STUDY ON SOME PROTOCOLS FOR THE INDUCTION OF LACTATION IN UNPRODUCTIVE CATTLE

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CERTIFICATE

This is to certify that the thesis entitled ***"STUDY ON SOME PROTOCOLS FOR THE INDUCTION OF LACTATION IN UNPRODUCTIVE CATTLE"*** submitted by **Mr. DARSHAN, C. N., I.D. No. MVHK 1520** in partial fulfilment of the requirements for the award of degree of **MASTER OF VETERINARY SCIENCE** in **VETERINARY GYNAECOLOGY AND OBSTETRICS** of the Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar is a record of bonafide research work carried out by him during the period of his study in this University under my guidance and supervision and the thesis has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles.

Place: Bengaluru

Date: August, 2017

(Dr. G. SUDHA)
Major Advisor

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(Dr. G. P. KALMATH)

TO

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LIST OF ABBREVIATIONS

±	Plus or minus
<	Less than
>	More than
Inj	Injection
IM	Intra- muscular
TD	Total Dose
S/C	Subcutaneous q
Mg	Milligram
mg/dL	Milligram per deci litre
mg/kg	Milligram per kilogram
mL	Milli litre
ng/mL	Nanogram per milli litre
pg/mL	Picogram per milli litre
$PGF_{2\alpha}$	Prostaglandin F_2 alpha
SE	Standard Error
GH	Growth Hormone
HF	Holstein Friesian
Mm	Milli Meter
PTHrp	Parathyroid Hormone-related peptide
rbST	recombinant bovine Somatotropin
bST	bovine Somatotropin
^{o} C	Degree Celsius
RIA	Radioimmunoassay

Pvt.	Private
Ltd.	Limited
Rpm	Rotations per minute
BCS	Body Condition Scoring
TGF-α	Transforming Growth Factor-α
CBG	Corticosteroid Binding Globulin
PTH	Parathyroid hormone
Bw	Body weight
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
IGF – 1	Insulin-like growth factor 1

Introduction

I. INTRODUCTION

Milk, a complex mixture of various constituents, is secreted by the mammary glands after parturition to nourish the young ones. Since milk is a major animal product being broadly consumed by human communities irrespective of age and religion, there is an extreme need to enhance the production limits in order to satisfy the ever growing demand for milk and its by-products by the consumers.

Though India stands first in world milk production, the individual production potential does not correspond to the total livestock population. The main reasons can be quoted as reproductive failure or repeat breeding and other disease conditions of udder. Until now the solution to this problem has been culling or slaughtering of the unproductive animals. Now, since there is a ban on cow slaughter for socio-religious reasons by Government of Karnataka, under Karnataka Prevention of Cow Slaughter and Preservation Act, 1964, the aged unproductive cows become economic burden to the farmers, therefore it is necessary to find an alternate way to reduce the economic burden of heavy maintenance costs on farmers. Hence it is proposed to induce lactation in dry or unproductive animals.

There are evidences that indicate the significant influence of endocrine hormones on the growth and development of mammary gland (Tucker, 2000). In animals suffering from any reproductive disorder, the udder development is not prominent due to preponderant negative balance of sex hormones concentration. In such conditions, exogenous administration of steroid hormones stimulated lactation and also restore the fertility of the animal (Verma *et al*., 2004).

Mammary duct growth is stimulated by estrogens, while estrogen and progesterone in combination stimulate lobule-alveolar development of the mammary gland (Tucker, 2000). Low average plasma concentrations of estrogen and progesterone before treatment were found in successfully induced cows (Erb *et al*., 1976).

Many techniques over the past 70 years have utilized the ovarian hormones estrogen and progesterone, alone or in combination, to develop the mammary gland and initiate lactation (Collier *et al.*, 1976; Chakriyarat *et al.*, 1978; Peel *et al.*, 1978).

Early attempts to induce lactation used 120 to 180 days' injection of estrogen and progesterone (Turner *et al.,* 1956). These long-term treatments generally resulted in low milk yields and low rates of success. Smith and Schanbacher (1973) developed a seven-day estrogen and progesterone protocol and successfully induced lactation in 60 per cent of treated animals. Although the new protocol was successful in 60 per cent of treated animals, there was still substantial variability in milk yields between lactation induced cows. Since the development of shorter protocol, many modifications have been employed to improve success rates and reduce the variability in milk yields.

The addition of reserpine, a tranquilizer that increases blood prolactin levels for several hours and dexamethasone, a synthetic glucocorticoid, to induction protocols have increased success rates, but have not reduced the variation in milk yields recorded from induced cows (Collier *et al.,* 1977; Chakriyarat *et al.,* 1978; Peel *et al.,* 1978). Receptivity to reserpine and dexamethasone administration following the initial estrogen and progesterone protocol can further be increased by administering $PGF_{2\alpha}$ which removes the corpus luteum, the progesterone source.

The use of $PGF_{2\alpha}$ for synchronization before induction is employed in order to improve the success rate, increase in milk yields and eliminate the variability in milk yields between cows. Keeping these facts in view, the present study was designed with following objectives:

1. To induce lactation in unproductive dairy cattle by using two different protocols.
2. To study the estrogen and progesterone concentrations in lactation induced animals.

Review of Literature

II. REVIEW OF LITERATURE

2.1 The anatomy of the mammary gland

The mammary distinguishes the mammalian class from the other taxonomic classes in the ranking system. It is involved in synthesis and secretion of milk. The udder/mammary gland consist of teats, ducts, alveoli that contain the secretory cells, and supporting tissue (Akers, 2002). The udder of cows is split into two halves with each containing two mammary glands and a teat. The median suspensory ligament helps in the attachment of udder to the body wall.

The gland cistern located at the base of the teat continues as the teat cistern. Further, it leads to the teat opening which is also known as a streak canal. The milk drains from the secretory tissue into the gland cistern from the primary mammary ducts (Akers, 2002). Mammary fat pad (adipose tissue), stroma (connective tissue) and parenchyma (secretory tissue) are the three basic supporting tissues in the mammary gland. The duct system of mammary gland grows within the mammary fat pad (Neville *et al.*, 1998). The stroma surrounds the alveoli and provides structural support and anchorage to the mammary gland (Akers, 2002). The parenchyma contains a duct system and lobes. The lobes are made up of several lobules, which include groups of alveoli and their surrounding ducts. The alveoli are the functional unit of the mammary gland that synthesize and secrete milk (Akers, 2002).

2.1.1 Fetal Development

The mammary gland development is a continuous process that occurs in five main stages namely, fetal, pre-pubertal and post-pubertal, gestational, and lactational development (Akers, 2002; Robinson, 2007). The fetal stage of mammary development takes place around day 30 of bovine embryo during which the mammary band, the thickening of the ectoderms is formed (Akers, 2002). Later the mammary band develops into the mammary streak that becomes the mammary line by the fifth week. Mammary crest forms from the mammary line due to the repeated proliferation of the ectoderm cells and mesenchymal cells and transformed into the mammary bud at day 43. The formation of these buds marks stage in which different developmental pattern between the species and the sexes can occur. Bovine have four mammary buds with each corresponding with the four parts of the udder (Akers, 2002). The mammary bud leads to development of teat and gland cistern through formation of primary sprout which form the secondary sprout and later as major ducts (Robinson, 2007). The streak canal is formed by an invagination of the tip of the teat (Akers, 2002). Many secondary sprouts develop from the mammary pit and as the canalization continues, the lumen of teat, the gland cistern, teat cistern, and streak canal are developed (Akers, 2002).

2.1.2 Pre-pubertal and post-pubertal mammary gland development

The development of the mammary gland that occurs between birth of a female calf and her capability to conceive is associated with the increasing duct system, adipose tissue and connective tissue in development (Akers, 2002). The post-natal development of the mammary gland is split into pre-pubertal and post-pubertal development. Before attainment of puberty, growth of the mammary gland occurs isometrically, but about 3 months before puberty, the growth of the mammary gland becomes allometric due to the stimulation by the ovarian activity that occurs before and during puberty (Akers, 2002; Hovey *et al*., 2002).

Isometric growth of mammary gland occurs again following the first few estrous cycles (Akers, 2002). The estrogen secreted by the ovary during each estrous cycle stimulates the development of the mammary gland duct system making it more intricate with each subsequent estrous cycle (Tyler and Ensminger, 2006). Along with estrogen, growth hormone (GH) also stimulates mammary development. During this developmental time, even though the duct system becomes more complex, the growth and development of the lobulo-alveolar system is minimal (Hovey *et al*., 2002).

2.1.3 Development of mammary gland during Pregnancy

Majority of the development of the mammary gland occurs during pregnancy (Akers, 2002). Significant development of the duct system is observed during the first three to four months of gestation (Tyler and Ensminger, 2006) whereas the lobulo-alveolar system development starts to after five months with extensive alveolar hyperplasia and hypertrophy. High concentrations of mammogenic hormones, such as GH, estrogen, and progesterone stimulate maturation of alveoli (Topper and Freeman, 1980). Both estrogen and progesterone are maintained at very high levels throughout pregnancy and are reported to be essential for final duct growth (Akers, 2002). In addition, the reproductive hormones estrogen, progesterone and prolactin have vital role for lobulo-alveolar development (Brisken, 2002). By seventh month of gestation the mammary gland develops completely to be capable of producing milk (Tyler and Ensminger, 2006).

2.1.4 Development of mammary gland during lactation

The lactation cycle, in the adult animals, can be divided into mammogenesis, lactogenesis, galactopoiesis and involution. These stages are regulated by a strict hormonal control and can be grouped under three categories (Neville *et al.*, 2002). Firstly, the reproductive hormones such as estrogen, progesterone, placental lactogen, prolactin and oxytocin act directly on the mammary gland and bring about the growth and development of mammary tissue. Secondly, metabolic hormones which includes, GH, corticosteroids, thyroid hormones, insulin and GI hormones have direct effects on the mammary gland in early life and also during pregnancy. Finally, locally secreted hormones like GH, prolactin, parathyroid hormone-related peptide (PTHrp) and leptin have role in the development of mammary gland. Prolactin is reported to have role in growth and differentiation of the alveoli and ductal structures. PTHrp is expressed in the mammary epithelial cells during lactation and its secretion may be related to the extracellular calcium concentration. This peptide is important for the transport of calcium from blood to milk. Recently, concentration of leptin, which is synthesized in the adipose tissue and mammary gland, is reported to be increased during pregnancy (VanHouten *et al.*, 2004).

