during the first seven days than would be expected for cows after parturition. They have attributed this to continued development of alveoli. Total protein was 1 per cent higher on the seventh day than those reported for Holstein cows seven days after calving but was within the range on the 42nd day.

Eventhough Pinheiro et al. (1974) observed that there was no colostrum in induced lactation most of the workers have reported the presence of colostrum.

Hormones in milk of induced lactation.

Much work has been done to determine the level of injected hormones that may be exereted through milk as residue. The hormones 17 beta-oestradiol and progesterone, that are commonly used for induction of lactation, are normally present in the milk of cows. It has been found that the quantities are more in colostrum, in the milk of pregnant cows and cows in oestrus. The study of Willet et al. (1976) making use of radio active labelled hormones for the induction of lactation showed that a major portion of the hormones were excreted in the dung and urine and that the excretion in milk was in traces. The concentration

of oestrogen and progesterone in the milk of cows induced to lactation were on no occasion greater than the concentration of the hormones in the milk of cows in normal lactation. In cows with induced lactation the concentration of these hormones in milk seemed to decrease significantly during the initial period of about two weeks after the commencement of milking (Erb et al. 1976a,b). The values of oestrogen and progesterone in the milk of cows induced to lactation were similar to those in the milk of non-induced cows immediately post-partum and luteal phase of oestrus cycle respectively (Hacker and Smith, 1976 and Narendran et al. 1976).

Effect of Induction of Lactation on Blood Values

As the basis of hormonal induction of lactation is to simulate the hormonal set up in the animal body during pregnancy, the changes in blood values must be similar to those that occur in pregnancy and early lactation. Several workers have estimated the changes in blood values during pregnancy and lactation. Data regarding the blood picture during induced lactation are not much.

The red blood corpuscles count in cattle varies depending on the breed and age. The normal ranges found by Greatorex (1957) in adult cattle were RBC 4.1-7 x $10^6/mm^3$; packed cell volume 21-55 per cent and haemoglobin 12 $\pm$ 1.5 g/100 ml. Schalm (1972) found the ranges to be RBC 5.0-10.0 x $10^6/mm^3$; PCV 24-46 per cent; haemoglobin 8.0-15.0 g/100 ml and plasma protein 6.5-8.0 g/100 ml.

Nirmalan and Nair (1971) observed that there was considerable decrease in the RBC count in pregnant cows. The PCV, RBC and haemoglobin were found to decrease during the first 10 days of lactation (Treacher et al. 1975). Rowlands et al. (1975) observed that there were changes in all the blood constituents except phosphorus and potassium during the period of three months on either side of parturition.

Effect of Induction of Lactation on Health and Reproduction

Folley and Malpress (1944) observed symptoms of nymphomania in cows given oestrogen alone for induction of lactation. Raised tailhead, relaxation of pelvic ligaments, tendency to mount or to be mounted by other animals were observed. The animals with oestrogen treatment were found to gain in weight after the treatment (Turner et al. 1956). But in spite of normal growth reproductive problems were found in the animals (Naito et al. 1968). Thus the animals are rendered unsuitable for future reproduction.

In contrast to the treatment with oestrogen alone, treatment with oestrogen and progesterone did not show any of the undesirable effects (Turner et al. 1956). Meites (1961) reported that implantation of oestrogen and progesterone did not cause any untoward symptom except that the animals came to oestrus. Pinheiro et al. (1974) gave the hormone combination for 120 days to infertile cows and induced lactation. After the starting of

lactation, the cows came to heat and four out of the six cows conceived. They concluded that this was due to the hormones used.

Smith and Schanbacher (1973) observed that oestrus activity in the treated animals appeared to be transient. Oestrus cycle of normal length started 30-50 days after the lactation began and they conceived.

Erb et al. (1973) observed that during the seven days treatment period and for 2-4 weeks the vulva was enlarged and there was vaginal discharge and the uterus was turgid. The ovaries were found to be regressed but started follicle development 19 ± 4 days after the last injection. The cows came to heat 45 ± 5 days after the last injection. Ovarian cystic follicles were observed in some animals.

Collier et al. (1975b) observed that the cows artificially induced to lactation came to heat 43 days after the last injection. Out of a group of 11 cows, which were culled due to reproductive failure, used for the experiment, nine cows conceived. They suggested that this procedure could be used in problem breeders to bring them back to production.

MATERIALS AND METHODS

MATERIALS AND METHODS

Experimental Animals

The animals used for the experiment consisted of six cows and six heifers of the University Livestock Farm, Mannuthy. All the cows were dry and non-pregnant and their age varied from 5-11 years and their body weight ranged from 198-325 kg. The age of the heifers ranged from 2-4 years. All the heifers were non-pregnant and their body weight ranged from 197-274 kg. The details regarding age, body weight before and after the experiment of all the animals and the number of calvings in the case of cows are presented in Table 1. All the heifers used for the study had history of reproductive problems.

The animals were maintained under same management conditions in the farm. The animals were given pelleted feed and sufficient amount of silage to meet the maintenance requirements. During the treatment period the animals were given an additional one kg of the pelleted feed to meet the allowances for the udder development.

The animals were observed both in the morning and in the evening everyday for the symptoms of heat till each animal had two oestrus cycles prior to the beginning of the experiment.

The six cows were divided into two blocks, the first block consisting of three animals with the highest body weight and the other the remaining three. Similar division of the heifers into

two blocks was made. Taking one animal at random from each block, the three experimental groups were formed. The three treatments were: (1) natural oestrogen and progesterone twice daily for seven days (2) synthetic oestrogen and progesterone twice daily for seven days and (3) synthetic oestrogen and progesterone on alternate days during 14 days.

The Hormones

The natural oestrogen used was oestradiol dipropionate (Ovocyclin-CIBA) in sterile oily solution containing 5 mg of the active principle per ml. The synthetic oestrogen used was stilboestrol dipropionate (Vetoestrol-M&B) containing 10 mg of the hormone per ml. Progesterone supplied by the Organon (India) Limited, Calcutta in crystalline form was dissolved in olive oil to get a concentration of 25 mg per ml. To find out the effect of olive oil on the blood values four animals were injected with olive oil alone and the blood values were estimated before and after the injections.

The treatments were allotted at random to the three groups of animals. Photographs were taken on the day prior to the commencement of the experiment to record the pre-treatment condition of the mammary gland.

The animals of the first group consisting of two cows and two heifers were injected with natural oestrogen 'Ovocyclin' and

progesterone at the rate of 0.05 mg and 0.125 mg per kg body weight respectively at 12 hour intervals for seven consecutive days.

The animals in the second group were injected with synthetic oestrogen 'Vetoestrol' and progesterone at the rate of 0.05 mg and 0.125 mg respectively per kg body weight at 12 hour intervals for seven consecutive days.

The animals in the third group were injected with synthetic oestrogen 'Vetoestrol' and progesterone at the rate of 0.10 mg and 0.25 mg respectively per kg body weight on alternate days during a period of 14 days.

All the injections were given intramuscularly in the gluteal muscle alternatively on the left and right sides. The animals in groups I and II received the injections at 9.30 A.M. and 9.30 P.M. and those of the third group at 9.30 A.M. only.

Photographs to show the udder development in the treated animals were taken before the commencement of milking. Milking was started when the udder became engorged with secretion or on the 21st day after the first injection whichever was earlier. The animals were milked twice daily.

Collection of Milk Samples

The animals were completely milked in the morning and in the evening and the yield was recorded. Samples of milk were

collected from individual animals from the morning milking after thoroughly mixing the entire quantity of milk produced. The sampling procedure described in the Indian Standards IS 1479 Part I (1960) was strictly followed. The samples were collected everyday till they became negative for clot-on-boiling test. The samples collected on the first, eighth and 21st day of milking were analysed for fat, protein, lactose, chloride, total solids and ash. Similarly milk samples were collected for analyses from four cows on the first, eighth and 21st day of their parturition and they served as the control. The composition of the milk obtained from the experimental animals was compared with that of the control animals.

The lactometer reading of the samples of milk immediately after bringing the samples to the laboratory was taken at 29°C using a Zeal lactometer. The clot-on-boiling test was done as per the procedure described in Indian Standards specification, IS 1479 Part I (1960).

Analysis of Milk

The fat content in the milk was estimated using the Gerber method as described in Indian Standards, IS 1224 (1958). The protein, total solids and ash content in the milk were estimated as per the procedure described in Indian Standards, IS 1479 Part II (1960). Bock's method as described by Oser (1964) was employed for the estimation of lactose. The chloride content was estimated by direct titration method as described by Ling(1956).

Collection of Blood

Before the commencement of the hormone injection, blood samples were collected thrice at intervals of three days and the blood values such as erythrocyte count, haemoglobin concentration, packed cell volume and plasma protein were estimated. These values were used as the pre-treatment blood values. During the period of treatment blood samples were collected from the animals of the first and the second groups on the second and fifth day after the first injection. Samples of blood from the third group of animals were collected on the second and the eighth day after the first injection when they had received the same dose of hormone as of the animals in groups I and II. After the treatment, samples of blood were collected from all the animals thrice at intervals of three days. Blood samples were collected in the morning half an hour before giving the hormone injection.

Ten ml of blood was collected from each animal in test tubes using Disodium salt of Ethylene diamine tetra acetic acid as anticoagulant (20 mg/10 ml). The blood values were estimated on the same day.

Estimation of Blood Values

The erythrocyte count was made as per the procedure prescribed by Coffin (1953). The haemoglobin content was estimated

by Wongs method as described by Oser (1964). The Wintrobe method was described by Kolmer et al. (1969) was used for the estimation of packed cell volume. Plasma protein was estimated by the Microkjeldahl method as described in A.O.A.C. (1960).

Statistical Analyses

The statistical analyses of the data on blood and milk were done according to Standard methods (Snedecor and Cochran, 1967).

RESULTS

R E S U L T S

Udder Development

The development of the udder in response to treatment with the hormones was judged on the basis of the increase in size as evinced by external appearance. During the treatment period no appreciable increase in size of the mammary gland could be seen in many of the animals which received natural or synthetic oestrogen combined with progesterone for seven days. But during the last phase of the treatment an appreciable increase in udder size was seen in animals which received synthetic oestrogen combined with progesterone on alternate days during a period of 14 days. However, after the termination of the treatment and before the milking was initiated, a marked increase in the size of the udder was noticed in the case of all the animals in the three groups. Since one of the animals in group III died on the 17th day after the commencement of the experiment due to trypanosomiasis the complete data of that animal could not be collected. Photographs showing an increase in the size of the udder due to treatment are presented in Plates I to XI. It was seen that the development was more conspicuous in heifers than in cows.

The animals of group I which comprised of cows (Nos. 953 & 584) and heifers (Nos. 473 & 486) showed comparatively better udder development due to the treatment than the animals of the

other two groups. In cow No. 953 the mammary gland was flabby and pendulous and the change in size was not appreciable(Plate I). But in cow No. 584 there was appreciable udder development by the 21st day (Plate II). In heifer No. 473 the udder development started earlier than in the other animals of the group (Plate III). By the 16th day after the first injection the udder was well developed and on the 19th day the udder became turgid. A colostrum-like fluid oozed out from the teats when the animal was lying down. So milking was commenced on the 20th day itself. In the case of heifer No. 486 eventhough there was not much udder development during the treatment period, there was marked udder development and engorgement of the teats by the 21st day (Plate IV).

The group II consisted of two cows (Nos. 408 & 203) and two heifers (Nos. 614 & 480). In this group the development of the udder was comparatively poor. In cow No. 408 the udder was very pendulous and appreciable change was not present during the treatment period. But there was appreciable udder development around the 21st day after the first injection (Plate V). In cow No. 203 there was only little udder development even on the 21st day when milking was started in all the animals (Plate VI). Contrary to the other animals of this group heifer No. 614 started showing udder development even during the last few days of the injection period. By the 12th day the mammary gland was fully engorged and there was oozing of colostrum-like fluid from the teats when the animal was lying down (Plate VII). Therefore this animal was

milked from the 13th day onwards. In the case of heifer No. 480 the udder development during the treatment period was negligible but around the 21st day there was marked development (Plate VIII).

The group III consisted of cows (Nos. 903 & 308) and heifers (Nos. 586 & 642). The animals of this group received oestrogen and progesterone on alternate days during the 14 days period. As cow No. 308 died on the 17th day after the commencement of the experiment, complete data regarding the animal could not be collected. In the case of cow No. 903 the change in udder size was not appreciable even on the 21st day (Plate IX). In heifers (Nos. 586 & 642) there was appreciable enlargement of the udder during the last phase of the treatment. On the 21st day after the first injection heifer No. 586 (Plate X) and heifer No. 642 (Plate XI) showed fully developed mammary glands.

Lactation

Excepting the two heifers, which came into lactation early, all the other animals were milked twice daily from the 21st day onwards. During the first 60 days of milking the daily yield of individual animal varied from 140 ml (Cow No. 203) to 4300 ml (Heifer No. 473). The weekwise average daily milk yield of the animals for the first eight weeks period is presented in Table 2.

During the 60 days period the peak yield of the individual

animals of group I varied from 1010 ml for cow No. 584, to 4300 ml for heifer No. 473. The peak yield of cow No. 953 was 2600 ml and for the heifer No. 486 it was 1650 ml. In one cow of this group the milk yield was found to be on the increase even after the eighth week. In the heifer Nos. 473 and 486 the peak yield was attained during the second week and fifth week respectively.

In the animals of the second group the peak milk yield during the first 60 days varied from 140 ml for cow No. 203, to 3430 ml for heifer No. 614. Cow No. 408 gave a peak yield of 600 ml and for heifer No. 480 the peak yield was 700 ml during this period. The peak yield was attained during the fourth week in both the heifers as well as in cow No. 203, whereas in cow No. 408 it was during the seventh week.

The peak yield of individual animals of group III varied from 520 ml for cow No. 903, to 3350 ml for heifer No. 586 during the 60 days period. The highest yield for heifer No. 642 was 2570 ml. Cow No. 903 reached the peak yield during the seventh week. The milk yield of heifer Nos. 586 and 642 were on the increase even after the eighth week.

Statistical analysis of the data on milk yield during the 60 days period showed that there were highly significant differences between the groups I and II as well as between II and III. There was no significant difference between groups I and III. The

milk yield from the group II was significantly lower than from the other two groups (Table 3). Similarly as a group the heifers yielded significantly higher quantity of milk than the cows. The groupwise average daily yield per animal during the eight weeks period was found to be 1766 ml, 942 ml and 1638 ml for groups I, II and III respectively. A weekwise average daily milk yield of the experimental animals of the three groups are presented in Fig. 1.

Properties of Milk

The physical properties of milk obtained by induced lactation were studied daily upto the 21st day. The properties were studied with reference to colour, lactometer reading and clot-on-boiling.

Colour.

On the first day of the lactation the milk from all the heifers except heifer No. 486 of group I was yellowish in colour resembling the colostrum of normal cows. The colour gradually changed to white by the fourth day of lactation. In cow Nos. 584, 408 and 203 the milk was lighter in colour and was white by the second day of lactation. The milk of heifer No. 486 and cow Nos. 953 and 903 was blood-tinged. The colour persisted upto the fourth day of lactation in heifer No. 486 and seventh day in cow Nos. 953 and 903 and thereafter the colour became normal.

Lactometer reading.

The lactometer reading of the samples of milk collected from the individual animals of the experimental groups varied from 26-42 on the first day of lactation. These values were found to be less than the values obtained for the milk samples collected on the first day of lactation from the control animals. The lactometer readings for the samples of the control animals ranged from 32-50. It was observed that by about the eighth day the lactometer readings of the milk of experimental animals were within the range obtained for normal cow's milk.

Clot-on-boiling.

As compared to the samples of milk from the control animals the samples collected from experimental animals gave a positive reaction to clot-on-boiling test for a longer period of time. The number of days taken for the milk to become negative for the test was less in heifers than in the cows. In heifers the period ranged from 2-5 days for milk to become negative for clot-on-boiling whereas in the cows the period ranged from 4-19 days.

Composition of Milk

Samples of milk from individual animals of the experiment collected on the first, eighth and twenty-first day of lactation were analysed for fat, protein, lactose, chloride, total solids and ash. The values obtained were compared with those samples

of milk collected on the same days from normal lactating cows. The values obtained are presented in Tables 4, 5 and 6.

In the samples of milk collected on the first day the fat percentage showed much variation among the animals. The average fat percentage in the three treatment and the control groups was 5.85, 3.37, 4.73 and 6.65 respectively (Table 4). On statistical analysis it was found that there was no significant difference among the four groups with regard to the fat percentage in the first day milk ($F = 1.047$).