Lactogenesis is the onset of milk secretion and includes all the changes in the mammary epithelium necessary for transformation of undifferentiated mammary gland of early pregnancy to full lactation after parturition. The cytoskeletal modification of mammary cells from a non-secretory to a secretory state consists of a cascade of events. The process consists of cytologic and enzymatic differentiation of alveolar cells which coincides with limited milk secretion before parturition, whereas the other stage consists of copious secretion of milk that begins within four days prior to parturition and that may extend for next few days following parturition (Tucker, 1981).

Galactopoiesis or lactation is the maintenance of milk production, is regulated by hormones like prolactin and GH during lactogenesis and galactopoiesis. Both prolactin and GH act synergistically to potentiate the proliferative mammary gland to a lactating tissue. However, GH supersedes prolactin during galactopoiesis in cows. Prolactin acts either directly and/or through mammary epithelial factors and it also activates several transcription factors which is a major signalling molecule being STAT5a (Lamote *et al*., 2004).

2.1.5 Mammary gland involution

Involution of the mammary gland takes place with ceasing of milk removal from the gland which is characterized by apoptosis of the alveolar epithelial cells and proteolytic degradation (Accorsi *et al.,* 2002). The decrease in prolactin, GH and IGF-I are correlated with apoptosis caused by DNA fragmentation (Accorsi *et al*., 2002). Tissue proteinases restructure the mammary gland to prepare for next reproductive cycle. The tissue closer to the teat gets degenerated more than the farther tissue (Akers, 2002). The alveolar tissues are eventually regenerated to prepare for the next gestation and lactation (Tyler and Ensminger, 2006).

2.2 Hormones and mammary function in lactation induction

Blaxter *et al*. (1949) found that reduced secretion of milk in experimentally reduced thyroid hormone secretion but the administration of thyroid hormones or iodinated casein did not increase production of milk as galactopoietic agents as their effects were transient. Lyons (1958) reported that prolactin and growth hormone are mammogenic. The steroid hormones were reported to require prolactin and/or growth hormone for mammogenic activity.

Estrogen stimulates mammary duct growth whereas both estrogen and progesterone synergistically stimulates lobule-alveolar development of the mammary gland (Cowie and Folley, 1961). Exogenous prolactin and growth hormone were observed to stimulate milk synthesis in cows (Benson, *et al*., 1958; Cowie, *et al*., 1980).

2.2.1 Estrogen

Rivera (1964) reported that *in vitro* studies using estrogen along with prolactin and growth hormone stimulated mammary growth especially when the medium was supplemented with five per cent serum. The estrogen *in vivo* was noticed to cause secretion of growth factors from the pituitary, kidney and mammary tumor cells (Sirbasku, 1978). It was hypothesized that growth factors secreted from extra mammary tissues into serum may act through an endocrine mechanism to mediate the mammogenic effects of estrogen. Further, the growth factors secreted locally from mammary tissue also may mediate estrogenic effects on mammogenesis through paracrine/autocrine mechanism (Dembinski and Shiu., 1987).

Specific estrogen receptors in mammary tissue contributed for the estrogen regulation of mammary growth (Puca *et al*., 1969). Estrogen initiates lactation either by causing release of prolactin from the anterior pituitary gland into blood which in turn initiates lactation (Nagasawa *et al*., 1969) or by increasing the number of prolactin receptors in mammary cells (Sheth *et al*., 1978), which is another lactogenic event. Estrogen is observed to initiate lactation in the periparturient period and it is the first hormone to increase in blood during periparturient period (Smith *et al.*, 1973). Woodward *et al*. (1993) reported that either adipocytes or fibroblasts of the mammary stroma did not proliferate in response to estrogen in cattle. Though Yang *et al*. (1980) observed the failure of estrogen alone to stimulate growth *in vitro* a study reported by McGrath (1983) proved that estrogen stimulated epithelial cell growth in mixed cultures of epithelial and stromal cells which was supported by the findings of Silberstein *et al*. (1982) and Hovey *et al*. (1999) in heifers. The estrogens interact with growth factors in serum and stimulate secretion of factor(s) from stromal cells of the mammary gland to cause growth of mammary epithelial cells (Dembinski *and* Shiu, 1987; Imagawa *et al*., 1994).

2.2.2 Growth Factors

The stromal cells of the mammary gland secrete IGF-I major growth factor with its role in mammogenesis along with other growth factors such as epidermal growth factor, fibroblast growth factors, transforming growth factor-α (TGF-α), hepatocyte growth factor, and macrophage colony stimulating factor (Campbell *et al*., 1991; Hovey *et al*., 1999; Imagawa *et al*., 1994). In addition, combination of other growth factors and binding proteins has also been identified in mammogenesis as the receptors for several growth factors have been detected in mammary tissue of cows (Forsyth, 1996).

2.2.3 Progesterone

Bresciani (1968) observed that progesterone induces DNA synthesis at the end buds along the walls of the mammary ducts at progesterone receptors sites (Haslam and Shyamala, 1979(a)), while Haslam and Shyamala (1979(b)) and Sud *et al.* (1968) observed that estrogen increased the number of progesterone receptors. The observation was fortified by the exogenous administration of progesterone which acted synergistically with estrogen to induce lobulo-alveolar growth (Randel and Erb, 1971) during pregnancy in cattle. A rapid decline in secretion of progesterone in the periparturient period in cattle is reported to initiate secretion of copious quantities of milk (Smith *et al*., 1973) which provides evidence for the vital role of progesterone that suppress lactogenesis.

Djiane and Durand (1977) reported that the progesterone suppresses the ability of prolactin to increase the number of prolactin receptors in the mammary glands and also blocks glucocorticoid receptors in mammary tissue that would suppress the lactogenic activity of glucocorticoids (Collier and Tucker, 1978). Smith and Schanbacher (1973) showed that administration of a combination of estradiol-17β and progesterone for seven days induced lactation in about 70 per cent of treated sterile cows with 70 per cent of normal milk production. However, progesterone inhibits initiation of lactation, once lactation gets established as its administration had no effect on milk yield (Herrenkohl, 1972).

2.2.4 Prolactin

Prolactin is known to initiate copious secretion of milk and markedly increase the development of the lobulo-alveolar system of the mammary glands. However, the prolonged elevation of serum prolactin concentrations was not recorded during normal pregnancy in cattle though a large portion of mammary growth takes place (Amenomori *et al*., 1970; Oxender *et al*., 1972). Although estrogen and progesterone fails to stimulate mammogenesis in the absence of prolactin and/or growth hormone, prolactin signaling was reported to be essential for mammogenesis and differentiation of the mammary gland during pregnancy (Topper *et al*., 1980). The prolactin through systemic effects stimulates the ductal side branching and terminal end bud regression, while it directly increases the lobulo-alveolar development at the mammary epithelium during pregnancy (Brisken *et al.*, 1999).

Prolactin is critically important for initiation of lactation in the periparturient period in cattle. An *in vitro* study by Juergens *et al.* (1965) proved that prolactin in combination with insulin and cortisol is required to induce secretion of milk proteins. Prolactin binding to its receptor was noted to initiate the lactogenic response for a cascade of events that initiate to stimulate the transcription of genes to regulate secretion of milk proteins. Further, STAT5 transcription factors seem to mediate the signal from the prolactin receptor to the genes involved in phosphorylation of protein kinases. In cattle prolactin secretion does not limit the secretion of milk as prolactin concentration in blood of cows is slightly correlated with milk yield (Koprowski and Tucker, 1973).

2.2.5 Growth Hormone (GH)

Oxender *et al*. (1972) observed a little change in the concentrations of serum growth hormone for the growth of mammary gland during normal gestation. In cattle, though a parturition-induced surge of growth hormone occurs (Ingalls *et al*., 1973), but the surge of GH during the time of delivery of the calf and the first stage of lactogenesis could not be correlated. However, Keys and Djiane (1988) observed that growth hormone failed to bind to receptors in the mammary gland of cattle even though mRNA for its receptors was expressed in this tissue (Glimm *et al.*, 1990).

Bauman (1991) proposed that the role of growth hormone in coordinating the partitioning of nutrients toward the mammary gland during lactation. More nutrients are made available to the mammary gland during early lactation wherein the adipocytes become less sensitive to insulin and GH receptors are probably involved in mediation of this action (Bauman and Vernon, 1993). Growth hormone induces the secretion of IGF-I, from either the liver or cells in the mammary stroma, and IGF-I may mediate the mammogenic action of growth hormone via endocrine, paracrine or autocrine mechanisms (Holly and Wass, 1989; Forsyth, 1996).

It has been established that the individual effects of either growth hormone or prolactin could only be observed in the absence of the other (Flint, 1995). Radcliff *et al*. (1997) demonstrated that GH administration induced a marked increase in mammary parenchymal mass and total mammary cell numbers normal heifers.

2.2.6 Placental lactogen

Placental lactogen, a peptide hormone synthesized in and secreted from the placenta of many species including humans (Josimovich and MacLaren, 1962). The concentration of placental lactogen was reported to be very low in maternal serum as compared to that in the fetus (Wallace, 1993). Placental lactogen is reported to stimulate mammogenesis by binding primarily to the prolactin receptor in rodents (Forsyth, 1994). Byatt *et al. (*1992) found that the administration of placental lactogen had little effect on metabolism of lactating cows and thus, its role on normal mammary physiology of cattle is not clear.