On the eighth day the variation among the animals in the fat percentage of milk was much reduced and was within the normal range. The average values were 4.25, 4.35, 4.42 and 5.05 for groups I, II, III and control respectively (Table 5). Statistical analysis showed that there was no significant difference among the four groups ($F = 0.751$).

The fat percentages of the milk obtained on the 21st day of lactation from the groups I, II, III and control averaged 5.25, 4.8, 4.9 and 4.95 respectively (Table 6). Eventhough the fat percentage in some animals in the experimental groups were slightly higher than that in the control group there was no statistically significant difference ($F = 0.085$). The percentage of fat in the samples of milk of the different groups is presented in Fig. 2.

Protein.

The average percentage of protein in the samples of milk collected on the first day was 7.55, 8.84, 9.96 and 12.07 for groups I, II, III and control respectively (Table 4). On statistical analysis it was found that the protein percentage was significantly higher in the control group than in the groups I and II. In group III eventhough the average protein percentage was lower than that of the control the difference was not found to be statistically significant. Group III gave the highest protein percentage which was significantly different from group I but not from group II (Table 7).

On the eighth day of lactation the protein content in all the groups decreased. The average value for the percentage of protein was 4.75, 4.89, 5.12 and 4.55 for groups I, II, III and control respectively (Table 5). The differences among the four groups were not statistically significant ($F = 0.672$).

Similarly on the 21st day of lactation the protein percentage in milk averaged 4.2, 4.4, 4.28 and 3.85 in groups I, II, III and control respectively (Table 6). Eventhough the protein percentage in the experimental groups were slightly higher than that in the control group there was no statistically significant difference among the four groups ($F = 1.167$). The average percentage of protein in the different groups is presented in Fig.3.

Lactose.

The average percentage of lactose in the first day milk was 2.4, 1.72, 1.92 and 2.24 for groups I, II, III and control respectively (Table 4). There was no statistically significant difference among the groups in the percentage of lactose ($F = 0.334$).

On the eighth day the percentage of lactose in milk was higher in all the groups. The percentage of lactose was 4.52, 3.92, 4.50 and 4.56 for groups I, II, III and control respectively (Table 5). These values for the four groups were not statistically different from each other ($F = 0.781$).

The lactose content in the milk obtained on the 21st day of lactation averaged 4.81, 4.56, 4.49 and 4.8 for groups I, II, III and control respectively (Table 6). At this stage also the values were not significantly different for the different groups ($F = 1.311$). The average percentage of lactose in different groups is presented in Fig. 4.

Chloride.

The chloride content in the samples of milk obtained on the first day of lactation showed that in the induced lactation milk the values were slightly higher than those in the milk from the animals of the control group. The average percentage of chloride for groups I, II, III and control was 0.23, 0.24, 0.25 and 0.20 respectively (Table 4). On statistical analysis it was

found that the difference in the values for the different groups was not significant (F = 0.062).

The samples of milk collected on the eighth day from all the groups contained lesser amounts of chloride. The percentage of chloride was 0.15, 0.18, 0.17 and 0.15 for groups I, II, III and control respectively (Table 5). There was no statistically significant difference among the groups (F = 0.381).

The chloride content in the milk collected on the 21st day averaged 0.14, 0.15, 0.15 and 0.13 per cent for groups I, II, III and control respectively (Table 6). There was no significant difference among the groups in their chloride content (F = 0.357).

Total solids.

The total solids content in the samples of milk of the first day averaged 18.37, 17.41, 18.17 and 21.91 per cent for groups I, II, III and control respectively (Table 4). The percentage of total solids in the control group was significantly higher than that in the experimental groups (Table 8).

On the eighth day the percentage of total solids in the samples of milk was more uniform. The average value was 15.41, 14.24, 13.73 and 15.09 per cent for groups I, II, III and control respectively (Table 5). On statistical analysis of these values it was found that the differences among the values were not significant (F = 1.297).

Similarly in the samples obtained on the 21st day of lactation the total solids averaged 14.74, 14.14, 13.83 and 14.14 per cent for groups I, II, III and control respectively (Table 6). There was no significant difference among the total solids content in the samples of milk of the different groups on the 21st day of lactation. The average percentage of total solids in different groups is presented in Fig. 5.

Ash.

The ash content in the samples of first day averaged 0.49, 0.52, 0.60 and 0.77 per cent for the groups I, II, III and control respectively (Table 4). There was no statistically significant difference in the ash content among the groups (F = 2.265).

In the samples of milk obtained on the eighth day the ash content averaged 0.55, 0.51, 0.69 and 0.66 per cent for the I, II, III and control groups respectively (Table 5). The ash content in the samples of milk of the different groups was not statistically significant (F = 1.236).

On the 21st day of lactation the average value for the ash content was 0.54, 0.45, 0.62 and 0.63 per cent for groups I, II, III and control respectively(Table 6). The difference among the groups was not statistically significant (F = 1.238).

Effect of Hormones on the Blood Values

Erythrocyte count.

The enumeration of the erythrocytes was done before, during and after the injections (Table 9). During the pre-treatment period the average value for groups I, II and III was 6.44, 6.74 and 6.78 x $10^6/mm^3$ respectively. During the treatment period the count was 5.45, 6.05 and 6.09 x $10^6/mm^3$, for groups I, II and III respectively. During the first seven days of the post-treatment period the average erythrocyte count was 5.06, 5.69 and 5.97 x $10^6/mm^3$ for groups I, II and III respectively. Statistical analysis showed that there was a significant reduction RBC count during the treatment as well as the post-treatment periods (Table 10). In group I the decrease in RBC count was significantly greater as compared to the other two groups. Between the groups II and III there was no significant difference in the count. The difference in the RBC count obtained during the treatment and post-treatment periods was not statistically significant.

Analysis of variance also showed significant difference in RBC count between the cows and the heifers during the entire period. So it was necessary to establish whether this was due to the difference in response of these two catagories of animals to the hormone treatment. For this purpose the normal deviate 'u' was calculated using the formula,

$$u = \frac{\left\{\text{Mean for heifers (Treatment)} - \text{Mean for heifers (Pretreatment)}\right\} - \left\{\text{Mean for cows (Treatment)} - \text{Mean for cows (Pretreatment)}\right\}}{\sqrt{\left(\frac{1}{r1} + \frac{1}{r2} + \frac{1}{r3} + \frac{1}{r4}\right) \text{MESS}}}$$

On analysis it was found that the difference was due to the normal difference in the REC count of cows and heifers (u = 0.551).

Haemoglobin.

The determined values of haemoglobin of the three groups of animals for the different periods are tabulated in Table 11. The average value during the pre-treatment period was 11.81, 11.93 and 12.01 g/100 ml and during the treatment period 10.45, 10.69 and 10.99 g/100 ml for groups I, II and III respectively. During the post-treatment period the value was 10.51, 11.05 and 10.98 g/100 ml for groups I, II and III respectively. Statistical analysis showed that in all the three groups there was a significant decrease in haemoglobin concentration during the treatment and post-treatment periods as compared to the pre-treatment period (Table 12). It was also seen that there was no significant difference in haemoglobin concentration between treatment and post-treatment periods. There was no significant difference among the groups in their response to the hormones with respect to haemoglobin concentration. Significant difference in the amount of haemoglobin was noticed between the cows and heifers. This difference was due to the normal difference in the haemoglobin concentration between cows and heifers and not due to the treatment (u = 1.824).

Plasma protein.

The concentration of plasma proteins of the animals during the pre-treatment, treatment and post-treatment periods is presented in Table 13. During the pre-treatment period the average plasma protein concentration was 7.57, 7.62 and 8.05 g/100 ml and during the treatment period the values averaged 8.23, 8.09 and 8.87 g/100 ml for groups I, II and III respectively. During the post-treatment period the value was 8.87, 7.89 and 8.45 g/100ml for groups I, II and III respectively. Statistical analysis showed that there was a significant increase in plasma protein during the treatment as well as the post-treatment periods in comparison to the pre-treatment period. But there was no significant difference between the values obtained during the treatment and post-treatment periods (Table 14). Among the groups there was no significant difference in their response to the hormones in respect of plasma proteins. There was a significant difference in plasma protein concentration between the cows and the heifers and this difference was found to be due to the normal difference that existed before the experiment (u = 0.765).

Packed cell volume.

The data on the packed cell volume of blood of different animals during the three periods is presented in Table 15. The average value of 38.87, 40.30 and 40.80 per cent obtained during the pre-treatment period changed to 34.5, 37.6 and 38.3 per cent

during the treatment period for groups I, II and III respectively. For groups I, II and III the value was 34.8, 38.0 and 39.2 per cent respectively during the post-treatment period. Statistical analysis showed that there was a significant decrease in the packed cell volume in all the three groups during the treatment as well as the post-treatment periods (Table 16). Similarly there was a significant increase in packed cell volume during the post-treatment period as compared to the treatment period. There was a significant difference in PCV among the three treatment groups. As compared to the values obtained during the pre-treatment period the reduction of PCV in group I during the treatment period was 4.37 per cent whereas in groups II and III the reduction was 2.7 and 2.5 per cent respectively. During the post-treatment period the reduction in comparison to the pre-treatment value was 4.07, 2.3 and 1.6 per cent respectively for groups I, II and III. Statistical analysis of the data revealed that the difference in PCV obtained during the treatment and post-treatment period between cows and heifers was due to the difference that existed during the pre-treatment period ($u = 0.145$). It was observed that the injection of olive oil alone had no effect on any of these blood values.

Effect of Induction of Lactation on Health and Reproduction of Animals

The behaviour of the injected animals was observed daily. Swelling of vulva and mucous discharge from the vagina, relaxation

of the pelvic ligaments and general excitement were noticed during the treatment period. There was noticeable elevation of the tailhead in four animals. Oestrus behaviour was detected on the second day of injection in almost all animals. In two animals oestrus behaviour of transient nature was observed throughout the treatment period. Regular oestrus cycle was noticed in two cows and four heifers from 42-57 days after the commencement of the milking. One cow and three heifers were inseminated when they came in regular heat for a second time and all of them became pregnant.

The body weights of the animals before starting the experiment and after the experiment are presented in Table 1. Slight decrease in body weight was noticed in heifers immediately after the treatment period.

TABLES

Table 1. Details of animals used for the induction of lactation.

Group	Tattoo no. of animals	Age	Body wt. at the commencement of experiment (kg)	Body wt. at close of experiment (kg)	No. of previous lactations	No. of A.I. after last calving	Interval between last heat and commencement of experiment (days)
I	953	9 Y 4 M	265	264	4	6	4
	584	4 Y 3 M	213	211	1	2	7
	473*	2 Y 5 M	274	265	-	4	11
	486*	2 Y 2 M	197	187	-	-	9
II	408	8 Y 9 M	292	293	4	1	15
	203	5 Y 1 M	240	239	1	1	13
	614*	3 Y 3 M	228	223	-	5	8
	480*	2 Y 4 M	194	187	-	-	6
III	903	10 Y 5 M	324	324	3	5	13
	308	11 Y 1 M	198	193	6	6	14
	586*	4 Y 1 M	250	235	-	9	13
	642*	2 Y 2 M	227	220	-	3	3

* Denotes heifers.

Table 2. Weekwise average daily milk yield for the first eight weeks (in ml).

Week	Group I Tattoo no. of animals				Group II Tattoo no. of animals				Group III Tattoo no. of animals		
	953	584	473	486	408	203	614	480	903	586	642
1	554	291	3893	539	216	73	1586	333	188	1335	734
2	1130	486	4079	974	193	60	3119	495	315	1997	1588
3	1268	583	3909	1097	281	77	3057	420	340	2300	1747
4	1488	659	3716	1446	349	98	3277	513	310	2764	2044
5	1811	741	3680	1619	446	97	3043	415	350	3015	2300
6	1971	829	3621	1129	514	97	3057	445	385	3085	2395
7	2071	904	3457	950	533	81	2970	440	410	3185	2570
8	2264	929	3307	1129	471	79	2830	500	383	3207	2571
Av.	1570	678	3708	1110	375	83	2867	445	335	2611	1968

Table 3. Analysis of milk yield data of different groups of animals.

Analysis of variance table

Source	df	SS	MSS	F
Between groups	2	90.5358	45.2679	61.280**
Between cows and heifers	1	381.4696	381.4696	516.406**
Error	656	484.5559	0.7387	
Total	659	956.5613		

Pairwise comparison

Between groups	I and II	=	10.345**
"	I and III	=	1.164
"	II and III	=	8.352**

** Significant P / 0.01.

Table 4. Milk yield and composition on the first day of lactation.

Group	Animal number	Milk yield (ml)	Lacto-meter reading	Fat (%)	Protein (%)	Lactose (%)	Chloride (%)	Total solids (%)	Ash (%)
I	953	160	41	2.00	10.49	2.71	0.23	17.48	0.41
	584	240	27	7.30	8.12	1.87	0.24	20.76	0.47
	473	3620	26	9.00	5.15	3.53	0.23	17.15	0.46
	486	730	27	5.10	6.14	1.49	0.23	18.08	0.65
	Average	1187	30.25	5.85	7.55	2.40	0.23	18.37	0.49
II	408	590	34	2.30	9.31	1.44	0.24	18.89	0.40
	203	140	42	2.00	10.62	0.96	0.24	16.26	0.35
	614	1200	35	7.00	6.53	3.25	0.21	19.14	0.73
	480	700	39	2.20	8.91	1.24	0.24	15.36	0.63
	Average	657	37.50	3.37	8.84	1.72	0.24	17.41	0.52
III	903	280	33	1.90	9.90	1.60	0.23	14.79	0.53
	586	1300	40	3.30	8.70	3.53	0.23	18.18	0.61
	642	780	39	9.00	11.28	0.62	0.24	21.55	0.87
	Average	786	37.30	4.73	9.96	1.92	0.23	18.17	0.60
Control	475	4500	50	5.50	12.87	2.50	0.21	22.01	0.66
	037	3800	49	5.60	12.26	2.71	0.21	21.51	0.89
	013	800	32	9.50	11.28	1.01	0.22	22.56	0.84
	471	2000	46	6.00	11.88	2.75	0.19	21.55	0.71
	Average	2775	44.20	6.65	12.07	2.24	0.20	21.91	0.77

Table 5. Milk yield and composition on the eighth day of lactation.

Group	Animal number	Milk yield (ml)	Lacto-meter reading	Fat (%)	Protein (%)	Lactose (%)	Chloride (%)	Total solids (%)	Ash (%)
I	953	920	32	4.0	5.54	4.39	0.15	17.01	0.50
	584	430	27	4.0	4.15	4.28	0.21	14.94	0.81
	473	4200	29	4.1	4.35	4.58	0.11	15.14	0.60
	486	890	30	4.9	4.95	4.85	0.15	14.57	0.49
	Average	1610	29.50	4.25	4.75	4.52	0.15	15.41	0.60
II	408	170	31	3.3	5.54	4.22	0.23	14.10	0.49
	203	60	23	5.0	3.96	2.42	0.24	12.93	0.61
	614	3030	29	5.1	4.55	4.65	0.11	12.94	0.64
	480	440	32	4.0	5.54	4.39	0.15	17.01	0.51
	Average	925	28.75	4.35	4.89	3.92	0.18	14.24	0.55
III	903	300	28	4.6	5.54	3.82	0.23	13.32	0.82
	586	1780	31	4.2	5.15	4.85	0.14	13.83	0.58
	642	1220	30	4.9	4.95	4.85	0.15	14.05	0.68
	Average	1100	29.60	4.42	5.12	4.50	0.17	13.73	0.69
Control	475	7700	29	4.9	4.35	4.64	0.12	14.67	0.59
	037	9500	27	4.5	5.14	4.64	0.17	16.46	0.66
	013	3900	27	5.5	4.95	4.58	0.17	14.18	0.73
	471	7800	28	5.3	3.76	4.39	0.15	15.08	0.66
	Average	7225	28	5.05	4.55	4.56	0.15	15.09	0.66

Table 6. Milk yield and composition on the twenty-first day of lactation.

Group	Animal number	Milk yield (ml)	Lactometer reading	Fat (%)	Protein (%)	Lactose (%)	Chloride (%)	Total solids (%)	Ash (%)
I	953	1330	27	5.3	4.75	4.85	0.15	15.96	0.42
	584	600	26	4.5	3.96	4.51	0.18	13.57	0.52
	473	3800	27	5.2	3.76	4.94	0.12	12.86	0.47
	486	1300	26	6.0	4.35	4.94	0.12	16.59	0.77
	Average	1757	26.50	5.25	4.20	4.81	0.14	14.74	0.54
II	408	290	29	3.4	4.95	4.58	0.21	13.59	0.40
	203	110	24	4.5	3.75	4.50	0.13	12.66	0.34
	614	3150	25	7.1	4.15	5.00	0.11	16.69	0.47
	480	420	28	4.2	4.75	4.15	0.15	13.63	0.59
	Average	992	26.50	4.8	4.40	4.56	0.15	14.14	0.45
III	903	320	26	3.7	4.35	3.53	0.22	12.40	0.89
	586	2580	28	5.1	4.35	4.94	0.13	13.86	0.43
	642	1830	27	5.3	4.15	5.00	0.11	15.25	0.55
	Average	1577	27	4.7	4.28	4.49	0.15	13.83	0.62
Control	475	9200	29	4.9	3.96	4.93	0.11	13.48	0.58
	037	9200	27	5.3	3.76	4.78	0.13	13.69	0.60
	013	5600	27	4.5	4.35	4.78	0.14	14.98	0.60
	471	9900	28	5.1	3.36	4.71	0.13	14.41	0.73
	Average	8475	27.75	4.95	3.85	4.80	0.13	14.14	0.63

Table 7. Percentage of protein in the first day milk of animals in different groups.

Analysis of variance table

Source	df	SS	MSS	F
Between groups	3	45.1786	15.0595	5.504*
Error	11	30.0943	2.7358	
Total	14	75.2729		

Pairwise comparison

Between groups	I and II	=	1.1692
"	I and III	=	1.967*
"	I and control	=	3.930*
"	II and III	=	0.8845
"	II and control	=	2.761*
"	III and control	=	1.672

* Significant P $\leq$ 0.05

Table 8. Percentage of total solids in the first day milk of animals in different groups.

Analysis of variance table

Source	df	SS	MSS	F
Between groups	3	47.5402	15.8467	4.115*
Error	11	42.3560	3.8505	
Total	14	89.8962		

Pairwise comparison

Between groups	I and II	=	0.688
"	I and III	=	0.129
"	I and control	=	2.551*
"	II and III	=	0.507
"	II and control	=	3.239*
"	III and control	=	2.491*

* Significant P / 0.05

Table 9. Effect of the hormone treatment on RBC count (millions/Cmm)

Group	Animal number	Pre-treatment period			Treatment period		Post-treatment period		
I	953	6.92	6.83	6.83	5.49	5.49	5.28	5.35	4.71
	584	4.67	4.76	4.82	4.61	8.95	4.49	4.19	4.33
	473	7.51	7.62	6.92	5.93	5.75	5.27	5.13	4.75
	486	6.79	6.74	6.87	6.38	5.98	6.32	5.80	5.21
II	408	5.28	5.21	5.37	5.12	5.30	5.12	5.47	5.33
	203	4.41	4.72	4.41	3.82	3.21	3.56	3.65	3.93
	614	8.80	9.27	9.04	8.31	8.06	7.72	6.40	7.25
	480	8.13	8.36	7.96	7.78	6.79	6.40	6.76	6.83
III	903	5.59	5.39	5.54	5.06	4.65	4.94	5.56	5.50
	308	4.35	4.96	4.56	3.90	3.94	3.97	-	-
	586	9.33	9.33	9.24	7.93	8.13	7.32	7.41	8.11
	642	7.59	7.75	7.76	7.15	6.98	6.86	7.15	6.88

Table 10. Effect of hormones on RBC count.

Analysis of variance table

Source	df	SS	MSS	F
Between groups	2	8.5002	4.2501	5.884**
Between periods	2	19.1375	9.5688	13.247**
Between cow-heifer types	1	131.2133	131.2133	181.660**
Error	88	63.5634	0.7223	
Total	93	222.4144		

Pairwise comparison

Between groups	I and II	=	2.354**
"	I and III	=	3.473**
"	II and III	=	1.157
Between periods	I and II	=	3.748**
"	I and III	=	4.822**
"	II and III	=	0.618

** Significant P / 0.01

Table 11. Effect of hormone treatment on haemoglobin (g/100 ml)

Group	Animal number	Pre-treatment period			Treatment period		Post-treatment period		
I	953	11.30	11.49	10.91	11.38	11.47	11.76	10.71	9.47
	584	10.27	11.50	10.17	9.20	9.80	9.45	10.29	9.60
	473	13.30	12.81	12.44	9.00	10.59	10.71	10.21	9.47
	486	12.02	12.66	12.86	9.64	12.53	12.60	10.92	10.92
II	408	11.70	11.67	12.02	11.25	10.37	12.18	10.92	10.50
	203	8.16	8.24	8.36	8.02	7.78	7.98	8.19	8.24
	614	13.36	14.23	14.46	12.40	13.40	13.15	12.60	12.60
	480	13.53	14.00	13.56	10.79	12.10	11.76	12.18	12.59
III	903	11.80	11.30	11.80	10.34	10.34	10.50	10.92	9.16
	308	9.56	9.60	9.50	8.25	8.61	8.56	-	-
	586	14.46	14.46	14.70	12.40	13.86	13.02	13.02	13.72
	642	11.67	12.91	12.66	11.94	12.18	12.60	12.18	10.57

Table 12. Effect of hormones on haemoglobin concentration.

Analysis of variance table

Source	df	SS	MSS	F
Between groups	2	5.1017	2.5509	1.664
Between periods	2	25.5103	12.7552	8.324**
Between cow-heifer types	1	125.0488	125.0488	81.608**
Error	88	134.8393	1.5323	
Total	93	290.5001		

Pairwise comparison

Between periods	I and II	=	5.327**
"	I and III	=	3.955**
"	II and III	=	1.046

** Significant P < 0.01

Table 13. Effect of hormone treatment on plasma protein (g/100 ml)

Group	Animal number	Pre-treatment period			Treatment period		Post-treatment period		
I	953	8.53	8.31	8.75	8.75	8.96	10.28	11.81	10.50
	584	7.43	7.43	7.65	9.53	8.75	8.75	10.50	8.09
	473	7.00	7.00	8.09	8.12	8.09	7.00	7.43	7.21
	486	6.30	7.21	7.21	6.78	7.00	8.53	8.09	8.31
II	408	7.65	7.43	7.65	7.87	8.09	8.31	8.31	8.75
	203	8.09	7.87	7.87	7.87	7.65	7.00	8.31	7.65
	614	9.05	7.87	7.65	8.75	8.53	10.28	8.31	8.68
	480	6.56	6.56	7.21	8.31	7.65	7.00	7.65	7.43
III	905	7.56	7.65	7.43	8.09	8.96	8.53	8.31	7.87
	308	8.96	9.18	8.96	10.28	11.37	10.06	-	-
	586	8.09	8.31	8.53	7.87	8.31	8.53	7.65	7.78
	642	7.21	7.38	7.43	8.06	8.09	7.43	7.50	7.70

Table 14. Effect of hormone treatment on plasma protein.

Analysis of variance table

Source	df	SS	MSS	F
Between groups	2	2.4133	1.2067	0.839
Between periods	2	9.3722	4.6861	3.260*
Between cow-heifer type	1	14.7088	14.7088	10.234**
Error	88	126.4748	1.4372	
Total	93	152.9691		

Pairwise comparison

Between periods	I and II	=	5.327*
"	I and III	=	3.955*
"	II and III	=	1.046

* Significant P $<$ 0.05

** Significant P $<$ 0.01

Table 15. Effect of hormone treatment on PCV (%)

Group	Animal number	Pre-treatment period			Treatment period		Post-treatment period		
I	953	42	40	40	36	36	36	38	35
	584	28	33	29	28	28	32	32	28
	473	43	46	44	36	34	36	35	30
	486	39	39	44	37	41	42	39	35
II	408	37	42	43	36	36	37	39	37
	203	23	23	23	22	22	23	23	23
	614	53	54	53	53	47	49	46	46
	480	46	46	51	43	42	42	48	43
III	903	38	36	34	33	34	33	36	36
	308	27	26	26	25	25	26	-	-
	586	56	56	54	49	52	52	49	53
	642	47	46	46	45	43	40	43	48

Table 16. Effect of hormone treatment on PCV.

Analysis of variance table

Source	df	SS	MSS	F
Between groups	2	284.324	142.162	3.272*
Between periods	2	528.165	264.082	6.079**
Between cow-heifer types	1	4076.064	4076.064	93.839**
Error	88	3822.423	43.436	
Total	93	8710.978		

Pairwise comparison

Between groups	I and II	=	13.241*
"	I and III	=	19.385*
"	II and III	=	6.359*
Between periods	I and II	=	15.926**
"	I and III	=	11.909**
"	II and III	=	5.074*

* Significant P / 0.05

** Significant P / 0.01

ILLUSTRATIONS

Fig. 1. WEEKWISE AVERAGE DAILY MILK YIELD OF THE THREE GROUPS OF ANIMALS.

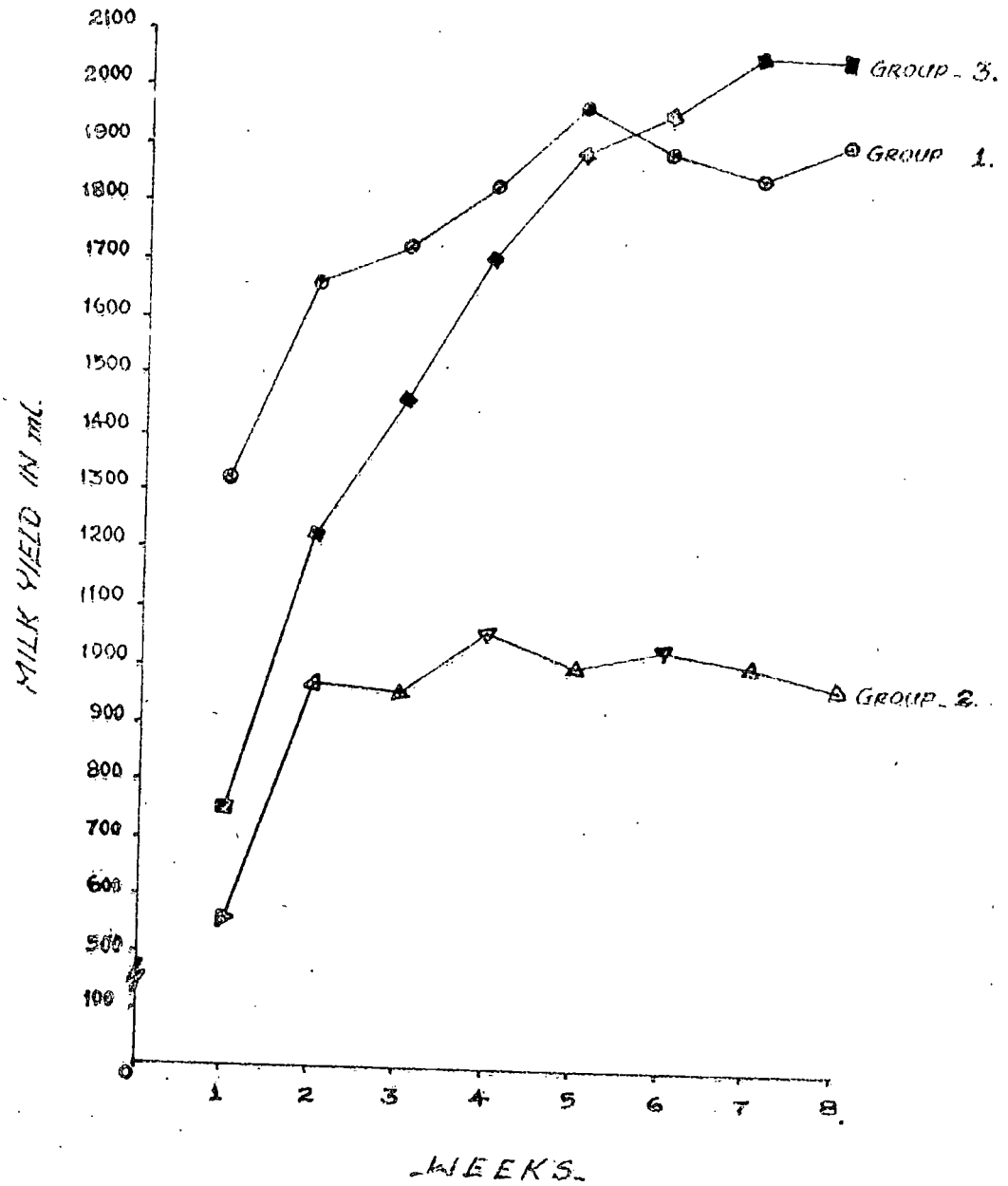


Fig. 2. AVERAGE PERCENTAGE OF FAT IN THE MILK OF EXPERIMENTAL AND CONTROL ANIMALS.

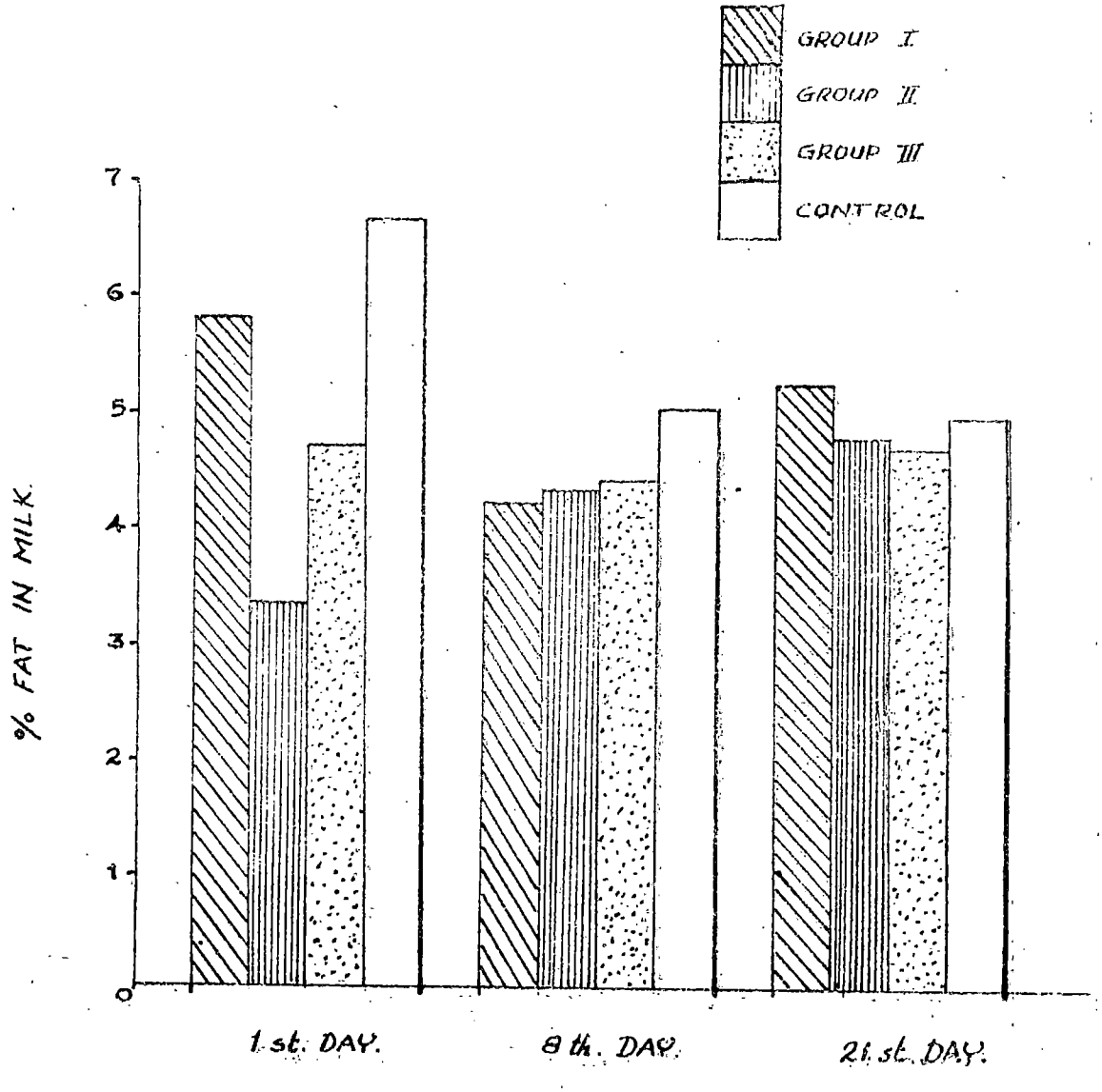


Fig. 3. AVERAGE PERCENTAGE OF PROTEIN IN THE MILK OF EXPERIMENTAL AND CONTROL ANIMALS.