2.2.7 Glucocorticoids

Cortisol, a predominant endogenous glucocorticoid of cattle plays a vital role in differentiation of the lobulo-alveolar system of mammary gland. Glucocorticoid-induced differentiation is essential to allow prolactin to induce subsequent synthesis of milk proteins. Following the injections of glucocorticoids into non-lactating cows with well-developed lobulo-alveolar systems induced onset of lactation (Tucker and Meites, 1965), although the quantity of milk subsequently produced was greater when prolactin secretion was also increased (Collier *et al.,* 1977). This supported the evidence for synergy existed among the hormones required for lactogenesis. Glucocorticoid concentrations in blood remained low for the greater part of gestation until just prior to parturition and its peak coincided with delivery of the offspring (Edgerton and Hafs, 1973). Binding of serum glucocorticoids to corticosteroid binding globulin (CBG) protein, they get inactivated (Gala and Westphal, 1965). CBG decreases during the periparturient period and free glucocorticoids markedly increase. Gorewit and Tucker (1976) determined that glucocorticoids bind to specific receptors in mammary tissue which noncoordinately regulated secretion of α-lactalbumin and β - casein (Ray *et al.*, 1986).

Therapeutic doses of synthetic glucocorticoids suppressed milk yields (Braun *et al.*, 1970). However, glucocorticoids were found to be strongly galactopoietics in rats (Thatcher and Tucker, 1970). Kehrli *et al.* (1999) suggested that elevated secretion of glucocorticoids in the periparturient period is associated with the immune suppression contributing for the increased incidence of mastitis and other diseases in early lactation. Administration of adrenocorticotropic hormone increased the secretion of glucocorticoids and reduced secretion of milk in cattle.

2.2.8 Thyroid Hormones

The mammary gland is in a euthyroid state during lactation and the rest of the body is hypothyroid which are required to enhance the metabolic priority of the mammary gland. Large quantities of iodine were lost in milk which might be contributed to the overall hypothyroid condition of the lactating animal. The secretion rate of thyroxine was suppressed as milk yield increased during the lactation (Lorscheider and Reineke, 1971). However, it is established that thyroxine in blood had little biological activity and regarded as a prohormone that undergoes enzymatic 5′deiodination within the thyroid and peripheral tissues to become biologically active hormone, triiodothyronine. During lactation there was decreased conversion of thyroxine to triiodothyronine in liver and kidneys and increased conversion in the mammary gland (Kahl *et al.*, 1991; Jack *et al.*, 1994).

2.2.9 Insulin

Koprowski and Tucker (1973) reported that insulin, a polypeptide hormone was involved in mechanisms partitioning nutrients away from synthesis of milk and toward body tissues in cattle which binds to the IGF-I receptor (Kasuga *et al.,* 1981) and mimic the mammogenic effects of IGF-I. However, the mammary uptake of glucose, acetate, *β*-hydroxybutyrate, triglycerides and amino acids were reported to be independent of insulin in cattle (Laarveld *et al.,* 1985). Griinari *et al.* (1997) demonstrated that sustained increase in insulin concentrations in blood along with glucose concentrations significantly increases the protein concentrations in milk. This suggested the possibility to manipulate hormones, diet and genetics for milk production with a desired composition.

2.3 Hormonal induction of lactation

Artificial induction of lactation in animals has been a highly researched field since more than eight decades. On perusal of literature, the reported results of such experiments by various scientists were yet to be concurred due to the variability. Hence, even to this day the importance to this topic has not faded (Kensinger, 2000).

Administration of exogenous hormones to mimic mammary gland development during pregnancy has led to research to understand how the hormones work synergistically to promote lactogenesis. During the attempts to mimic mammary development with varied dosages of exogenous hormones, it was observed that induced-lactations actually occurred in treated animals, and these results have led to the use of exogenous hormones to induce lactations in non-pregnant cattle.

Techniques to reduce culling rates and replacement costs through the use of hormonal induction of lactation in dairy cattle have been attempted with varied success for past eight decades (Turner *et al.*, 1956; Smith and Schanbacher 1973). Development of a reliable cost efficient protocol to artificially induce lactation would allow dairy farmers to reduce culling losses of infertile cows and lower replacement costs by retaining such animals would make the farmer sustainable.

As role of hormones on mammary gland function was established through *in vivo* and *in vitro* studies, the use of hormones for induced lactation is the most tested and proved biotechnological technique. Various hormones and their analogues with different dosages have been administered exogenously for induction of lactation in barren cattle.

Vital role hormones in the development and function of the mammary gland were studied through *in vivo* and *in vitro* (Williams and Turner, 1960; Tucker, 2000). The studies revealed the role of estrogen in growth of mammary duct and in combination with progesterone it stimulates lobulo-alveolar development of mammary gland in dairy cows (Tucker, 2000). Estrogen is also involved in initiating lactogenesis in cattle at parturition. It initiates lactation in two ways namely, it causes release of prolactin from the anterior pituitary gland into blood (Nagasawa, 1969; Tucker, 2000) and it increases the number of prolactin receptors in mammary cells (Sheth, 1978; Tucker, 2000).

Although combinations of exogenous progesterone and estrogen work synergistically to stimulate lobulo-alveolar growth, it is the high levels of progesterone during pregnancy that helps to regulate lobular-alveolar growth and block lactogenesis. Progesterone blocks lactogenesis by binding the glucocorticoid receptors in mammary tissues which would suppress the lactogenic activity of glucocorticoids (Tucker, 2000). The exact mechanism of lactogenesis block by the progesterone is unclear, but removal of the progesterone block by luteolysis and a decline in progesterone as parturition approaches which allow the onset of lactogenesis would explain the mechanism (Fulkerson, 1979; Tucker, 2000).

The ovarian hormones, estrogen and progesterone, are not the only hormones required for lactogenesis, as prolactin is also needed. Blood prolactin levels in cattle surge several hours prior to parturition, (Ingalls *et al*., 1973; Tucker, 2000) and this surge in prolactin is apparently necessary for full lactogenesis. The surge in prolactin can be blocked with the use of bromocriptine, reducing milk yield, but this effect can be reversed with administration of prolactin (Akers *et al.,* 1981).

Glucocorticoids, cortisol being the major glucocorticoid in cattle, also play a role in mammary gland development leading to alveolar cell differentiation of the gland. Glucocorticoids compete with progesterone for mammary epithelial cell binding sites. Administering glucocorticoids to non-lactating cows with a developed mammary gland aids in the induction of lactation because the increase in glucocorticoids displaces progesterone from mammary cell receptors, thus reducing the progesterone block to prolactin receptor synthesis (Tucker, 1965; Fulkerson and McDowell, 1975).

Milk yields from these induced-lactations can be enhanced with the addition of prolactin offering additional evidence that the hormones (estrogen, progesterone, prolactin, and glucocorticoids) work synergistically in the onset of lactogenesis (Tucker, 2000; Akers, 2002). Cortisol has a general effect on metabolism in the lactating animal, but it is also required to maintain the secretory activity of the epithelial cells. However, cortisol has no identified role in milk synthesis (Sjaastad *et al.,* 2003).

2.4.1 Lactation induction protocols

The scientists have used various doses and duration of hormones to induce lactogenesis (Fulkerson and McDowell, 1975; Erb, 1976; Peel *et al*., 1978). They started from the months of hormone therapy to modern therapy, which is for a very short period that is reduced to a few days of therapy. Several protocols were designed with several combinations of drugs according to the need of animal.

2.4.1.1 Long term hormone treatment for inducing lactation

In earlier studies, the induction of lactation by long term (3 to 6 months) hormonal treatment either single or in combination was attempted by several workers (Reece, 1943; Narayan and Bhalerao, 1947; Yamamoto and Turner, 1955) with varied and inconsistent results.

Reece (1943) reported that development of udder and teat with subsequent milk yield of 11.5 liters per day in heifers following subcutaneous injection of five mg of diethyl stilbesterol dipropionate and five mg of testosterone propionate subcutaneously twice weekly for ***6*** weeks. Subcutaneous injection of 20 mg of stilbesterol per animal for

90 days which yielded 3.9 to 15.1 litres /cow/day (Narayan and Bhalerao, 1947). By implanting 10 mg to 2 g DES along with progesterone up to 4 mg resulted in enhanced mammary gland growth (Meites, 1950).

Turner *et al.* (1956) subcutaneously administered a mixture of 100 mg of progesterone and 100 mg of estradiol benzoate for a period of 180 days for induction of lobulo-alveolar growth in a group of heifers. Further, they administered 3 mg of estradiol benzoate daily for 14 days to induce lactation and reported lower milk yield and success rate.

Steroid hormone therapies for long term were tested on the udder development in order to stimulate the plasma hormone level during the pre-partum period. Williams *et al.* (1955) initially injected estrogen and progesterone daily for five months followed by estradiol injection for 10 days. Similarly, Yamamoto and Turner (1955) injected 100 mg of estrogen and progesterone daily for 6 months. But, the success rate was very less in both the experiments.

In contrary, Sud (1972) reported that ovariectomized heifers produced more milk than the intact heifer by the administration of hormones, estrogen and progesterone (1: 2.5) for 20 weeks. However, hormones at different doses given for longer duration up to 30 weeks resulted in very low milk yield (Garg and Nangia, 1972).

2.4.1.2 Short term hormone treatment for inducing lactation

Smith and Schanbacher (1973) designed a relatively high dosage based hormone treatment which was reported to mimick the high levels of estrogen and progesterone during the last trimester of pregnancy. They injected a combination of the hormones, estradiol - 17 β (0.1 mg/kg bw/day) and progesterone (0.25 mg/kg bw/day) for 7 days to induce lactation in non-breeding and non-lactating dairy cattle. The lactation was initiated in 60 per cent (6 out of 10) of treated animals after the first series of injections with this protocol.

The postponement in onset of lactogenesis by 10 days when additional oestradiol-17 β and progesterone was administered from day 16 to day 22and from day 8 to day 21 reflected the inhibitory role of high plasma progesterone on lactogenesis (Kuhn 1969; Hartmann *et al.*, 1973).

Erb (1976) and Peel *et al*. (1978) demonstrated that milk yields did not improve from the 7 days treatment even after following the same treatment up to 11days or 12 days. Chakriyarat *et al.* (1978) reported that the injections of estrogen alone or in combination with progesterone were reported to be important in mimicking pregnancy in cattle although a long term steroid stimulation was also necessary for the complete mammary cell differentiation and development.