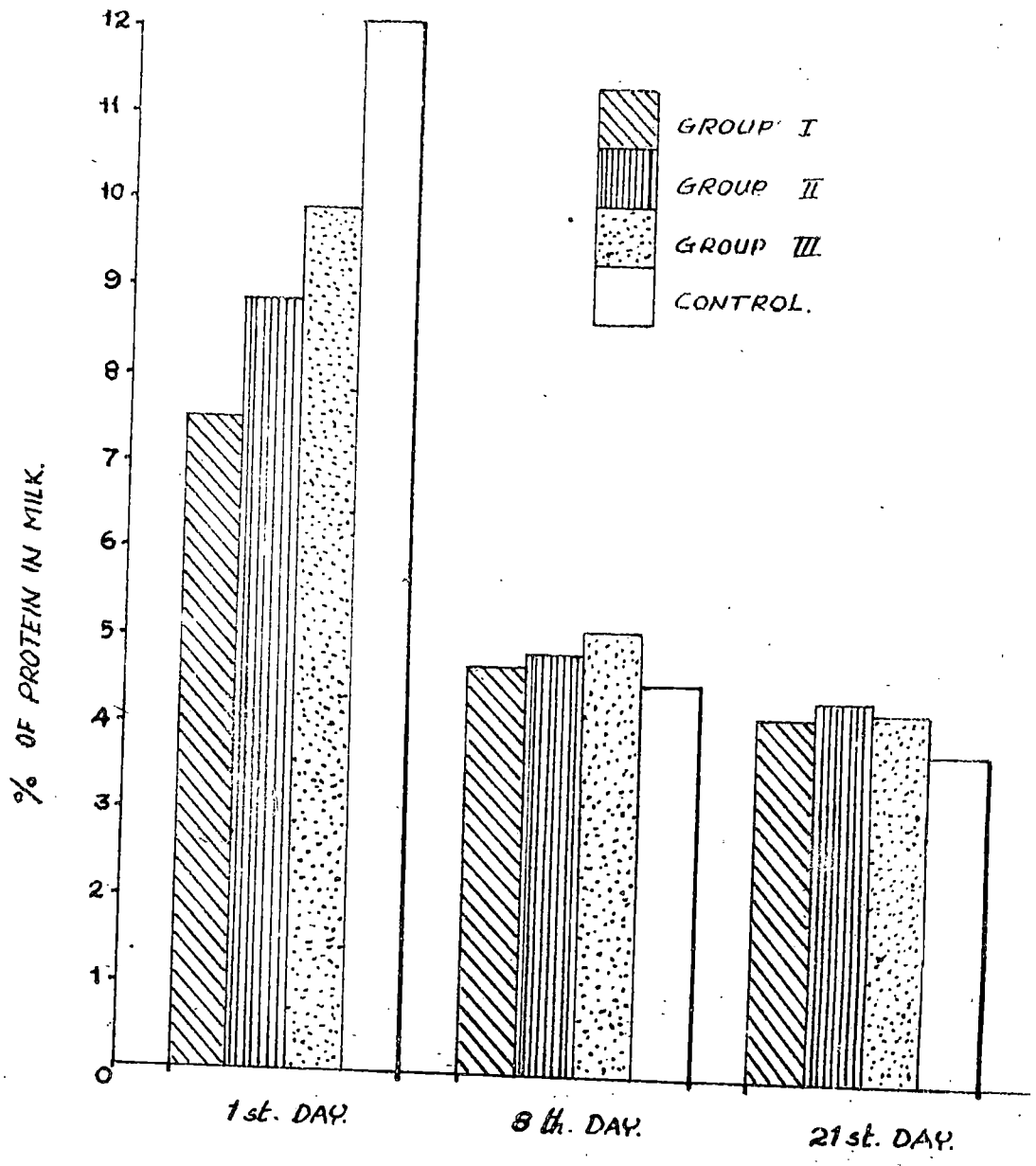


Fig-4. AVERAGE PERCENTAGE OF LACTOSE IN THE MILK OF EXPERIMENTAL AND CONTROL ANIMALS.

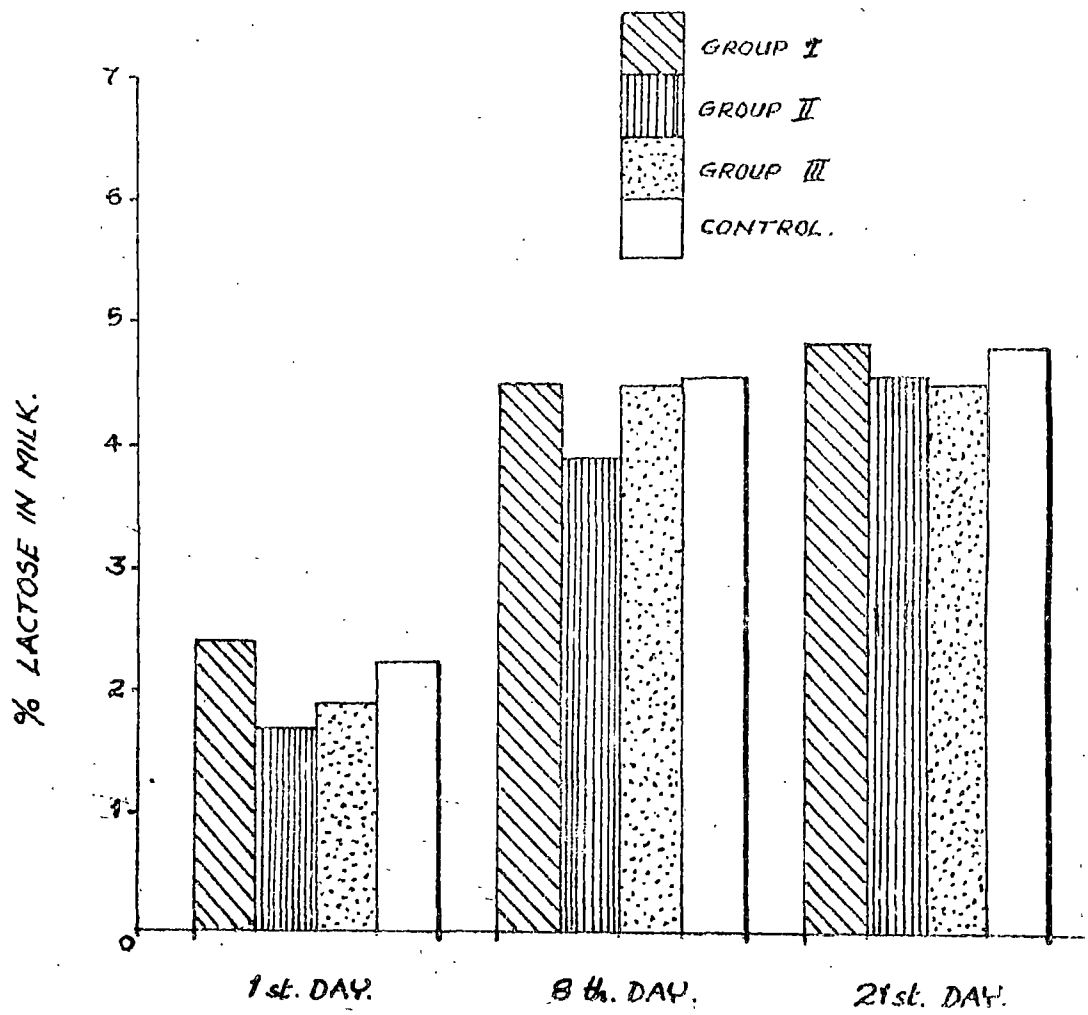


Fig. 5. AVERAGE PERCENTAGE OF TOTAL SOLIDS IN THE MILK OF EXPERIMENTAL AND CONTROL ANIMALS.

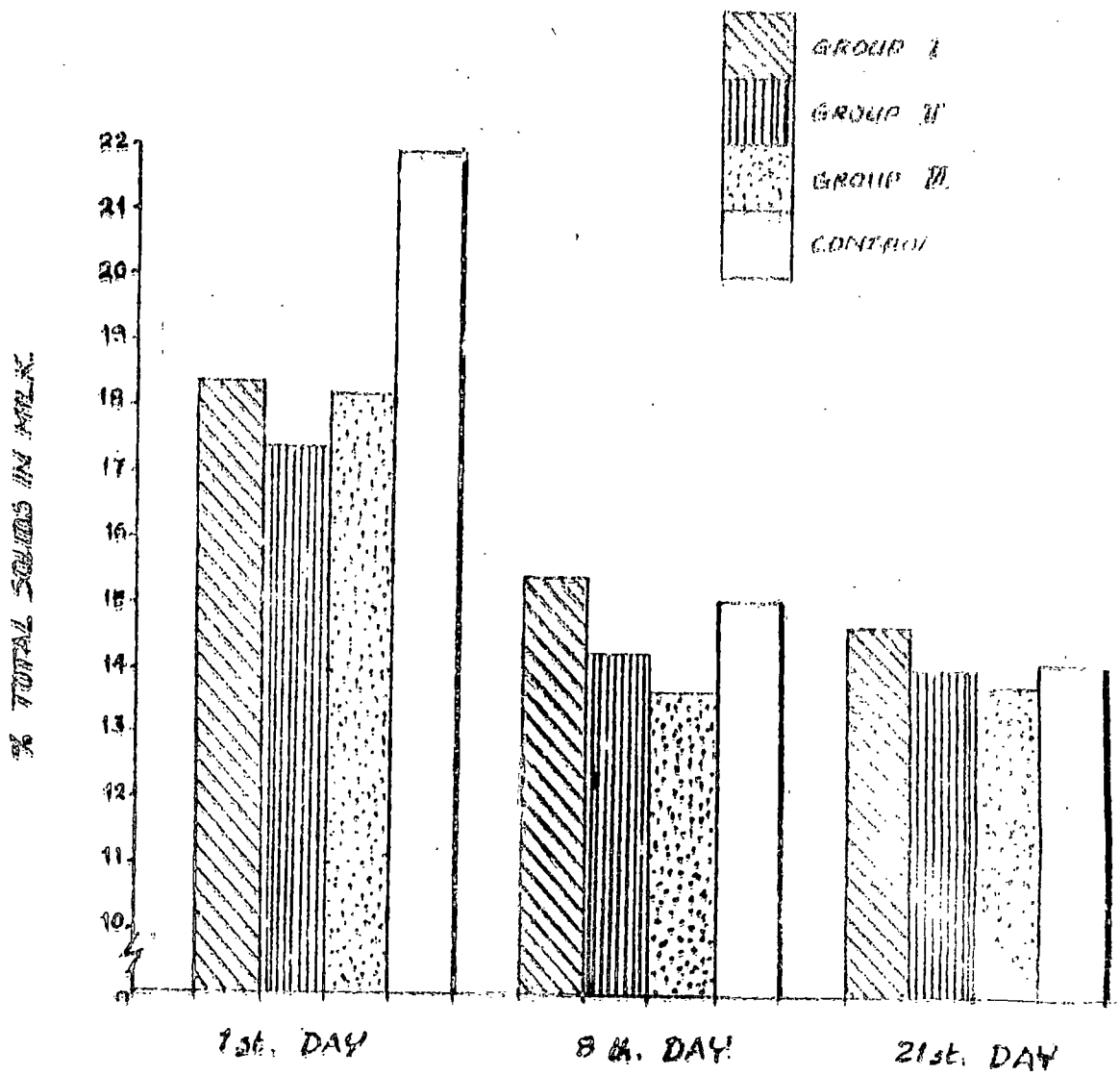
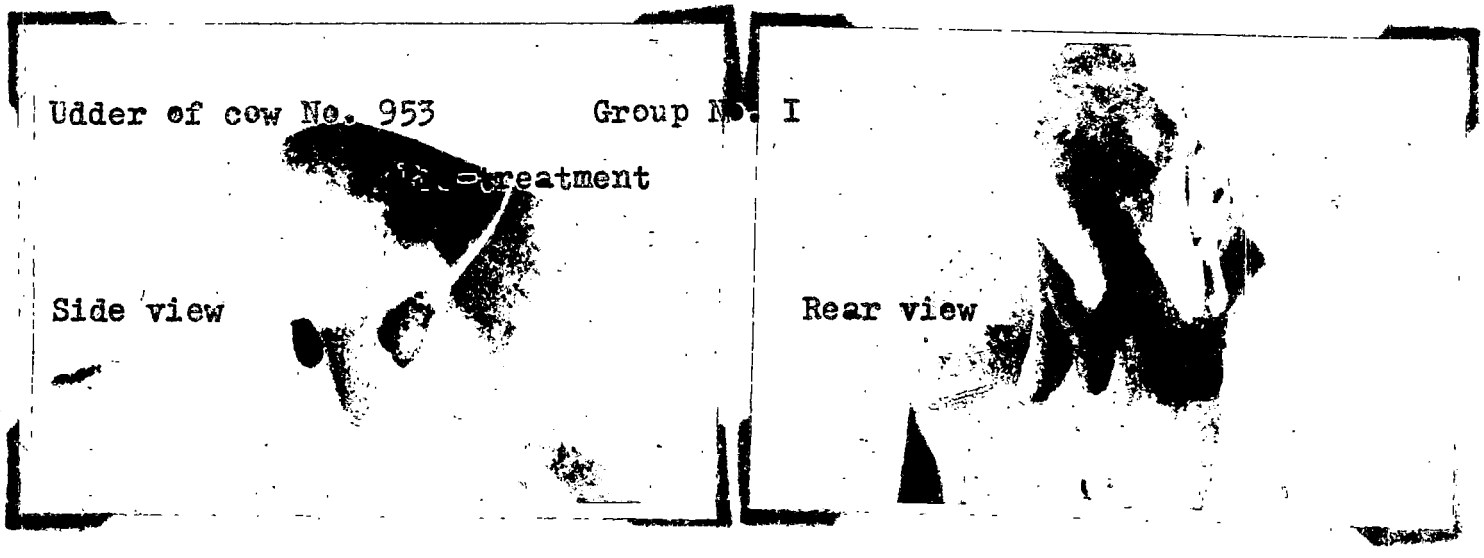
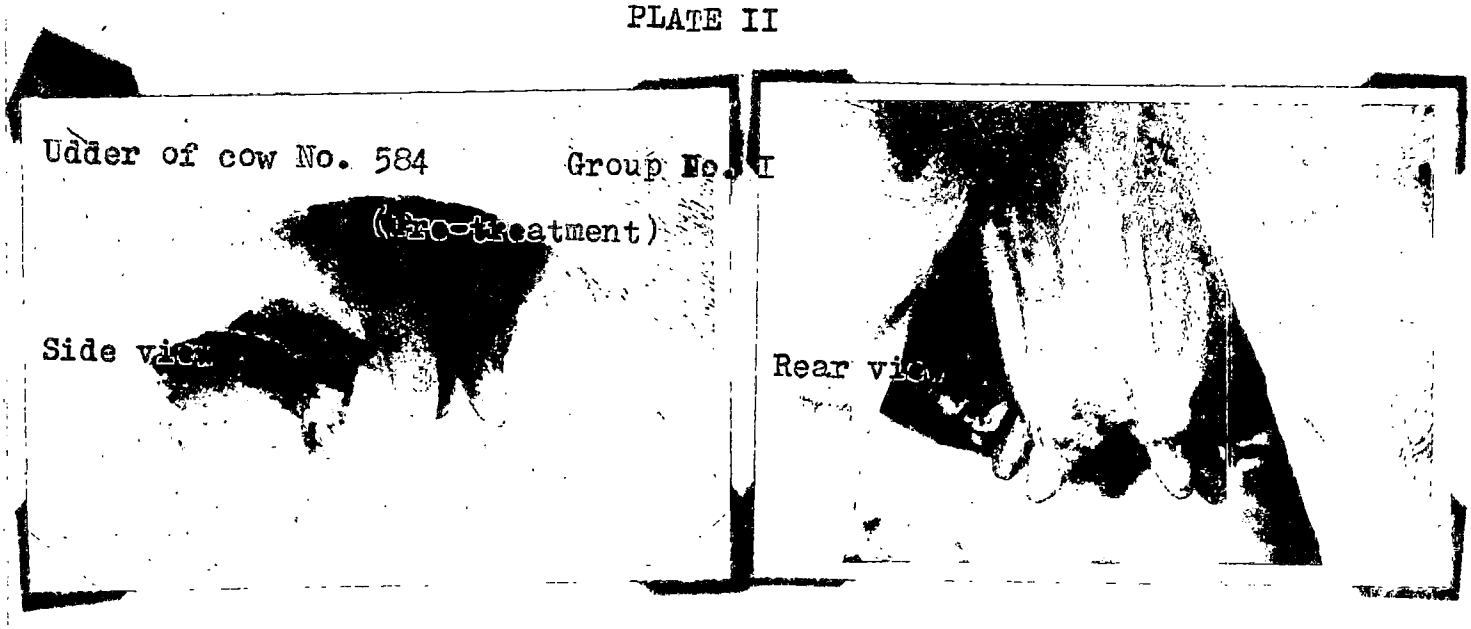


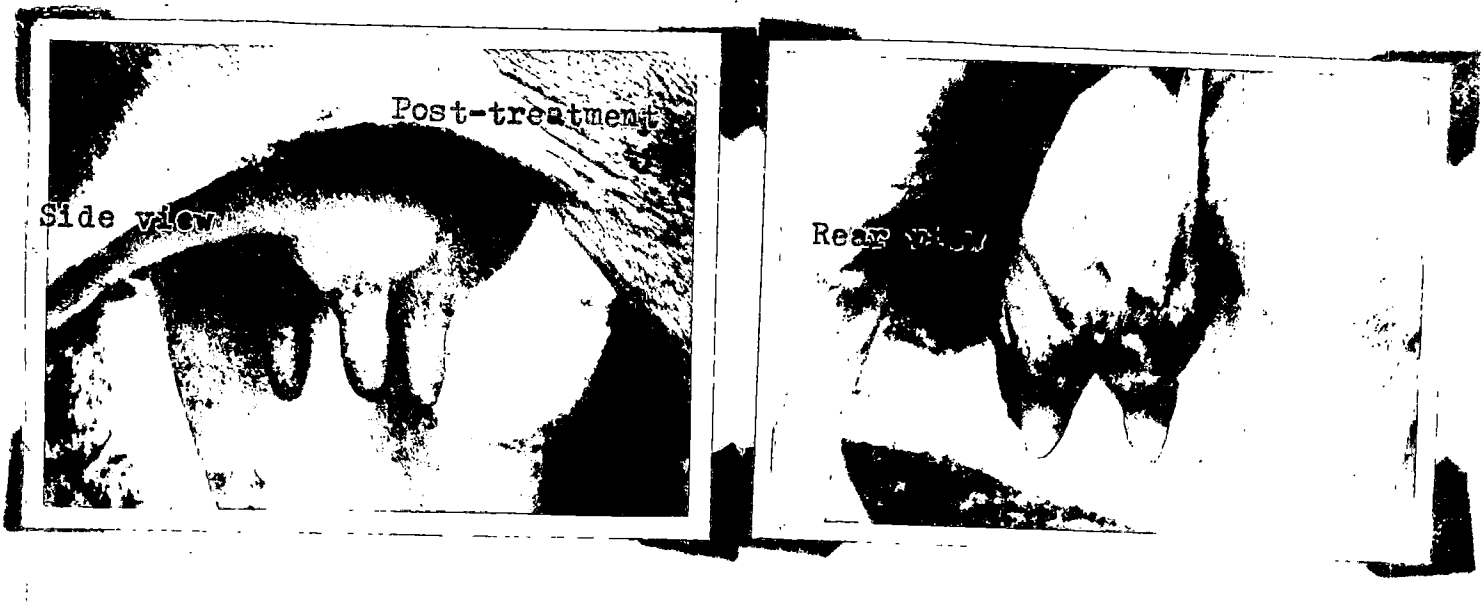
PLATE I


Udder of cow No. 953 Group No. I
Post-treatment
Side view
Rear view


Post-treatment
Side view
Rear view

PLATE II


Udder of cow No. 584
Group No. I
(Pre-treatment)
Side view
Rear view


Post-treatment
Side view
Rear view

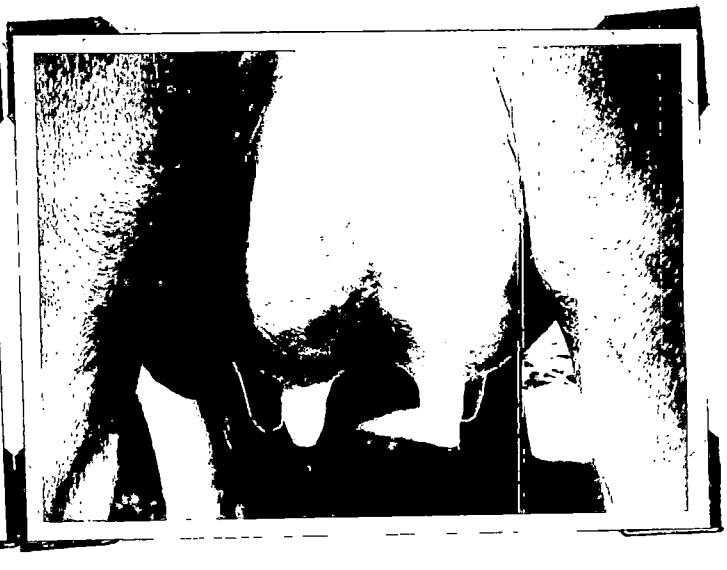
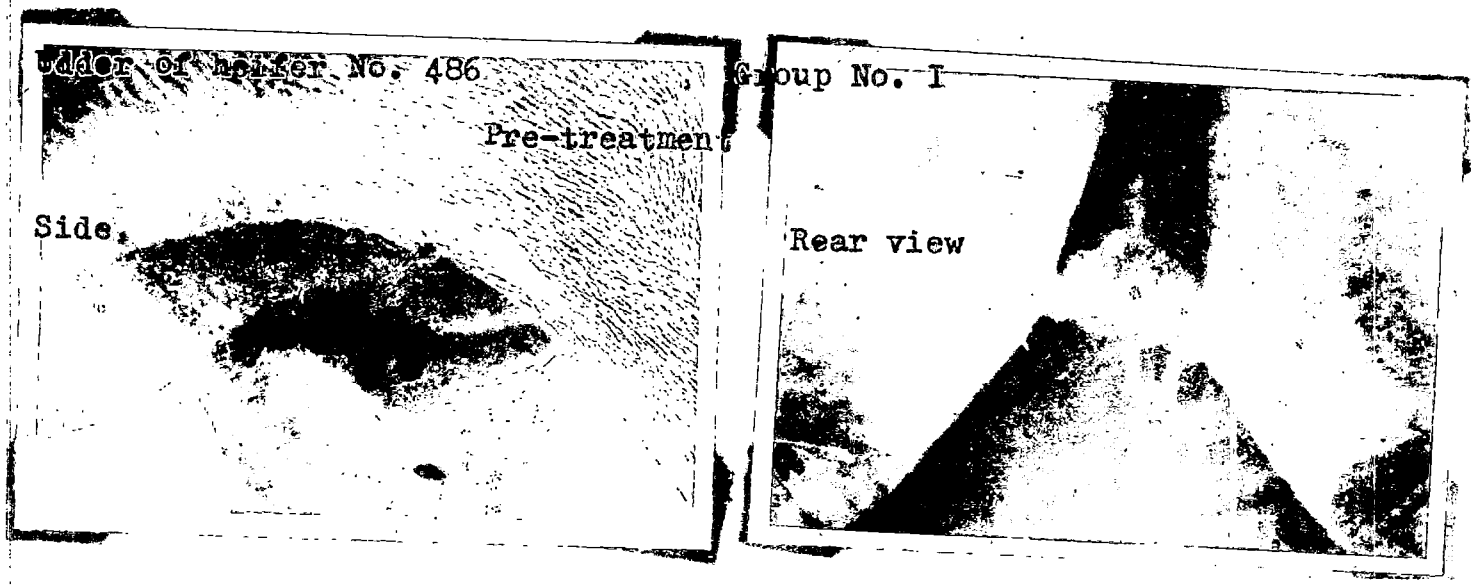
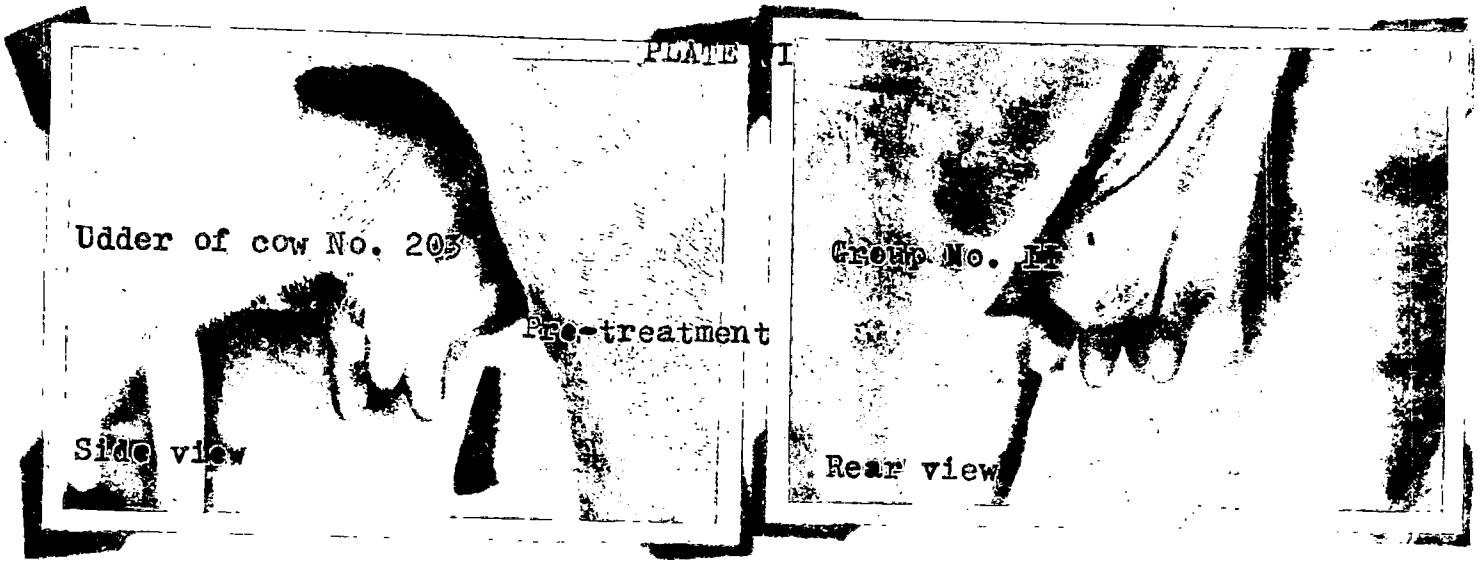


PLATE IV


Heifer No. 486
Group No. I
Pre-treatment
Side
Rear view


Post-treatment
Side view
Rear view

PLATE I


Udder of cow No. 203
Group No. II
Pre-treatment
Side view
Rear view

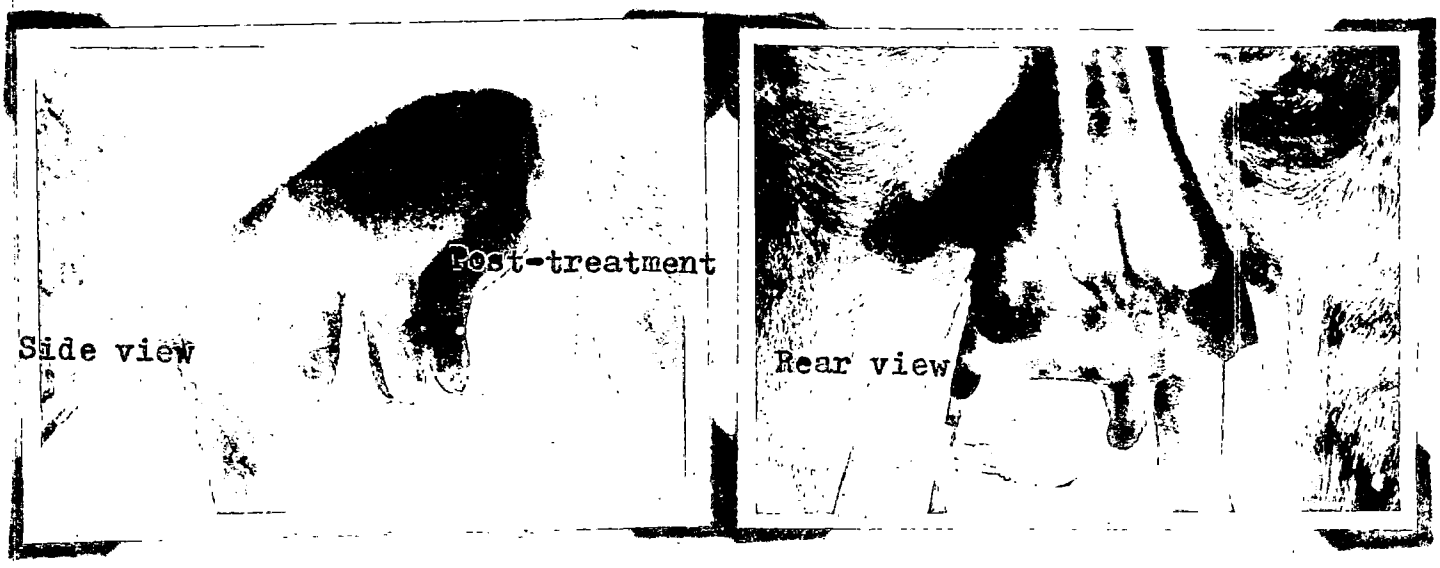
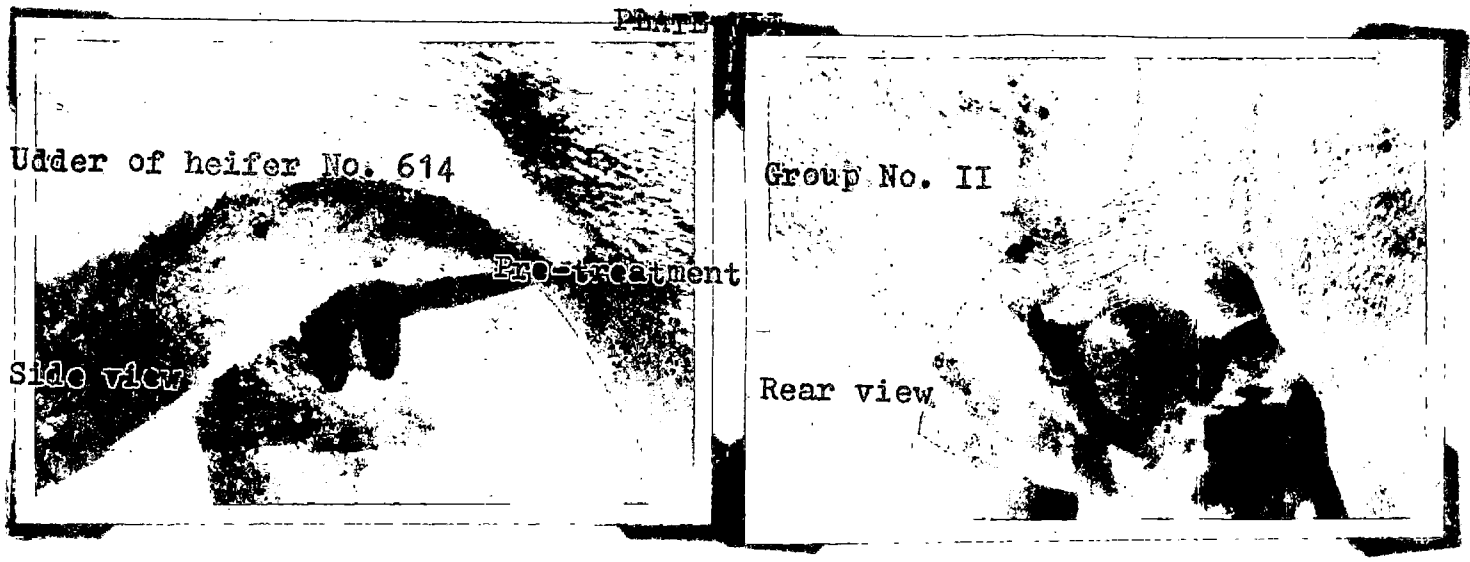