Short period of insertion of intravaginal sponge of estrogen and progesterone for 10 days was also found to positively induce lactation in dry cattle (Davis *et al.*, 1983).

2.4.1.3 Short term hormone treatment with combination of other drugs for inducing lactation

Although Smith and Schanbacher (1973) reported that the successful induction of lactation in a majority of animals with the shorter protocol but they also experienced 40 per cent failure. This failure rate left opportunities for other scientists to examine protocols to improve success rate of artificial induction of lactation in non-pregnant and non-lactating dairy cows.

To improve success rate of artificial induction of lactation in non-pregnant and non-lactating dairy cows the researchers attempted either an extension of the duration of hormonal treatment (Erb *et al.,* 1976a; Erb *et al.,* 1976; Peel *et al.,* 1978) or doubled the daily dose of steroids used (DeLouis *et al.,* 1978). Nonetheless, the variation in milk yields was left unexplained. Several researchers included use of either exogenous dexamethasone (Collier *et al.,* 1975; Collier *et al.*, 1976; Chakriyarat *et al.*, 1978) and/or reserpine (Collier *et al.,* 1977; Peel *et al*., 1978; Lembowicz *et al.*, 1978) in the induction regimes as a strategy to improve the success rate of induced lactation.

Exogenous reserpine can elicit an increase in blood prolactin concentration that lasts for several hours in cattle (Bauman *et al.*, 1977), thus imitating the dramatic increase in prolactin seen several days prepartum in pregnant cows (Convey, 1974). It is also well documented that plasma glucocorticoids increase dramatically at the time of parturition in ruminant species (Convey, 1974; Heald, 1974; Collier *et al.*, 1975) and administration of the synthetic glucocorticoid, dexamethasone, can mimic increased glucocorticoid levels (Collier *et al.*, 1975).

Lembowicz *et al.* (1978) were able to successfully reduce the 7 day estrogen and progesterone protocol to 5.5 day, or even 3.5 day, in multiparous Polish Black and White cows, so that its application would be more practical in commercial herds. The doses of 17-β estradiol and progesterone, as outlined by Smith and Schanbacher (1973), were used in addition to eight single injections of reserpine between days 9 to 16 at a dose of 22.5 mg/cow/day. The lower milk yields resulting from this modified procedure were not significantly different from those of cows induced into lactation with the 7 day treatment, but the advantages were its simplicity and reduction of estrus-like excitement (mounting) that would be beneficial to producers (Lembowicz *et al.,* 1978).

Estrogen and progesterone in plasma increases during day 2 to 7 of steroid treatment (Head *et al.,* 1982) and decreased by day 16 to 19 as prolactin increased. Injection of thyrotrophic releasing hormone increased prolactin release after 10 days of steroid treatment.

2.4.1.3.1 Glucocorticoids in induction of lactation

Glucocorticoids, cortisol being the major glucocorticoid in cattle, also play a role in mammary gland development leading to alveolar cell differentiation of the gland. Glucocorticoids compete with progesterone for mammary epithelial cell binding sites.

Increase in the circulating glucocorticoids at parturition is a necessary factor in initiating lactogenesis (Collier *et al.,* 1975), thus leading researchers to examine the addition of exogenous synthetic glucocorticoid, dexamethasone, to induction protocols. Collier *et al*. (1975) modified the 7 day treatment designed by Smith and Schanbacher (1973), by injecting dexamethasone thrice (20 mg/cow/day) to 6 heifers and 10 cows on day 18, 19, and 20 of the induction protocol and resulted in a 69 per cent success (success more than 9 kg milk/day at peak yield). Further, they found that dexamethasone administration did not substantially improve the success rate. However, the same protocol followed by Ball *et al*. (2000) in Holstein heifers notably increased the success rate and they also put forward that the fertility and subsequent lactation were not affected by the induction procedure.

Chakriyarat *et al*. (1978), using 19 dairy cows of varied breed and age, examined the addition of 3 single injections of dexamethasone (0.028 mg/kg bw/day) on day 18, 19, and 20 of the 7 day estrogen - progesterone induction protocol. They reported that addition of dexamethasone injections increased the success rate of 82 per cent (9 of 11 cows) as compared to that of induced lactation without dexamethasone (3 of 11 cows) in which they recorded 27 per cent success.

In a similar study carried out by Fleming *et al.* (1986), where they administered 17-β estradiol (0.10 mg/kg bw/day) and progesterone (0.25 mg/kg bw/day) for 21 day, along with 3 single injections of dexamethasone (0.028 mg/kg bw/day) on days 31 to 34, in 11 Holstein cows and 9 Guernsey cows for lactation induction. They reported improvement in success rate (success more than 5 kg milk/day) but the milk yields did not increase as compared with that of cow's not receiving dexamethasone injections.

Administering glucocorticoids to non-lactating cows with a developed mammary gland aids in the induction of lactation because the increase in glucocorticoids displaces progesterone from mammary cell receptors, thus reducing the progesterone block to prolactin receptor synthesis (Tucker and Meitis, 1965; Fulkerson and McDowell, 1975). Milk yields from these induced-lactations can be enhanced with the addition of prolactin, offering additional evidence that the hormones (estrogen, progesterone, prolactin, and glucocorticoids) work synergistically in the onset of lactogenesis (Tucker, 2000; Akers, 2002).

A preliminary experiment in maiden heifers was conducted using a series of injections of oestrogen plus progesterone then a synthetic glucocorticoid treatment gave encouraging results (Fulkerson and McDowell, 1975).

Injections of dexamethasone trimethyl acetate initiated lactation in nulliparous Ayrshire heifers that were previously given a series of injections of estradiol benzoate plus progesterone to develop mammary glands. Essentially normal lactation occurred following injection of 20 mg/day dexamethasone for 3 days, whereas injection of 40 mg/day for 4 days initiated secretion of smaller volumes of milk-like fluid containing relatively high levels of lipid.

Most milk was produced by animals injected with 10 mg estrogen and 200 mg progesterone every 3 days over 28 days to prime their mammary glands and then treated with dexamethasone (either alone or in combination with cloprostenol). When a lower dose of estrogen was used to develop mammary glands, the same hormones were the most effective at initiating lactation (Sawyer *et al.*, 1986).

Recently, Macrina *et al.* (2014) studied the effect of dexamethasone on the stage of lactation and age at lactation induction. They reported that dexamethasone though increased the milk yield initially, did not persist throughout the milking period. Although both the groups in their study similar dexamethasone treatment, the milk yield was recorded to be more in the 18 months old aged heifers than the 14 months old which was reasonably attributed to greater mammary epithelial mass of older heifers.

2.4.1.3.2 Prolactin analogues in induction of lactation

Since most of the methods employed 7 days treatment with a combination of estrogen and progesterone using similar or identical doses of the steroids, the reason for the variation in milk yield was considered to lie somewhere else. In pregnant cows, prolactin levels always increase markedly during the last few days before parturition. However, the autumn calving cows have higher mid-pregnancy levels of prolactin than spring calving due to usual seasonal variation maintained in pregnancy (Cowie *et al.*, 1980).

Koprowski and Tucker (1973) indicated very low correlations between serum prolactin and milk yield in cows especially in early lactation. Their findings were similar with the results of Peel *et al.* (1978) which suggested that high plasma prolactin was not a prerequisite for induction of lactation in the cow and that prolactin was not noted to have any effect on milk yield. However, Smith and Wagner (1980) reported the essence of prolactin for both the onset and maintenance of lactation, by administration of a dopamine receptor agonist during lactation which suppresses the prolactin secretion. In cows, bromocriptine treatment from 12 days prepartum until 10 days postpartum reduced rather than prevented milk secretion, had no effect on mammary DNA content but did reduce tissue differentiation (Akers *et al.*, 1981) and these changes were retrogressed by treatment with prolactin. This reinforces the view that prolactin is lactogenic rather than mammogenic. As prolactin plays an essential role in lactogenesis, many studies indicated the use of prolactin in combination with progesterone and estrogen to combat the milk yield variation (Tucker, 2000).

Frequent milking up-regulates mammary prolactin receptors (Bauman *et al.*, 1999) thereby enhancing the lactogenesis. Flint *et al.* (1995) in their experiment to achieve extended lactation length used the combination of GH and prolactin as they possess anti-apoptotic property acting through stimulation of the cell survival factor IGF-1 and inhibition of one of its binding proteins, IGF-BP5, respectively.

2.4.1.3.2.1 Role of reserpine in induced lactation

It was hypothesized that prolactin may be a limiting component of the lactogenic complex, in cows that fail to lactate following the 7-day estrogen and progesterone treatment (Collier *et al.*, 1977). Collier *et al.* (1977) modified the 7-day estrogen/ progesterone protocol with the goal of improving success rates and decreasing the variation in milk yields among animals, by incorporating the addition of reserpine injections. Reserpine injections were administered to non-pregnant cows either on day 13, 14, 15, and 16, or day 8, 10, 12, and 14 of the experiment. Days 13, 14, 15, and 16 were chosen so that prolactin levels in induced cows would mimic the increase in prolactin that occurs in pregnant cows during the period immediately prior to parturition (Convey, 1974; Collier, 1977). Reserpine was administered on day 8, 10, 11, and 12 based on results of an earlier study that indicated that mammary tissue from induced undergoes cellular changes associated with lactogenesis by day 8 and continuing through day 16 of treatment (Collier *et al.*, 1976; Croom *et al.*, 1976; Collier *et al.*, 1977).The studies concluded that use of reserpine to cause prolactin release reduced variation in milk yields between animals and increased the success rate (success more than 9 kg milk/day at peak yield) from 40 to 100 per cent in cows administered reserpine on day 13 to 16, and from 75 to 100 per cent in cows receiving reserpine injections on day 8, 10, 12, and 14 (Collier *et al.*, 1977).