Post-treatment
Side view
Rear view

PLATE IV


Udder of heifer No. 614
Pre-treatment
Side view
Group No. II
Rear view

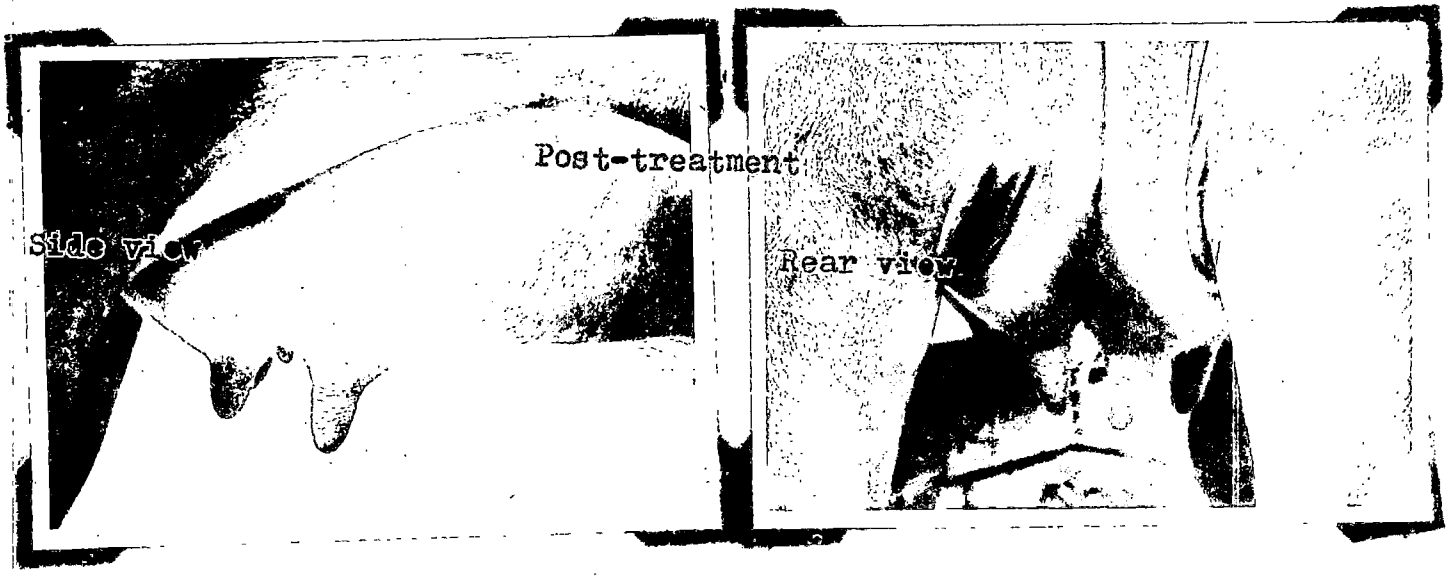
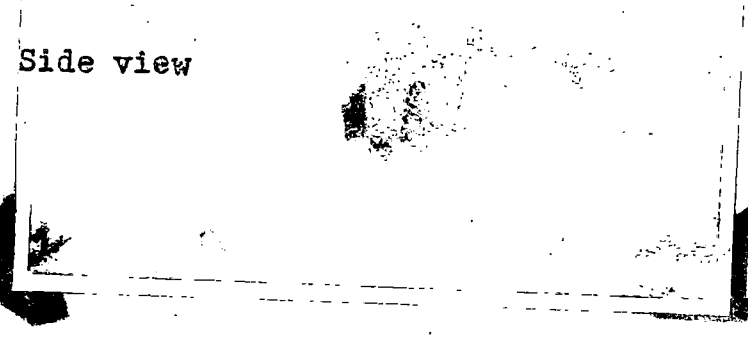