The administration of reserpine to non-lactating dairy cattle produced a marked increase in circulating serum prolactin levels. The intramuscular administration of reserpine to non-lactating cows which have been hormonally induced into lactation, by the intramuscular administration of estrogen and progesterone, and dexamethasone, resulted in a substantial rise in milk production (Bauman *et al.,* 1977). These studies showed that cows treated with the standard oestrogen-progesterone treatment plus reserpine had successfully induced lactations. Further, Dabas and Sud (1989) concluded the fact that reserpine, in combination with estrogen and progesterone in a 7-day treatment procedure was able to successfully re-induce the lactation in cattle.

However, Peel *et al*., (1978, 1979), utilizing dairy cows of mixed breed, demonstrated that administering reserpine (5 mg/day) on day 1, 6, 11, 16, and 21, in addition to the 7-day protocol described by Smith and Schanbacher (1973), did not increase milk yields, but increased the proportion of cows responding to lactation induction treatment when compared with controls receiving estradiol - 17 β and progesterone injections alone. Further, Peel *et al.* (1978) demonstrated that though reserpine elevates the plasma prolactin that reduced the variation in milk yields and increases the success rate in cows, lactation may also be hormonally induced by administration of bromocryptine, even when basal plasma prolactin levels were suppressed to lower than 20 ng/mL.

Though, several modifications to the 7-day estrogen and progesterone treatment protocol yielded higher success rates (success defined by the scientist, but usually more than 9 kg milk/day peak yield), only a little improvement in reducing the variation in milk yields among animals was reported (Collier *et al.*, 1977; Chakriyarat *et al.*, 1978; Peel *et al.*, 1978; Fleming *et al.*, 1986).

Further, in the absence of estrogen and progesterone treatment, a short period of reserpine treatment followed by dexamethasone trimethyl acetate failed to induce lactation in cows indicated that the ovarian steroids may be essential for the induction of lactation in the cows (Collier *et al.*, 1977; Peel *et al.*, 1978).

Though high plasma progesterone has an inhibitory effect on lactogenesis, this effect could be overcome by the administration of reserpine to stimulate lactogenesis (Kuhn 1969; Hartmann *et al.*, 1973; Peel *et al.*, 1979).

Deshmukh *et al.* (1993) induced lactation in 6 infertile cows with estrogen and progesterone (1:2.5) for 7 days. Reserpine was also injected (5mg/mL) on day 8, 10, 12 and 14 and dexamethasone (20mg/mL) on days 18, 19 and 20. This resulted in increased success rate of lactation induction programme.

2.4.1.3 Metoclopramide in induction of lactation

Metoclopramide is a dopaminergic receptor antagonist which induces the release of prolactin from the anterior pituitary by blocking dopamine’s action as an inhibitor of prolactin secretion. It causes an increase in the circulating prolactin concentrations up to

3 to 8 times than the normal concentrations within one hour of an oral dose and circulating prolactin remain elevated for up to 8 hours (Gupta and Gupta, 1985).

Shridhar and Narayana (2006), reported that metoclopramide markedly increases the milk yield and fertility of dairy cattle induced to lactate with a combination of estrogen and progesterone. Similarly, Mohan (2007) also reported that animals treated with metoclopramide, yielded more milk yield as compared to reserpine treated animals along with estrogen, progesterone and dexamethasone administration.

2.4.1.4 Bovine recombinant somatotropin (rbST)

Azimov and Krouze (1937) were the first researchers to demonstrate that injections of crude pituitary extracts from cattle, increased milk yield in dairy cows. Young (1947) demonstrated that the somatotropin was the galactopoietic factor in pituitary extracts that stimulated milk yield in dairy cows.

Bauman *et al.* (1985) examined the effect of long-term (188 day) daily administration of either rbST or pituitary derived bovine somatotropin on lactational performance in 30 Holstein cows in their second to fifth lactation, beginning at 84 ± 10 days postpartum. Cows treated with rbST, either 13.5, 27, or 40 to 50 mg/day, had an increase in the milk yield from 23 to 41 per cent as compared to the control cows, while cows treated with pituitary-derived bovine somatotropin (27mg/day) recorded a slight increase in milk yield (16 per cent). Further, Bauman *et al.* (1985) reported that increased milk yield associated with somatotropin treatment caused a decrease in energy balance while treatment with rbST was not found to affect the energy balance as voluntary feed intake increased with increasing dose of rbST to compensate for the decrease in energy balance. Similar to Bauman *et al.* (1985), Mellado *et al.* (2011) also studied the role of rbST on lactation length and yield in Holstein cows and they reported that regardless of lactation number and age, the animals managed for extended lactation, with emphasis on lactation persistency with sustained application of rbST.

Tucker (1981) reported that the role of growth hormone in lactogenesis appears to be one of synergism with prolactin and adrenocorticotropin. The effect of GH on milk yield was confirmed by the results of experiments in first calf heifers and multiparous cows (Akers and Cleale, 1990). As increased milking frequency is noted to enhance the number of prolactin receptors on the mammary epithelial cells, additive milk production to a combination of exogenous GH and increased milking frequency was observed in cows (Speicher *et al.,* 1994). Later research results indicated that GH together with prolactin influences the milk production as such in ruminants, where GH has an influence on fat synthesis and prolactin influences protein and lactose synthesis (Knight, 2001).

Exogenous administration of GH increases lactation production in cattle by 6 to 30 per cent and thus persistency too in a sequential manner. The increased milk production after administration of GH is not followed by elevated feed intake, as is the case when cows are exposed to treatment with thyroid hormones. The effect of exogenous GH is related to increased mobilization of body stores, whereby the level of body stores before treatment starts is significant for the treatment effect (Rose and Obara, 2000). Conclusively, a positive relationship was established between plasma GH concentrations and milk yields in dairy cows (Sorensson and Knight, 2002). No difference in milk composition and somatic cell counts was noticed between bST treated and control cows (Magliaro *et al.,* 2004) and also increased milk yield (14.7%) was noticed between bST treated and control heifers (Macrina *et al.,* 2011).

2.4.1.4 Role of $PGF_{2\alpha}$ in induction of lactation

The ability of $PGF_{2\alpha}$ to induce luteolysis in cattle has led to research and development of protocols that may be used on commercial farms to improve pregnancy rate. Use of $PGF_{2\alpha}$ in the induction protocols offers an attractive means of regulating the stage of the estrous cycle in order to initiate lactation in cows as the protocols seem similar in endocrine status. Also, since progesterone competes with glucocorticoids for binding sites in mammary tissue (Fulkerson, 1979), removing the progesterone source (corpus luteum) by administering $PGF_{2\alpha}$ may allow induced cows to be more responsive to reserpine and dexamethasone treatment following the initial estrogen and progesterone protocol. Induced luteolysis allows glucocorticoids to displace progesterone from binding sites in mammary tissue, thereby removing the progesterone block to lactogenesis (Fulkerson, 1979). Exogenous $PGF_{2\alpha}$ may then be used following the initial estrogen-progesterone therapy to insure luteolysis and depress circulating progesterone at the time of glucocorticoid and reserpine administration. This may offer the opportunity to reduce variation in number of animals responding to lactation induction and perhaps reduce variation in level of milk yield

Tracy (2002), in an attempt to improve success rates and reduce variability in milk yields, studied the role of combination of drugs to induce lactation and their effect on milk yield. While all the animals were subjected to an estrous synchronization protocol prior to the initiation of the lactation induction scheme, a slight difference in the days of administration of $PGF_{2\alpha}$ injection was followed between the groups i.e., Group 1 animals received $PGF_{2\alpha}$ injection 7 days prior to Group 2 animals and then second dose of $PGF_{2\alpha}$ injection was repeated after 11 days interval in the both the groups. This was followed by administration of estradiol – 17 β (0.1mg/kg bw/day) and progesterone (0.25 mg/kg bw/day) for 1 to 7 day; one more shot of $PGF_{2\alpha}$ (25mg) on day 13, and reserpine (5mg/day) and dexamethasone (20mg/day) injections on day 14 to 17. Although the milk yield was not improved, with the success rate of 60 to 70 per cent animals induced into lactation, this experiment indicated that more proportions of responded to the induction protocols when combined with other drugs that would eliminate the negative factors acting upon the induction status of the animal and also those which improve the response towards induction.

Mellado *et al.* (2006), in their study, increased the doses of estradiol and progesterone as well as the length of the protocol, which was 21 days. The protocol included doses of estrogen, progesterone, flumethasone, prostaglandin and bST.

Use of combination of Estradiol benzoate, acetate of medroxy progesterone, prostaglandin $F_{2\alpha}$ and isoflupredone acetate was shown to result in higher success rates of induced lactation (Frietas *et al.,* 2010).

Mohan *et al.,* (2007) compared the induction potential of various strategies by dividing the animals into four groups. Group 1 animals receiving 17βestradiol, progesterone, reserpine and dexamethasone injections; Group 2 animals receiving estradiol - 17β, progesterone, metoclopramide and dexamethasone injections while Group 3 and Group 4 animals received the same injections as Group 1 and Group 2, respectively along with initial administration of 2 doses of $PGF_{2\alpha}$. They found that a significantly higher milk yield was exhibited by Group 3 and Group 4 animals that were synchronized with $PGF_{2\alpha}$. They also remarked that use of $PGF_{2\alpha}$ with other hormones and drugs to induce lactation in repeat breeders helped in receiving higher profit.

Ramgattie *et al.* (2014) placed a 12- day lactation induction protocol using 2 injections of $PGF_{2\alpha}$ (25 mg) injections 11 days apart, estradiol - 17 β (0.1 mg/kg bw/day) and progesterone (0.25 mg/kg bw/day) from day 2 to 8, then on day 8 another injection of $PGF_{2\alpha}$ followed by reserpine (5 mg/day) and dexamethasone (20 mg/day) on each of the days 9 to 12. They concluded that by using the combination of commercially available drugs and hormones it was possible to induce lactation in a very short term in cows and heifers.

2.5 Hormones in lactation induced animals

Formation of normal colostrum can be achieved by injecting estrogen and progesterone and both the hormones are involved in control of selective transport of serum immunoglobulin into milk (Smith, 1971).