Post-treatment
Side view
Rear view

PLATE VIII

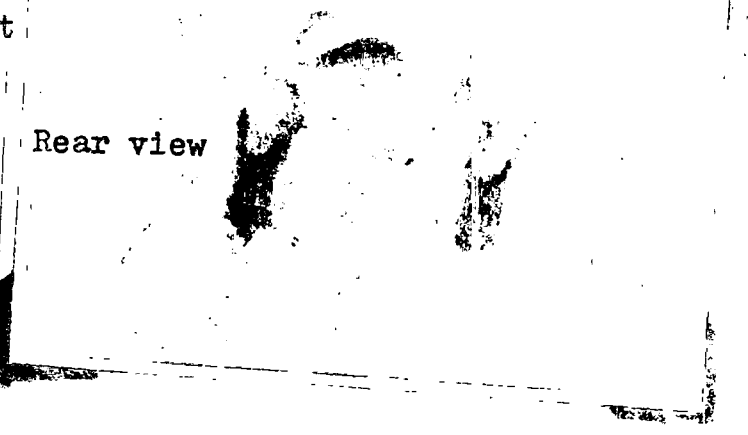
Udder of heifer No. 480 Group No. II

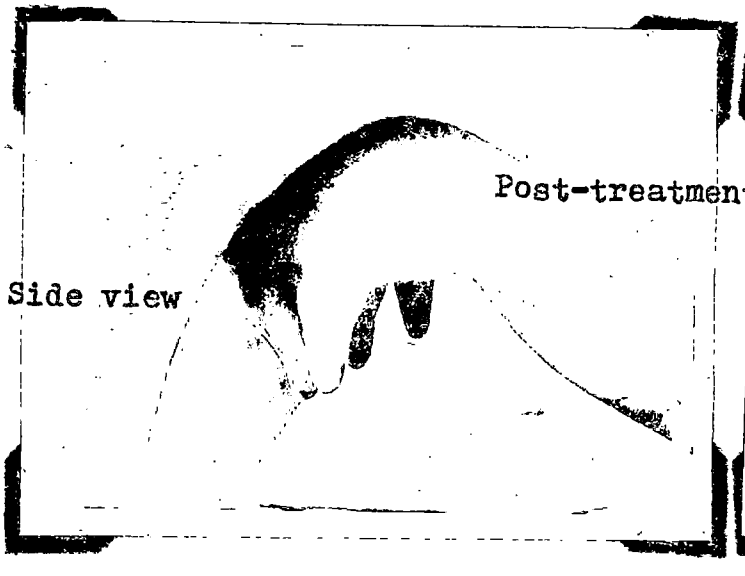
Pre-treatment

Side view



Rear view



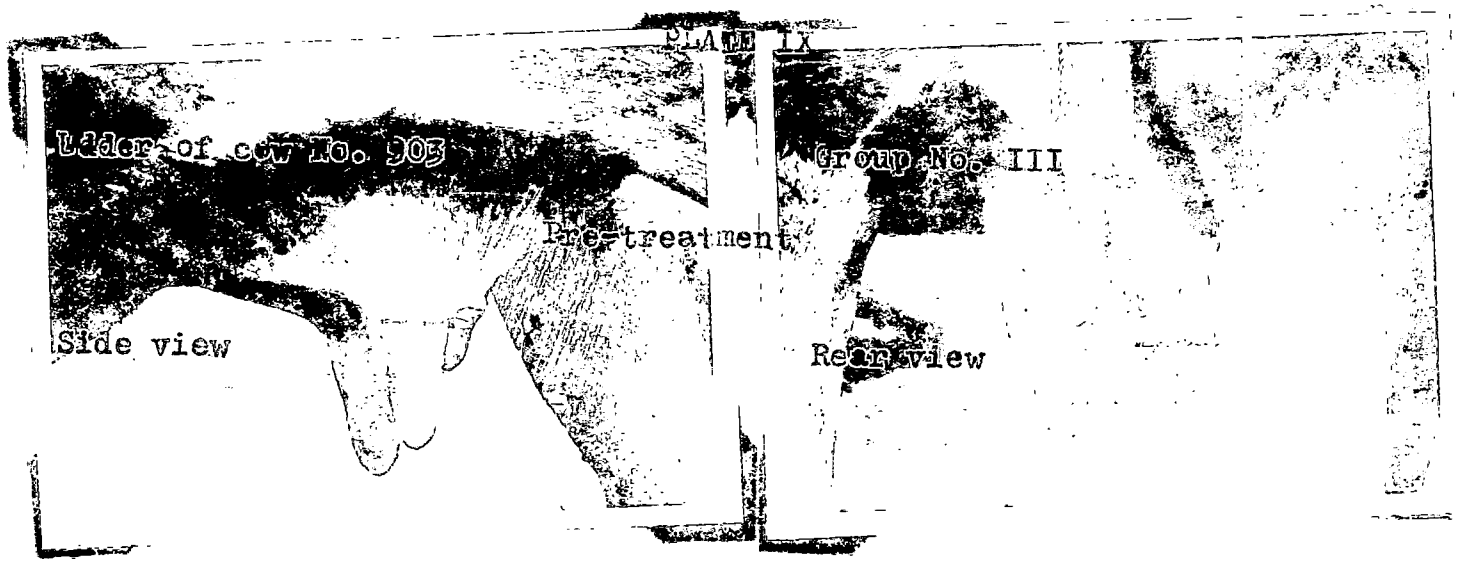


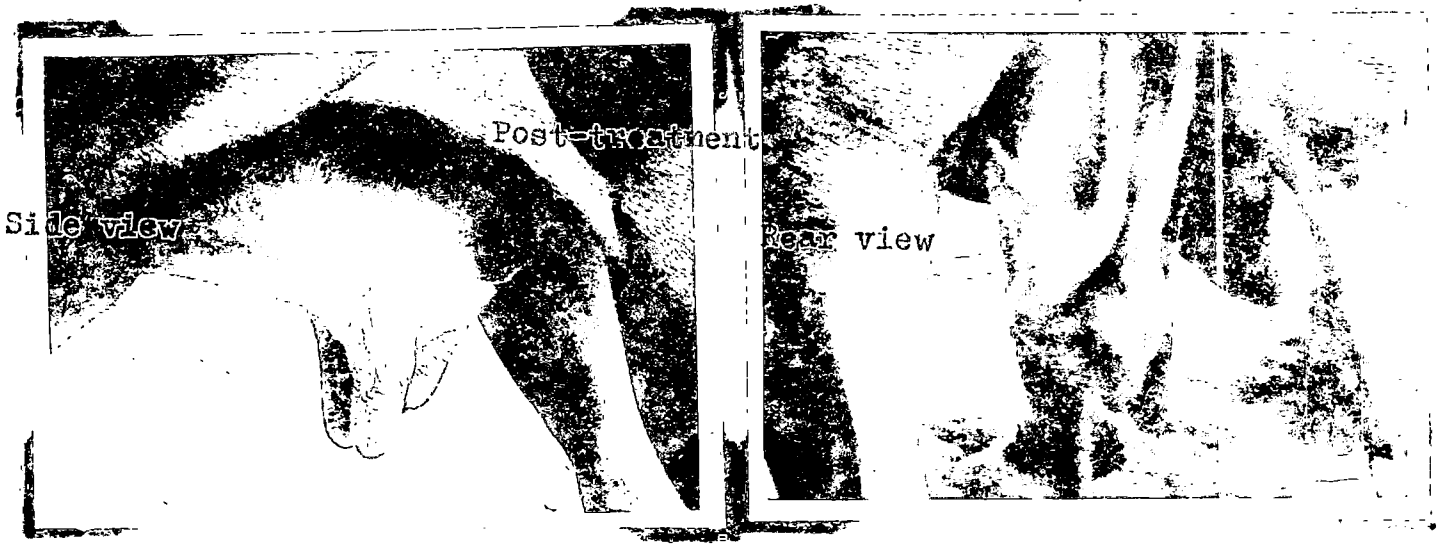
Post-treatment

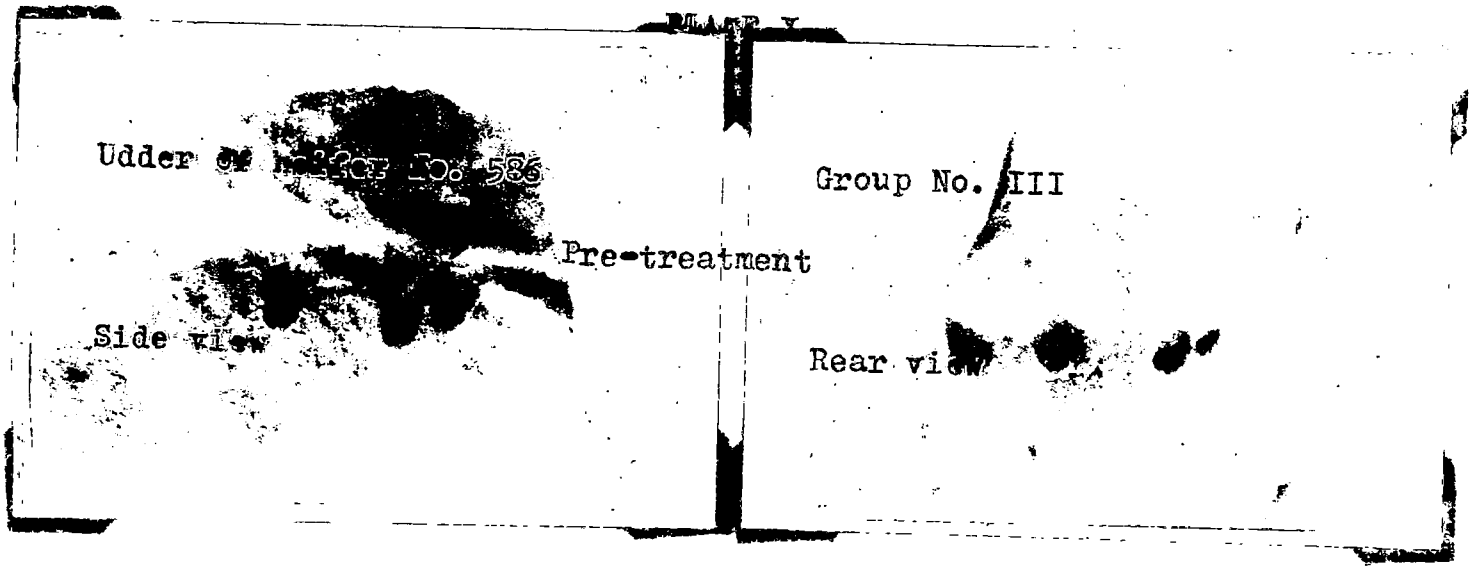
Side view

Rear view

PLATE IX


Udder of cow No. 903
Group No. III
Pre-treatment
Side view
Rear view


Post-treatment
Side view
Rear view


Udder of Heifer No. 586
Group No. III
Pre-treatment
Side view
Rear view

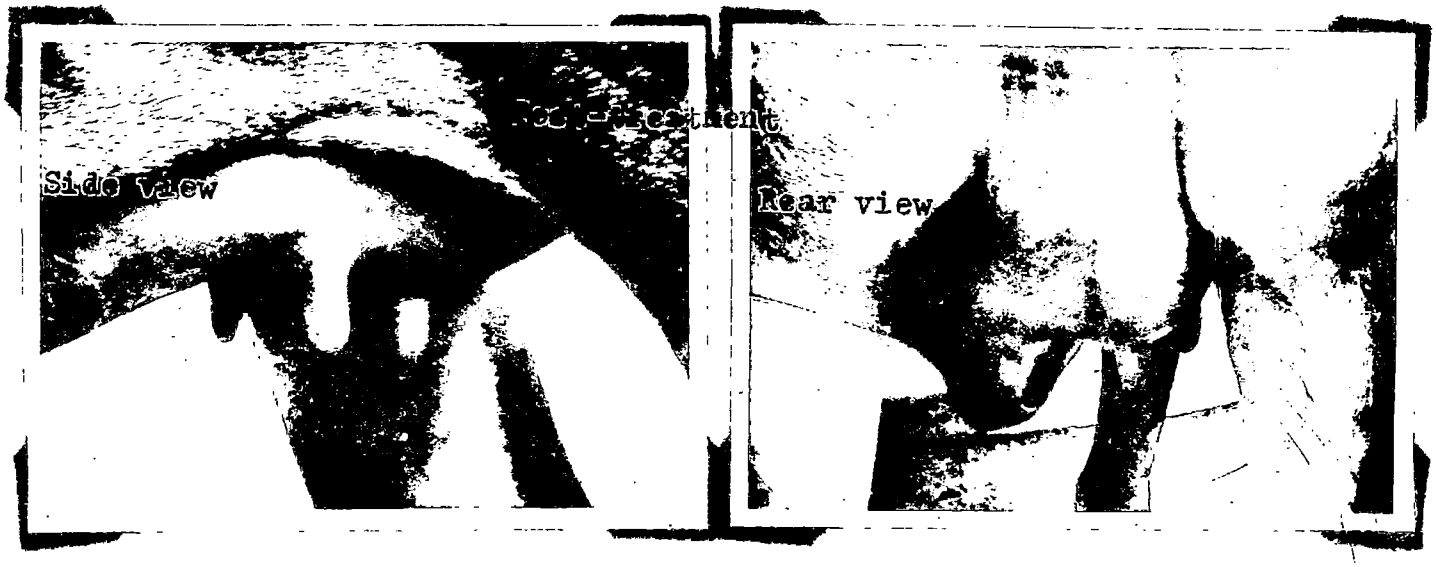
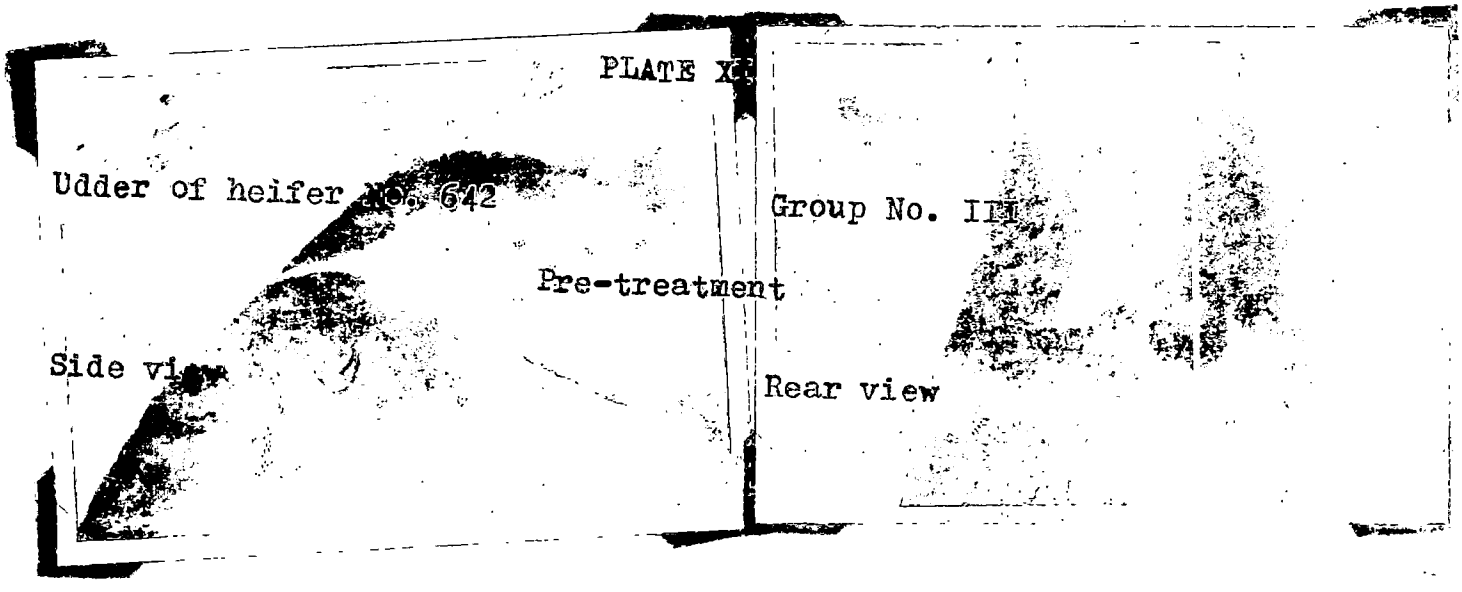

Pre-treatment
Side view
Rear view

PLATE X



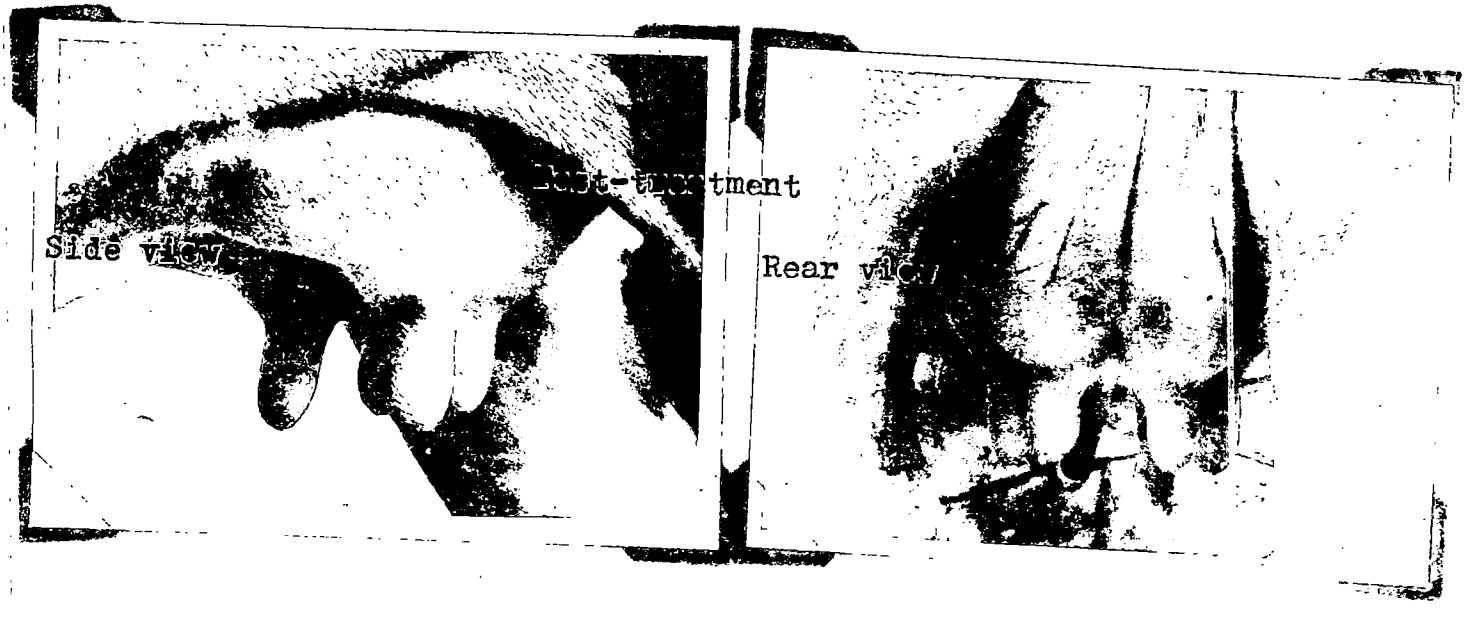
Udder of heifer No. 642

Group No. III

Pre-treatment

Side view

Rear view



Post-treatment

Side view

Rear view

DISCUSSION

DISCUSSION

The scientists in their studies made earlier were using prolonged treatments with oestrogen and progesterone for the induction of lactation (Turner et al. 1956 and Meites, 1961). The duration of the treatment varied from 60-180 days and this duration was thought to be required for the full development of the lobulo-alveolar system of the mammary gland. But from the work of Smith and Schanbacher (1973) it became evident that treatment for short period of seven days with a combination of natural oestrogen and progesterone was sufficient for successful induction of lactation. The procedure now commonly employed for the induction of lactation is based on the above work in which the combination of natural oestrogen and progesterone is given at the rate of 0.05 mg and 0.125 mg/kg body weight respectively at 12 hour intervals for seven days consecutively. The initiation of lactation began within 11-21 days after the first injection.

Development of the Udder

In the present work, the group I consisting of two cows and two heifers was injected with natural oestrogen and progesterone at the daily dose recommended by Smith and Schanbacher (1973). The udder development was judged based on the external appearance. It was seen that in this group there was not much udder development during the first seven days. But there was

good udder development in all the animals except in a cow at the commencement of milking on the 21st day. Perhaps due to the flabbiness of the mammary gland the udder development in this cow was not much appreciable. In general the findings were in agreement with the observations of previous workers with regard to mammary gland development (Smith and Schanbacher, 1973, 1974; Erb et al. 1973; Moss et al. 1975 and Collier, et al. 1975).

In the use of natural oestrogen preparations the cost is so prohibitive that it cannot be recommended for routine animal treatment. Williams and Turner (1961) established that the synthetic oestrogen was also effective for the induction of lactation. Information regarding the possibility of successful induction of lactation by the short duration treatment with synthetic oestrogen-progesterone combination is meagre. One of the objectives of the present investigation was to assess the efficacy of synthetic oestrogen combined with progesterone in inducing lactation by the short duration procedure. For this purpose the group II consisting of two cows and two heifers was given synthetic oestrogen and progesterone and the results obtained were compared with those of the animals receiving natural oestrogen-progesterone treatment. In this group also there was not much udder development during the period of seven days of the treatment. But appreciable development was noticed by about 21 days after the first injection. In heifer No. 614 there was some udder development even during the last few days

of the treatment and full development by about the 12th day after the last injection. In comparison to group I the response in the animals of this group was poor. It is possible that this might have been due to the higher potency of natural oestrogen given to the animals in group I as compared to the synthetic oestrogen given to the animals in group II.

Smith and Schanbacher (1973) recommended the administration of half the daily dose of hormones at 12 hour intervals. Since this was impracticable under field conditions a third group of animals consisting of two cows and two heifers was administered synthetic oestrogen and progesterone at the usual daily dose, but on alternate days, thus extending the duration of the treatment to 14 days. In this group the udder development was better in comparison to most of the animals in groups I and II. Udder development was evinced in the two heifers even before the termination of the treatment. On the 21st day there was full udder development in all the animals. The better development might have been due to the prolonged hormonal stimulation for the mammary gland development in comparison to the other two groups. The difference between the groups II and III was only in the duration of the treatment. The fact that the development was better in group III, revealed that a longer duration of treatment was conducive for better development of udder.

Meites (1961) reported that there was a difference between

cows and heifers in their response to the hormone treatment for mammary gland development and induction of lactation. He stated that probably the quantitative hormonal requirements for optimum udder growth might not be the same in cows and heifers. This observation pertained to the long duration treatment with the hormones. Recent studies using short duration treatment did not mention any difference in response between cows and heifers (Smith and Schanbacher, 1973; Smith et al. 1973 and Collier et al, 1975). In the present study, in order to assess the variation in response between cows and heifers, equal number of cows and heifers were included in each group. It was observed that in all the three groups the udder development was better in heifers than in the cows. The poor response shown by the cows as compared to the heifers indicated that the hormonal requirement was higher for cows than for heifers. Among the cows in the three groups, those in the group I showed better development. This indicated the higher efficacy of natural oestrogen, eventhough the number of animals used in this experiment was small.

In all the animals the mammary gland showed marked development during the early days of induced lactation. This was in agreement with the observations of Howe et al. (1975) who found further enlargement of the mammary gland during the initial stages of induction of lactation.

Lactation

Milking was commenced in all the animals on the 21st day

after the first injection except in two heifers (Nos. 614 & 473) in which it was commenced on the 13th and 20th day respectively. Smith and Schanbacher (1973) reported that the treated animals came into lactation within a period of 11-21 days after the first injection. Smith et al. (1973) also reported that the average time taken for the initiation of lactation was 19.6 days. In the present work the induced lactation began during the period of 13-21 days and this was in conformity with the findings reported earlier.

Lactogenesis, defined as yield of at least one kilogram of secretion in 24 hours, was noticed in seven out of the 11 animals used in this experiment. In other words, 63 per cent of the animals used for the experiment responded favourably to the treatment. Out of these seven animals, three yielded more than three litres of milk per day. The highest daily yield recorded was 4.3 litres, in heifer No. 473. The lowest yield in this experiment was 140 ml from cow No. 203.

Variations in response to the hormone treatment have been reported by several workers (Meites, 1961; Smith et al. 1973; and Collier et al. 1975b). Induction of lactation was observed in 38 out of 48 cows in a study made by Smith et al. (1973). Collier et al. (1975b) induced lactation in 14 out of the 16 cows used for the experiment. Variations in response were noticed in the present study also. Several factors were found to be responsible for the failure to respond. It was observed

that certain ovarian conditions like smooth inactive ovaries and the early stage of oestrus cycle at which the treatment of hormones was started might lead to successful initiation of lactation whereas persistant corpus luteum and midcycle ovaries with functioning corpus luteum were incompatible (Smith and Schanbacher, 1973). The functional status of the endocrine glands during the treatment period was stated to be a contributory factor for the variation in response. The data on oestrogen and progesterone levels in the peripheral blood during early lactation suggested that during normal lactogenesis in bovines, the progesterone level was low and the oestrogen level high (Smith et al. 1973). The opinion of Smith and Schanbacher (1973) that a large proportion of variability in response might be due to lack of control of internal progesterone synthesis seemed reasonable.

Erb et al. (1976) suggested that for obtaining better response in induced lactation the treatments were to begin on a day between third and eighth day post-oestrus. Out of the total of 11 animals used in the present study, five received the first injection of the hormones within 3-8 days post-oestrus and the remaining six had their first injection within 9-15 days. Four animals of the former group yielded more than one litre of milk per day whereas only three from the latter gave that much of milk. Therefore the results obtained in the present study were in agreement with the suggestion of Erb et al. (1976).

Another possible cause of variation was the rate of absorption of the hormones from the site of injection. Smith and Schanbacher (1974) observed that intramuscular injection of hormones in alcohol failed to induce lactation. Erb et al. (1976) injected oestrogen and progesterone in oil intramuscularly for a further period of five days and found that neither the interval from last steroid injection to first milking nor the yield was affected. In the present work response was obtained by the intramuscular injection of the steroids in oil. This indicated that intramuscular route was also effective for induction of lactation.

In the present work there were other factors responsible for the variations in performance. The three groups of animals received three different treatments and there was significant difference in response among these groups. Animals in group I were given natural oestrogen and progesterone and all the animals in this group gave more than one litre of milk per day during the eight weeks period. The animal that yielded the maximum quantity of milk was in this group.

The animals in the second group were given stilboestrol and progesterone for seven days. Except in one animal (heifer No. 614) all the other animals gave less than one litre of milk per day. This might be due to the low potency of synthetic oestrogen in comparison to natural oestrogen. On an equivalent weight basis oestradiol was reported to be about 10 times as

potent as stilboestrol (Roberts, 1971). The cow No. 203 which gave the lowest yield was from this group. Strangely enough heifer No. 614 not only gave better yield (3.42 litres) but also came into lactation earlier than all the other animals. Eventhough heifer No. 614 and 480 received the injections within 3-8 days post-oestrus the performance of heifer No. 614 was better thereby indicating that there were factors other than the stage of oestrous responsible for variation in performance. The functional status of other endocrine glands, the genetic potentiality of the animal for high milk production and better developed udder before the treatment might be the other factors contributory for the better performance.

Among the animals in group III, two heifers gave more than one litre of milk per day. In this group eventhough the cow did not perform well to the treatment the average performance of the animals was better than those of group II. Both these groups received stilboestrol but the duration of treatment was different. The steroid treatment given to this group for an extended period resulting in the prolonged hormonal stimulus for mammary gland development might be a cause for the better performance. The average daily milk yield of the different groups of animals showed that the yield in group III nearly equalled that in group I. There was one cow less in this group and added to this the fact that cows generally responded less favourably in this experiment might have been the contributory

factor for the better average daily milk yield in this group. Erb et al. (1976) estimated the excretion of hormones in the urine and concluded that intramuscular injection caused rapid absorption and excretion of oestrogen. So in the third group of animals the extended treatment period gave more time for the steroids to act unlike in the case of the other two groups. The animals in group III had a better response than the animals in group II. Both the groups were given the same steroid preparations but for different periods. The treatment for a period of 14 days was better than for a short period of seven days when synthetic oestrogen was used instead of natural oestrogen.

In induced lactation the period taken to reach the peak yield was longer. Turner et al. (1956) found that the animals on induced lactation reached the peak yield by about 14 weeks after the commencement of milking. Smith and Schanbacher (1973) and Narendran et al. (1974) reported that a period of 4-7 weeks was required to reach the peak yield. But Erb et al. (1973) observed that eight weeks lapsed before the animals reached the peak yield. In the present experiment the time taken by the different animals to reach the peak yield varied from 4-10 weeks except in heifer No. 473, in which the same was attained in two weeks. Cow No. 953 of group I and heifer Nos. 586 and 642 of group III took more time to reach the peak yield. Cowie et al. (1965) and Narendran et al. (1974) reported the presence of numerous immature alveoli in the mammary gland developed by

the injection of exogenous steroids. The prolonged time taken to attain the peak yield was attributed to the time taken for the maturation of these immature alveoli. The above finding was confirmed in the present work since some of the animals which were poor yielders in the beginning gave better yield subsequent to the regular milking stimulus.

Physical Properties of Milk

In general the colour of milk collected during the first few days of lactation from the experimental animals was similar to colostrum from the postpartum cows. However, in the cow Nos. 584, 408 and 203 the milk was lighter in colour. The milk from heifer No. 486 and cow Nos. 903 and 953 was blood tinged due to capillary bleeding which disappeared without any treatment. The yellowish colour of colostrum persisted more in heifers than in the postpartum cows. Folley and Malpress (1944) observed that secretion induced by treatment with steroid hormones remained colostral for longer periods and changed gradually to milk of normal composition.