Wiflet *et al.* (1976) carried out radioactive incorporation studies in cattle and quantified the endogenous hormones in urine, faeces, mammary secretion and plasma. Radioactivity from hydrogen- 3 and carbon-14 was detected in urine and feces but not in mammary secretions. Collier *et al.* (1977) reported higher prolactin concentration in reserpine treated groups compared to the control group. According to findings of Erb *et al.* (1976a) and Chakriyarat *et al.* (1978) inferior lactations were found to be associated with low concentration of plasma prolactin.

According to Harness *et al.* (1978) the variation in hormone concentrations in serum can be attributed to differences in the duration of treatment for induction of lactation, the carriers for the hormones (alcohol vs oil), the form of the hormone (estradiol – 17 β *vs* estradiol benzoate) and the intervals of injections. The other factors which may contribute to variability are the rate of absorption of the hormone from the injection site and existing functional status of the endocrine glands, pituitary and adrenals etc., during and following treatment (Smith and Schanbacher, 1973).

Neeru and Singh (1997) reported that there is no definite trend in plasma progesterone concentration of induced buffaloes but concluded that progesterone profile may be a critical factor to determine the success of induction of lactation in buffaloes. Inferior lactations were found to be associated with low concentration of plasma prolactin (Erb *et al*., 1976a; Chakriyarat *et al*., 1978)

Erb *et al.* (1976a) concluded that plasma concentration of total estrogen and progesterone hormone increased shortly after steroid injection in the induction protocols and the daily changes in estrogen and progesterone concentration were almost super imposable although the magnitude of fluctuations differed. Agarwal *et al*. (1993) reported that ovarian steroid used for induction of lactation are not excreted through the milk any marked amount and consumption of such milk may not cause any health hazard.

Tracy (2002) reported elevated concentrations of estrogen and progesterone in serum and milk when the animals were induced into lactation using a combination of $PGF_{2\alpha}$, estradiol – 17 β, progesterone, reserpine and dexamethasone. As reported by

McFadden *et al*. (1987), the serum lactalbumin concentration was also found to increase drastically in the experiment when reserpine and dexamethasone were administered.

2.6 Adverse effects on animals

An undesirable side-effect of large doses of estrogen is that cows may exhibit abnormal estrus behavior. Indeed, Smith and Schanbacher (1973, 1974) observed that the cows in their experiments exhibited increased estrus activity. Other workers using doses of estrogen similar to those used by Smith and his co-workers have also observed intense estros activity for as long as 20 to 50 days after cessation of estrogen injections (Paape and Guidry, 1973; Narendran and Hacker, 1974).

There were few treatment complications noticed in the induction protocols that included decreased feed intake, constipation, cystic follicles and development of hematomas which ultimately subsided itself. But in reserpine treated animals there was labored breathing, nasal congestion and drowsiness (Marrow, 1980). The adverse effects may be attributed to inhibitory effects on normal sympathetic activity by the drug in both the CNS and peripheral nervous system by binding to catecholamine storage vesicles, there by inhibits catecholamine synthesis by blocking the uptake of dopamine into the storage vesicle (Gilman *et al.*, 1990)

Other effects included nymphomania, relaxation of pelvic ligaments and fracture of pelvis and prolapse of vagina/uterus. Most of the treated cows exhibited decreased oestrous behaviour due to hormonal variations and hence precaution should be taken to avoid any possible injury to the treated animals (Verma *et al.*, 2004).

The repeated administration of hormones (estrogen and progesterone) to the animals caused the enhanced concentration of these hormones in the milk (Narendran *et al.*, 1979; Sawyer *et al.*, 1986; Deshmukh *et al.*, 1993).

Materials and Methods

III. MATERIALS AND METHODS

3.1 Experimental Animal

The study was conducted in 20 non-lactating and non-pregnant crossbred cattle with the history of repeat breeding and infertility which were apparently healthy. The animals for the study were selected from in and around the Bengaluru region. The present study was conducted in the geographic locality of 914 meters or 2998 feet above the sea level with 12°58′18″ latitude north and 77°35′37″ longitude east.

The study animals were selected based on the body condition scoring as per the methods described by Edmonson *et al.* (1989). The cows having BCS of 2.5 to 3.5 were randomly selected for the study. The reproductive organs of all the animals were examined per rectally prior to the inclusion into the study to rule out the reproductive disorders, if any. The body weight of the animals was calculated using the formula Body weight (in Kg) = LG^2 / 660 where L (length) – the distance in inches from point of shoulder to the point of rump, G (Girth) – is the circumference of chest in inches.

The cows selected for the study were dewormed with fenbendazole at the dose rate of 7.5 mg/ Kg bw (Panacur®, Intervet, India Pvt. Ltd. Pune, 1.5g/Bolus) PO and a single S/C injection of ivermectin at the dosage of 0.2 mg/kg bw (Bilivin®, Brilliant Bio Pharma Pvt. Ltd.) 10 days prior to initiation of study.

3.2 Drugs used in the study

3.2.1 Estradiol – 17 β and progesterone

Estradiol – 17 β (17β – Estra – 1, 3, 5(10) – triene – 3, 17 – diol) procured from Sigma chemicals, USA was used in the present study. Progesterone (Pregn - 4 - ene - 3, 20 – dione) which was also procured from Sigma chemicals, USA was used in the present study. The estradiol - 17 β and the progesterone were in the powder form and were dissolved in absolute ethanol to prepare a common stock solution for the administration to cattle in the study.

3.2.1.1 Preparation of common stock solutions

The common stock solution containing estradiol – 17 β and progesterone in the ratio of 1:2.5 was dissolved in absolute ethanol (Merck, Mumbai, India) on magnetic stirrer until they completely dissolve. The quantity of stock solution required for a cow was determined based on the body weight of the cow. The prepared hormone mixture was stored in air tight amber coloured glass container under refrigeration (4 ^{0}C) until further use. The mixture was used within a month of preparation.

3.2.2 Reserpine

Reserpine (Methyl-11, 17α – dimethoxy – 18-β 3, 4, 5 – trimethoxy benzolyl reserpate) an alkaloid extracted from *Rauwolfia serpintina* or *Romitoria vomitoria* plants. The reserpine was procured from Sigma Chemical USA. The procured reserpine was in the powder form which was later prepared by dissolving the required quantity (5 mg/ml) in 10 per cent glacial acetic acid to obtain a solution form and was stored in amber colored bottle prior to injection.

3.2.3 Glucocorticoids

The dexamethasone (Dexona®, 4mg/ml, KAPL, Pvt. Ltd., Bengaluru) was used in the present study at the total dosage of 20 mg/animal, IM.

3.2.4 Prostaglandins $F_{2\alpha}$

Dinoprost tromethamine, a natural prostaglandin $F_{2\alpha}$ (Lutalyse®, Zoetis, 5 mg/ml, 5 ml vial) was used in the study at the dose rate of 25 mg TD, IM.

3.3 Experimental Designs

The infertile cows selected were randomly divided into two groups comprising of 10 animals each.

3.3.1 Group I

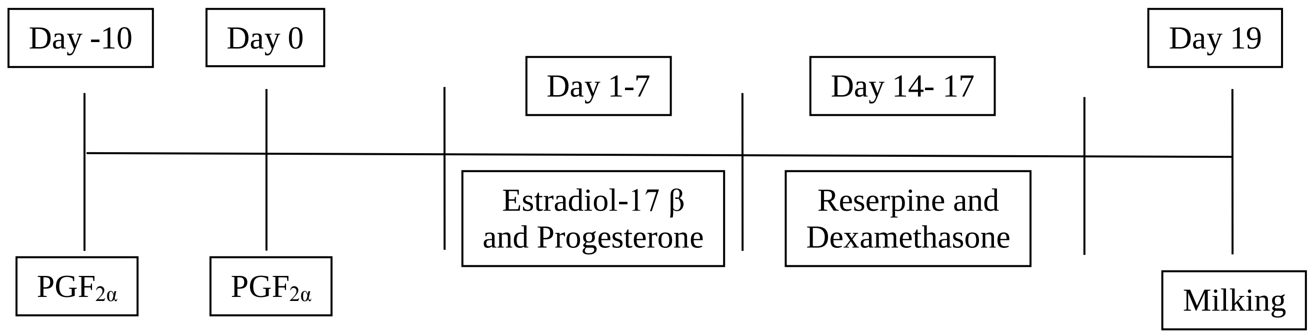
Group I comprised of 10 crossbred repeat breeder Holstein Friesian cows which were non - pregnant and non-lactating. The body weight of the cattle ranged from 295 to 390 kg with a mean body weight of 331.3 ± 8.89 kg and age of the cattle ranged from 60 to 90 months with average age of 70.8 ± 3.07 months. The schedule of drugs administration for induction of lactation for groups is depicted schematically in the fig.1. Cows in this group were synchronized with double $PGF_{2\alpha}$ protocol for the synchronization of estrus at 10 days apart, considering the day of second $PGF_{2\alpha}$ administration as day 0. From day 1 to 7, reconstituted estradiol-17β and progesterone were administered twice subcutaneously at a dose rate of 0.1mg/kg bw and 0.25 mg/kg bw, respectively. From day 14 to 17, reconstituted reserpine and dexamethasone were administered at a total dose of 5 mg per animal and 20 mg per animal, respectively. Milking was started from day 19 onwards.

3.3.2 Group II

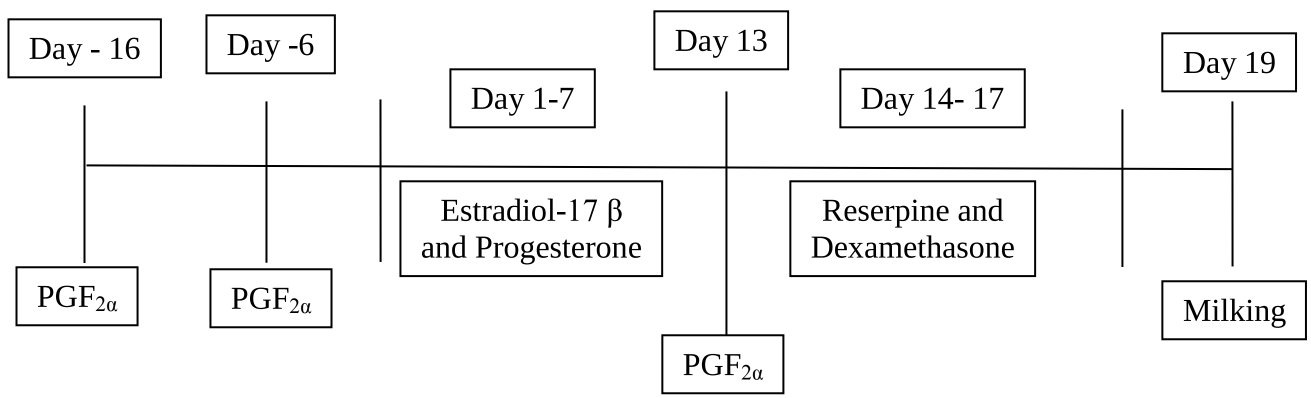
The selected animals comprised of seven crossbred Holstein Friesian cows, one Jersey crossbred heifer and two Jersey crossbred cows, which were non pregnant and non-lactating repeat breeders. The mean body weight of the cows ranged from 300 to 390 kg with a mean of 340.8 ± 11.22 kg and age ranged from 54 to 72 months with mean of 57 ± 2.24 months. The schedule of drugs administration for induction of lactation for Group II is depicted schematically in the fig.1.The cattle in this group were synchronized with double $PGF_{2\alpha}$ protocol for the synchronization of estrus at 10 days apart, considering the day of second $PGF_{2\alpha}$ administration as day 0. From day 1 to 7, estradiol – 17 β and progesterone was administered twice subcutaneously at a dose rate of 0.1mg/kg bw and 0.25 mg/kg bw, respectively. On day 13, an another dose of $PGF_{2\alpha}$ was administered followed by daily intramuscular injections of reserpine and dexamethasone from day 14 to 17 at a total dose of 5 mg/animal and 20 mg/animal, respectively. Milking was started from day 19 onwards.

Fig. 1. Protocols for induction of lactation in Group I and Group II

Group I



Group II



Dosages of drugs administered: Estradiol – 17 β 0.1 mg/kg bw twice daily S/C; Progesterone 0.25 mg/kg bw twice daily, S/C; Reserpine 5 mg/animal, TD, IM; Dexamethasone 20 mg/animal, TD, IM; $PGF_{2\alpha}$ 25 mg/animal, TD, IM.

3.4 Collection of blood sample

The blood samples were collected in vacutainer with clot activator by jugular venipuncture on day 0, 1, 4, 7, 13, 19 and 35 from the treated cows. The collected samples were allowed to clot and then were centrifuged at 3000 rpm for 20 minutes. Using micro pipette serum samples were collected and made into aliquots in duplicate and stored at -20º C pending the assay of concentrations of progesterone and estradiol – 17 β.

3.5 Hormonal assay by RIA

3.5.1 Progesterone estimation by radioimmunoassay

The progesterone was estimated as per the method described by Kaneko *et al*. (2008) using progesterone RIA kit (Beckman Coulter, Prague 10-Czech Republic, supplied by Anand Brothers, India).

The radioimmunoassay of progesterone is a competitive assay. Samples and calibrators were incubated with ^{125}I - labeled progesterone as tracer in antibody-coated tubes. After incubation the content of the tube was aspirated and bound radioactivity was measured. A calibration curve was established and unknown values were determined by interpolation from the curve.

Procedure:

1. RIA KIT (BECKMAN COULTER) was procured for the progesterone estimation.
2. Initially serum samples and all the reagents were brought to room temperature.

3. Both 50 micro liters of serum samples and 500 micro liters of tracer were mixed in progesterone coated tubes.

4. Then the tubes were incubated for one hour at 18-25 ºC temperature in a shaking incubator at 350 rpm, later the incubated tubes were subjected to decanting.

5. To measure the bound radioactivity, the decanted tubes were placed in a gamma counter for 60 seconds.

6. A calibration curve was established.

7. Progesterone values were then determined by interpolation from the curve by using Saprical data reduction software. For PRIA-1, the coefficients of variations were found below or equal to 8.15% for serum samples in intra-assay, whereas, for inter-assay it was 8.66% and the analytical sensitivity was 0.03 ng/ml). The antibody used in the immunoassay is highly specific for progesterone.

3.5.2 Estrogen estimation by radioimmunoassay

The estradiol was estimated as per the method described by Kaneko *et al*. (2008) using estradiol RIA kit (Beckman Coulter, Prague 10-Czech Republic, supplied by Anand Brothers, India).

The radioimmunoassay of estrogen is a competitive assay. Samples and calibrators were incubated 3 hours with ^{125}I - labeled estrogen as tracer in antibody-coated tubes. After incubation the content of the tube was aspirated and bound radioactivity is measured. A calibration curve was established and unknown values were determined by interpolation from the curve.

Procedure:

1. RIA KIT (BECKMAN COULTER) was procured for the estrogen estimation.
2. Initially, serum samples and all the reagents were brought to room temperature.
3. Both 50 micro liters of serum samples and 500 micro liters of tracer were mixed in estrogen coated tubes.
4. Then the tubes were incubated for 3 hour at 18 – 25° C temperature in a shaking incubator at 350 rpm and the incubated tubes were subjected to decanting.
5. For measuring the bound radioactivity, the decanted tubes were placed in a gamma counter for 60 seconds.
6. A calibration curve was established.
7. Estrogen values were determined by interpolation from the curve by using Saprical data reduction software. For PRIA-1, the coefficients of variations were found below or equal to 14.5% for serum samples in intra-assay and for inter-assay it was 14.5% and the analytical sensitivity was 9.58 pg / ml and functional sensitivity was 13.11 pg/ml.

Specificity – The antibody used in the immunoassay is high for estradiol. Low cross reactivities were obtained against other steroids (Estradiol-3 sulfate, Estrone, Estriol, Cortisol, etc.) or therapeutic drugs that may be present in the patient samples (Tamoxifene, etc.).

3.6 Statistical analysis

The data generated for different parameters were tabulated group wise and expressed as mean ± S.E. The data generated were analyzed using student t – test for milk yield and one way analysis of variance with Tukey's Multiple Comparison test for analysis of concentration of hormones using GraphPad Prism trial version 5.00, GraphPad Software, SanDiego, California, USA (Steel and Torrie, 1981).

Results

IV. RESULTS

4.1 Lactation induction

In the present study, the response of the cows to the induction of lactation in both the groups was recorded. Out of 10 cows that were induced to lactate eight cows were responded and 2 animals did not respond for the induction therapy in both the groups I and II. In group I, cow No. 7 and 8 did not respond to the therapy. Mid way through the treatment cow number seven in Group I was diagnosed with endometritis. Although cow No. 8 which was apparently healthy it did not elicit any response to therapy. Also in Group II 8 out of 10 animals responded, whereas the two animals although apparently healthy did not respond to therapy for the induction of lactation.

Few adverse effects like profuse salivation, lethargy and marked increase in the respiratory rate were observed during the reserpine administration in both the Groups (day 14 to 17), however the clinical signs/symptoms subsided following the completion of hormone treatment.

4.2 Milk Yield

Enlargement of the mammary gland in all the cows under treatment was noticed by day 7 and secretion in the mammary gland was observed between 9 and 14 of the induction therapy. The mammary gland was considerably enlarged by day 18 and 19 and the teats were engorged. Secretion of milk from teats was noticed on day 16 in Group II which was injected with $PGF_{2\alpha}$ on day 13 of induction of lactation whereas milk secretion was seen on day 18 in Group I. Irrespective of the day of secretion of milk observed milking was done in both the groups from day 19. The milk yield obtained on day one of milking from the induced cows ranged between 1.7 and 6.7 litres in Group I and 2.8 and 4.8 litres in Group II. The consistency of milk obtained on the first day was thicker than the normal milk and was slightly yellow in colour. The consistency of milk turned towards normal in all cows by 3 to 5 days of lactation.

The mean total milk yield and mean daily milk yield for the period of 100 days in both the groups is as shown in table 1 and fig. 2. The mean total milk yield for the period of 100 days in Group I and Group II were recorded as 508.5 ± 44.28 and 493.3 ± 21.6 litres, respectively. The mean daily milk yield recorded for the period of 100 days in Group I and Group II were 5.04 ± 0.44 and 4.88 ± 0.21 litres, respectively (Table 1 and fig. 2).

The weekly milk yield recorded in both the groups up to 14^{th} week is as shown in table 2 and fig. 3. In the present study, the mean daily milk yield was recorded to range from 4.16 ± 0.55 to 5.34 ± 0.48 litres in Group I whereas, in Group II it ranged from 4.15 ± 0.26 to 5.14 ± 0.28 litres during the 14 weeks of milk recording. The mean daily milk yield recorded on 0 day of the first recording was 4.16 ± 0.56 and 4.15 ± 0.27 litres, respectively in Group I and Group II which did not differ significantly ($P > 0.05$). By 4^{th} week the mean daily milk yield reached to 5.13 ± 0.43 and 5.13 ± 0.17 litres, respectively in Group I and Group II. During the14 week observation period the highest mean daily milk yield was achieved by 8^{th} week in both the groups. It was recorded as 5.43 ± 0.55 litres in Group I and 5.14 ± 0.28 litres in Group II. The highest mean daily milk yield recorded however, did not differ significantly ($P > 0.05$) between the groups. The mean

Table 1. Mean total milk yield in liters (100 days) and mean daily milk yield of Group I and Group II, induced to lactate

Group	Total Milk Yield (100 days)	Daily milk yield
Group I (n = 8)	508.50 ± 44.28[a]	5.04 ± 0.44[b]
Group II (n = 8)	493.30 ± 21.6[a]	4.88 ± 0.21[b]

Note: Figures in the parenthesis are the number of animals.

Mean values bearing different superscripts within a column are significantly different ($P < 0.05$).