On the first day of lactation the lactometer reading of the milk obtained by the induction of lactation was lower than that of the milk from the control animals. Variations due to individuals, low milk yield and low total solids content might be the reason for this. Similarly the milk of induced lactation took longer time to become negative for clot-on-boiling test.

In the cows this time was longer. The presence of blood and the high chloride content might be the reason for this in the cows.

Composition of Milk

Fat.

In the first day milk the fat content varied among the individuals within the group, but there was no significant difference between the groups. This wide variation seen on the first day got reduced on the eighth day onwards. Folley and Malpress (1944) and Perrin (1955) reported that the increase in fat percentage was slower during the first seven days as compared to the cows on normal lactation. The present work showed that the fat percentage in the first and eighth day milk of the induced lactation was slightly lower than that in the milk of control animals during the same period. But this difference was not significant. The fat percentage in the milk collected on the 21st day was slightly higher than that in the control cows. Narendran et al. (1974) reported that the difference in milk yield between the induced and normal lactations may account for some of the differences in the fat and protein percentages in milk. In the present work the fat percentage in induced lactation milk was not significantly different from that in the milk from postpartum control cows.

Protein.

The samples of milk collected from the animals of the first and second groups contained significantly lower percentage of protein as compared to those from the control group. Narendran et al. (1974) reported that short term hormone therapy and rapid lactational response might be the factors involved in the low protein percentage. In heifer Nos. 473 and 614 which came into lactation earlier than other animals, the protein percentage in milk was lower. In the third group of animals which received the treatment for a longer period the protein percentage was higher than in the other two groups. The findings in the present investigation were in agreement with those of Narendran et al. (1974). In the milk collected on the eighth day there was no statistically significant difference in the percentage of protein between the milk of control and experimental animals. Folley and Malpress (1944) and Perrin (1955) reported higher protein percentage in induced lactation milk. Erb et al. (1976) also reported that the total protein in the milk from induced lactation was higher by one per cent than that in the normal milk on the seventh day and this difference disappeared by the 42nd day. In the present trial the protein percentage was a little higher in the milk of animals on induced lactation on the 8th and 21st day. But this difference in the percentage of protein was not statistically significant.

Lactose.

The lactose content increased more slowly during the first seven days in the milk from induced lactation as compared to normal milk (Folley and Malpress, 1944). In the present investigation also it was found that the lactose content was lower in the first day milk. But the difference was not statistically significant. In the milk collected on the eighth and 21st day the lactose content was not different from that in the milk of the postpartum control animals for the corresponding period. So with regard to lactose content the milk from induced lactation was similar to that of the milk from the control animals. This was in agreement with the findings of Narendran et al. (1974) and Erb et al. (1976).

Total solids.

The total solids content in the milk collected on the first day of induced lactation was significantly lower than that in the first day milk of the control animals. This was attributed to the lower contents of protein and lactose in the induced lactation milk during the early stages. From the eighth day onwards no significant difference was present in the total solids content between the milk of induced lactation and the postpartum lactation. The milk from induced lactation was similar to that of the normal lactation in its total solids content.

Ash.

The ash content in milk of the first two groups was lower than that in the control. But this difference also was not statistically significant. On the eighth day of lactation the ash content in the milk from the induced lactation animals was equal to that of the milk from normal animals.

As regards the composition of milk in induced lactation it was observed that the type of changes that took place in the quantity of its components was similar to that in the milk from normal animals. Contrary to the observations made by Pinheiro et al. (1974) an initial colostral stage was present in all the animals which were induced to lactate. The observations made in the present study with regard to the fat, protein and lactose were in agreement with those of Shalash et al. (1963), Fulkerson and McDowell (1974), Narendran et al. (1974), Collier et al. (1975) and Erb et al. (1976).

Effect of Hormones on the Blood Values

Erythrocyte count.

Not much data was available regarding the effect of the hormones on blood values. It was expected that the changes in induced lactation would be similar to those during pregnancy and early lactation. The erythrocyte count during the entire period of gestation was estimated by Morris (1944). He found

that the erythrocyte count decreased in the latter half of gestation and returned to normal within 15 days after parturition. This decrease in the erythrocyte count resulted from an increase in both plasma and erythrocyte volumes. However, the increment in plasma volume was larger than that of the circulating erythrocytes. This disproportionate increase in plasma volume caused a decrease in the erythrocyte count (Assali and Brinkman, 1972). Nirmalan and Nair (1971) observed that pregnant and lactating cows had lower erythrocyte count than non-pregnant and non-lactating cows. In the present work it was seen that there was a significant decrease in the erythrocyte count during the treatment period in all the three groups of animals used for the experiment. This was in agreement with the findings of Morris (1944), Nirmalan and Nair (1971), Treacher et al. (1975) and Rawlands et al. (1975). The average reduction was 0.99, 0.69 and 0.69 x $10^6/mm^3$ in the groups I, II and III respectively. It was found that the reduction was significantly higher in group I than that in the other two groups. This might be due to the higher potency of natural oestrogen. The difference in the erythrocyte count during the treatment and the post-treatment periods was not significant. The fact that the post-treatment values were taken within a period of seven days after the last injection might have been the cause for it. There was significant difference between the cows and the heifers in the erythrocyte count taken during the different periods. Calculation of the normal

deviate 'u' showed that this difference was due to the difference that existed before the experiment. Variation in erythrocyte count due to age was reported by Holman (1955), Greatorex (1957) and Schalm (1965). It was observed that during induced lactation there was a significant decrease in the erythrocyte count both in the heifers and in the cows and that the decrease was higher when natural oestrogen was given.

Haemoglobin.

Eventhough Morris (1944) and Treacher (1975) reported a reduction in the haemoglobin content during pregnancy Nirmalan and Nair (1971) did not observe reduction in the haemoglobin concentration during pregnancy or lactation. In the present trial it was found that there was a significant reduction in the haemoglobin concentration during the treatment as well as the post-treatment periods. The reduction of haemoglobin content was more in the animals in group I, but this difference was not statistically significant. It was observed that both natural and synthetic oestrogen and progesterone reduced the haemoglobin concentration during the treatment period. Due to the differences in haemoglobin content that existed between the cows and the heifers before the experiment, there was a difference in the haemoglobin content in the same categories of animals during the period of treatment. Differences in the haemoglobin content in the blood of cows and heifers were reported by Holman (1956) and Schalm (1965). As the post-treatment

values were taken within a period of seven days there was no significant difference between the values during treatment and post-treatment periods.

Plasma protein.

All the animals in the three groups showed significant increase in the plasma protein concentration during the treatment period. Herak et al. (1975) reported an increase in the serum protein during the early days of lactation in sows. In birds the injection of oestrogen caused an increase in plasma protein. The increase was due to increased production of protein in the liver (van Tienhoven, 1968). The injection of steroids might have increased the protein synthesis in cows also. There was no significant difference in plasma protein during the treatment as well as the post-treatment periods. Similarly there was no significant difference between the effects of natural oestrogen and synthetic oestrogen on the concentration of plasma protein. However, statistical analysis revealed significant difference between the plasma protein content in the cows and in the heifers. This difference was found to be not due to the difference in response to the hormones ($u = 0.765$). It was noted that injection of both natural and synthetic oestrogen combined with progesterone for the induction of lactation caused an increase in the concentration of plasma proteins.

Packed cell volume.

Reduction in packed cell volume during pregnancy had been reported (Morris, 1944 and Treacher et al. 1975). In the present investigation it was found that there was a significant decrease in the packed cell volume during the treatment period in all the experimental animals. This was attributed to the reduction in the erythrocyte count. But during the post-treatment period as compared to the treatment period there was significant increase in packed cell volume. Since no corresponding increase in the erythrocyte count was noticed this might be due to variation in the corpuscular volume. The animals of the different groups had significant difference in the packed cell volume. During the treatment period the reduction in packed cell volume was 4.37, 2.70 and 2.50 per cent in groups I, II and III respectively. The decrease was more in the animals of the first group and this would have been due to the higher potency of natural oestrogen. Similarly there was a significant difference in packed cell volume between the cows and the heifers. This difference was found to be due to the difference that existed in the animals even before the commencement of the treatment. Packed cell volume was found to decrease during induction of lactation.

More data from a larger number of animals will be required to arrive at a definite conclusion regarding the changes in blood values that occur during induced lactation.

Effect of Induction of Lactation on Health and Reproduction

On the second day of the injection heat symptoms were manifested by most of the animals and persisted for two to three days. In two of the animals heat symptoms of a mild nature were seen intermittently throughout the treatment period. General excitement and relaxation of the sacro-sciatic ligament was seen even beyond the treatment period. In earlier works where oestrogen alone was given for induction of lactation many of the typical signs of nymphomania were elicited by the animals (Folley and Malpress, 1944 and Meites, 1961). When oestrogen and progesterone were given for long periods none of the undesirable features were exhibited (Turner et al. 1956). In the short duration treatment Smith and Schanbacher (1973) and Erb et al. (1973) observed heat symptoms of transient nature during the treatment period and for 2-4 weeks thereafter. Cystic ovaries were observed in some of the animals after the treatment. In the present work none of the animals developed cystic ovaries.

Regular oestrus of normal duration was seen in four heifers and two cows between 42 and 57 days. One cow and three heifers were inseminated when they came in regular heat for a second time and pregnancy was confirmed in all of them. Normal reproductive functions after induction of lactation had been reported by several authors (Pinheiro et al. 1974 and Collier et al. 1975b). These results indicated that induction of

lactation was not hazardous to the future reproductive performance of the animals. On the other hand it was seen that infertile animals, if induced to lactate, might come into regular reproductive cycles later.

During the induction of lactation an increase in body weight in the experimental animals had been reported (Renike et al. 1952 and Turner et al. 1956). Especially in beef cattle body weight gains had been observed by the feeding or implantation of oestrogen(Macdearmid and Preston, 1969; Robles and Cunshow, 1974 and Anderson et al. 1975). So it was quite logical to expect gain in weight in animals which were induced to lactate. In the present work it was seen that there was no increase in body weight in the cows. But there was a slight reduction in the body weight in all the heifers.

In general, the usage of hormones for the induction of lactation did not bring about any adverse effect on the health of the animals.

SUMMARY

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A study was conducted on induction of lactation in six each of cows and heifers using natural and synthetic oestrogen along with progesterone.

Three groups of animals, each comprising of two cows and two heifers, were used for the experiment. One group of animals were given natural oestrogen (oestradiol dipropionate) and progesterone at the rate of 0.05 mg and 0.125 mg per kg body weight respectively at 12 hour intervals consecutively for seven days. The second group of animals were given synthetic oestrogen (Stilboestrol dipropionate) and progesterone, the dose and duration being the same as in the first group. Synthetic oestrogen and progesterone at the rate of 0.10 mg and 0.25 mg per kg body weight respectively were given to the animals in the third group on alternate days during a period of 14 days. All the injections were given intramuscularly.

There was significant development of the udder in all the animals except one cow in group I. Appreciable development of udder was noticed in the animals in group II. In one of the heifers of this group the development was very marked. The development of udder in the animals in group III was remarkable except in one cow. The development of the udder in the heifers was better than in the cows.

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Lactation commenced on the 21st day after the first

injection in all the animals except in two heifers. One heifer from each of the groups I and II came into lactation earlier. The peak daily milk yield during the first 60 days ranged from 140 ml to 4300 ml. The highest milk yield was obtained from one of the heifers in group I whereas the lowest yield was from one of the cows in group II. The average daily milk yield of the three groups was 1766, 942 and 1638 ml for groups, I, II and III respectively. The average daily yield of the cows was 591 ml while that of the heifers was 2120 ml. Seven out of the eleven animals gave more than one litre of milk per day.

The peak yield was attained in the experimental animals within a period of 4-10 weeks except in one heifer which reached the peak yield in two weeks.

The composition and properties of the milk obtained from the experimental animals were compared with the milk from four postpartum cows. In general, the colour of the milk from the experimental animals on the first day of lactation was similar to that of the colostrum from the normal cows. From the eighth day onwards the colour of milk from all the experimental animals was similar to the normal milk. The milk from the experimental animals on the first day gave a lower lactometer reading but on the eighth day the lactometer reading was within the normal range. The milk of the experimental animals took more time to become negative for clot-on-boiling test and this was more marked in the cows than in the heifers.

The chemical composition of the milk with respect to fat, protein, lactose, chloride, total solids and ash were estimated and the values were compared with those of milk from postpartum cows. It was found that on the first day of lactation the milk from the experimental animals had less protein and total solids content. But on the eighth day of lactation the composition of milk from the experimental animals was not significantly different from that of the milk from normal cows. In the 21st day of lactation the milk from the animals oninduced lactation had a slightly higher protein and fat percentage eventhough the difference was not statistically significant. In general, the milk from the animals of induced lactation had similar qualities to that of the milk from postpartum cows.

The blood values of the animals were estimated before, during and after the treatment. There was a significant reduction in erythrocyte count, haemoglobin concentration and packed cell volume in all the animals in the three groups during and after the treatment period as compared to the pre-treatment period. The decrease in erythrocyte count was more in the animals in group I. The plasma protein was higher in the animals during the treatment as well as the post-treatment periods. The significant differences in blood values between the cows and the heifers noticed during the treatment and post-treatment periods, on statistical analysis, revealed that the differences were not due to the hormone treatment.

Heat symptoms were exhibited by the animals on the second day of the treatment. In some animals heat symptoms of transient nature were seen throughout the treatment period. Normal oestrus was seen in two cows and four heifers from 42-57 days after the commencement of lactation. Four animals were inseminated and all of them got conceived.

The body weight of the cows remained unchanged but slight decrease in body weight was observed in the heifers immediately after the treatment period.

It was observed that oestrogen, either in natural or synthetic form, together with progesterone was effective for inducing lactation in cattle. In the seven-days treatment method natural oestrogen was found to be more effective than synthetic oestrogen in the oestrogen-progesterone combination. Synthetic oestrogen and progesterone combination when given for a longer period produced a better response. Induction of lactation was more successful in the heifers than in the cows. The composition of milk from animals on induced lactation was not different from that of normal milk. The regaining of reproductive efficiency in some of the problem breeders was an added advantage of the technique.

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STUDIES ON
HORMONAL INDUCTION OF LACTATION IN COWS

BY

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ABSTRACT

A trial to induce lactation in cows and heifers using the hormones oestradiol dipropionate or stilboestrol dipropionate in combination with progesterone was conducted.

The experimental animals comprised of six each of heifers and cows divided into three groups of two each of cows and heifers. Natural oestrogen and progesterone were given to animals in the first group at the rate of 0.05 mg and 0.125 mg per kg body weight respectively at 12 hour intervals consecutively for seven days. The animals in the second group received synthetic oestrogen and progesterone at the same dose and for the same duration. Animals in the third group were given synthetic oestrogen and progesterone at the rate of 0.10 mg and 0.25 mg per kg body weight respectively, on alternate days, during a period of 14 days.

There was considerable udder development in all the animals. The development was more in the animals of group I and III in comparison to those of group II. The heifers exhibited pronounced udder development than the cows.

Lactation commenced within a period of 13-21 days after the first injection in all the animals. The milk yield reached the peak within a period of four to ten weeks. The peak yield varied from 140 ml to 4300 ml. The average milk yield per day in the three groups during the period of the

first 60 days was 1766, 942 and 1638 ml for groups I, II and III respectively. The yield obtained for group I and III was significantly higher than that for group II. The heifers gave better yield than the cows.

The composition and properties of the milk obtained from the induced lactation was compared with those of the milk from normal cows. It was found that on the eighth day onwards there was no significant difference in the composition and properties of milk obtained from the experimental animals as compared to those milk from postpartum cows. In general the milk from the animals of induced lactation had similar qualities to that of the milk from postpartum cows.

The erythrocyte count, haemoglobin concentration and packed cell volume decreased and the plasma protein increased during the treatment as well as the post-treatment periods in comparison to the pre-treatment period.

During the treatment period the animals exhibited symptoms of heat. Normal oestrus was observed in six animals within 42-57 days from the commencement of lactation. Four of them were inseminated and all of them conceived. The body weight remained unchanged in the cows but the heifers showed a slight decline in body weight.

Both natural and synthetic oestrogen along with progesterone were effective in inducing lactation. Natural oestrogen in combination with progesterone was more effective in short duration

treatment. When the synthetic oestrogen along with progesterone was given for a longer period better results were obtained than short duration. The induction was more successful in heifers than in cows. Regaining of the reproductive efficiency in some of the problem breeders was an added advantage of the technique adopted.