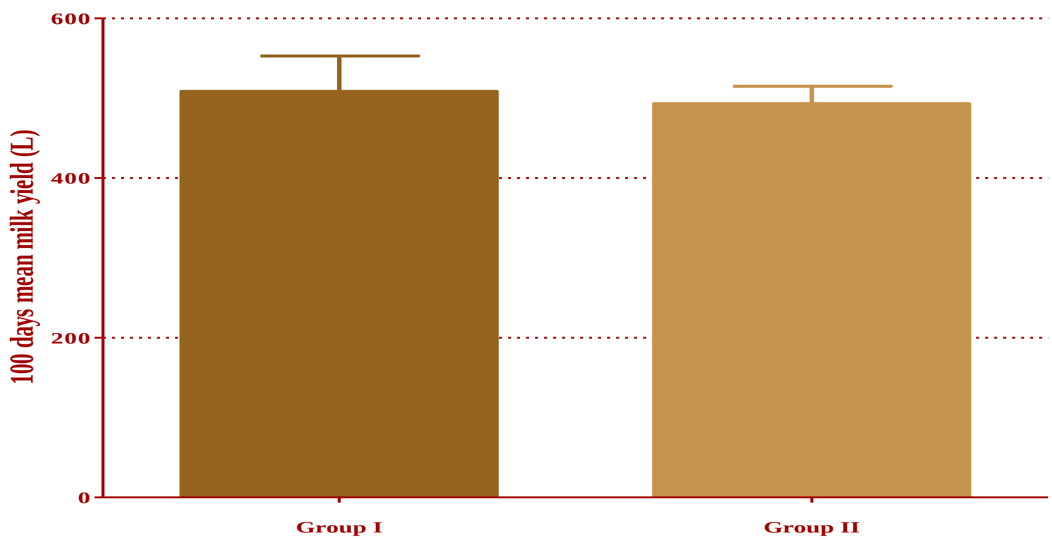


Fig. 2. Mean total milk yield (L) (100 days) of Group I and Group II, induced to lactate.

Table 2. Mean weekly milk yield in liters (mean ± S.E) of Group I and Group II, induced to lactate

Weeks	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Group 1 (n = 8)	4.16 ± 0.56 ^{a}	4.44 ± 0.45 ^{a}	4.64± 0.54 ^{a}	4.63 ± 0.64 ^{a}	5.13 ± 0.43 ^{a}	5.34 ± 0.48 ^{a}	5.33 ± 0.59 ^{a}	5.19 ± 0.48 ^{a}	5.43 ± 0.55 ^{a}	5.2 ± 0.49 ^{a}	5.13± 0.42 ^{a}	4.91 ± 0.43 ^{a}	5.14 ± 0.40 ^{a}	4.81 ± 0.34 ^{a}	4.74 ± 0.44 ^{a}
Group II (n = 8)	4.15 ± 0.27 ^{a}	4.53 ± 0.31 ^{a}	4.7 ± 0.23 ^{a}	4.89 ± 0.21 ^{a}	5.13 ± 0.17 ^{a}	4.91 ± 0.35 ^{a}	4.94 ± 0.17 ^{a}	4.99 ± 0.25 ^{a}	5.14 ± 0.28 ^{a}	4.99 ± 0.27 ^{a}	4.83 ± 0.30 ^{a}	5.04 ± 0.31 ^{a}	4.93 ± 0.29 ^{a}	4.844 ± 0.24 ^{a}	4.86 ± 0.29 ^{a}

Note: Figures in the parenthesis are the number of animals.

Mean values bearing different superscripts within a column are significantly different ($P < 0.05$).

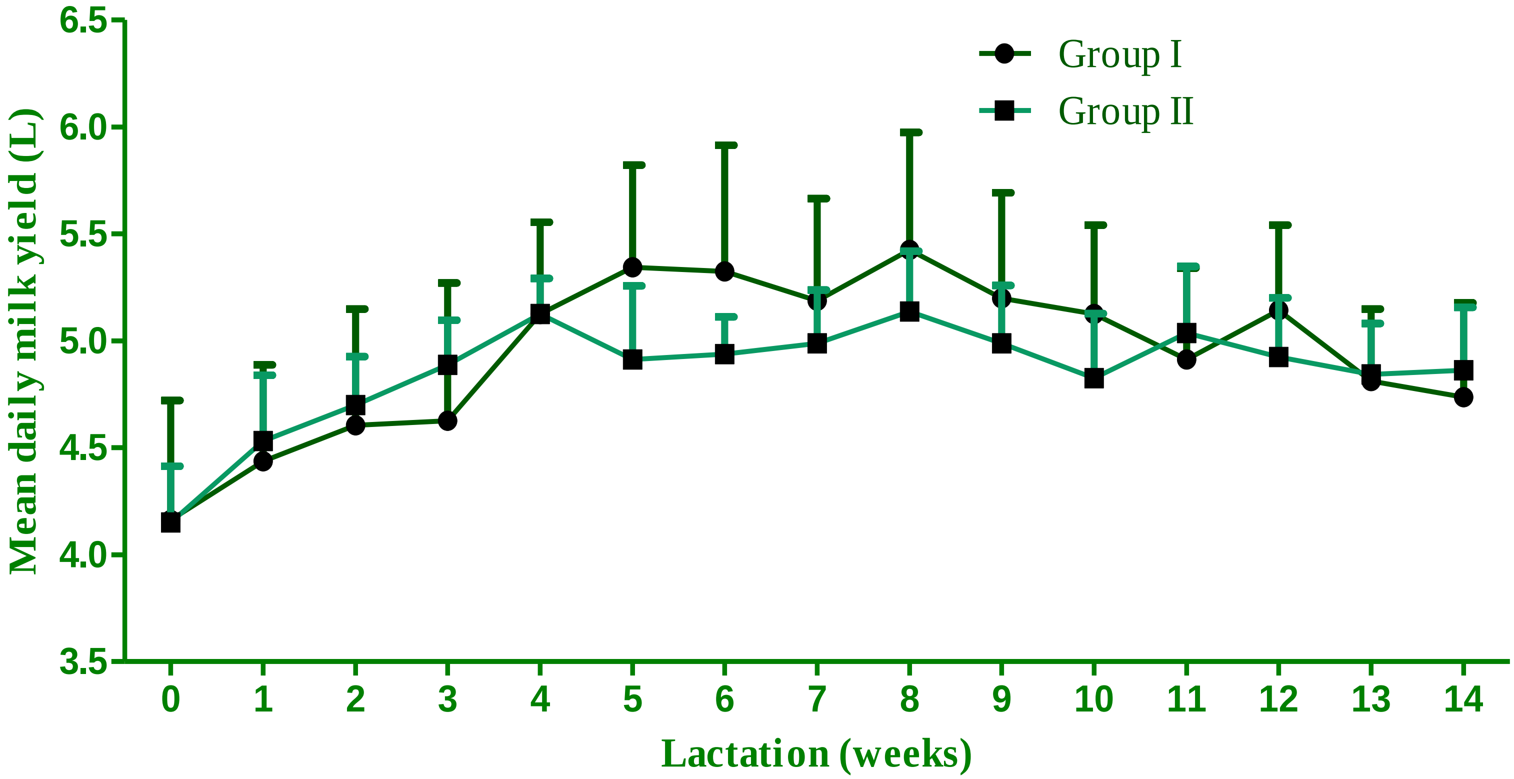


Fig. 3. Mean daily milk yield (L) at weekly intervals (14 weeks) of Group I and Group II, induced to lactate

daily milk yield obtained during the 14 weeks of milk yield recording in both the groups exhibited fluctuating trends. In Group I, however, the mean daily milk yield recorded by 8^{th} week (5.43 ± 0.55 litres) was significantly higher ($P < 0.05$) than that of the 0^{th} week (4.16 ± 0.55 litres). Similarly, the 8^{th} week mean daily milk yield (5.14 ± 0.28 litres) of Group II significantly differ as compare to the mean daily milk yield of 0^{th} week (4.15 ± 0.26 litres).

4.3 Hormone concentration

4.3.1 Progesterone concentration in serum

The mean serum progesterone concentration of Group I and Group II on days 0, 1, 4, 7, 13, 19 and 35 of induction of lactation is depicted in table 3 and fig. 4.

In Group I the mean progesterone concentration on the day 1 of lactation induction before the administration of estradiol – 17 β and progesterone was estimated to be 1.46 ± 0.36 ng/mL. Its concentration increased significantly ($P < 0.05$) from day 1values of 1.46 ± 0.36 ng/mL to 7.26 ±1.78 ng/mL on day 4 of lactation induction. The progesterone concentration reached its peak on day seven of estradiol 17 - β and progesterone administration. The day 7 value of 9.95 ± 1.91 ng/mL was statistically non - significant ($P > 0.05$) from the values of day 4 value. After the cession of estradiol 17 - β and progesterone administration (day 7) and the day before the injection of reserpine and dexamethasone, the mean progesterone concentration on day 13 was 4.63 ± 0.9 ng/mL. The mean serum progesterone concentration decreased significantly ($P > 0.05$) on the first day of milking (day 19 of induction protocol) to 1.64 ± 0.21 ng/mL from day 13

Table 3. The mean serum progesterone concentration (ng/mL) (Mean ± S.E) in lactation induced groups

Group (n = 10)	Treatment days						
	0	1	4	7	13	19	35
Group I	1.96 ± 0.26 [a]	1.46 ± 0.36 [a]	7.26 ± 1.78 [bc]	9.95 ± 1.91 [c]	4.63 ± 0.9 [ab]	1.64 ± 0.21 [a]	1.82 ± 0.22 [a]
Group II	1.96 ± 0.18 [a]	2.02 ± 0.26 [a]	6.55 ± 1.47 [b]	11.32 ± 1.93 [c]	6.65 ± 0.42 [b]	1.84 ± 0.16 [a]	1.96 ± 0.14 [a]

Note: Mean values bearing different superscripts within a row are significantly different (P <0.05).

One way Anova – Tukey’s Multiple Comparison test

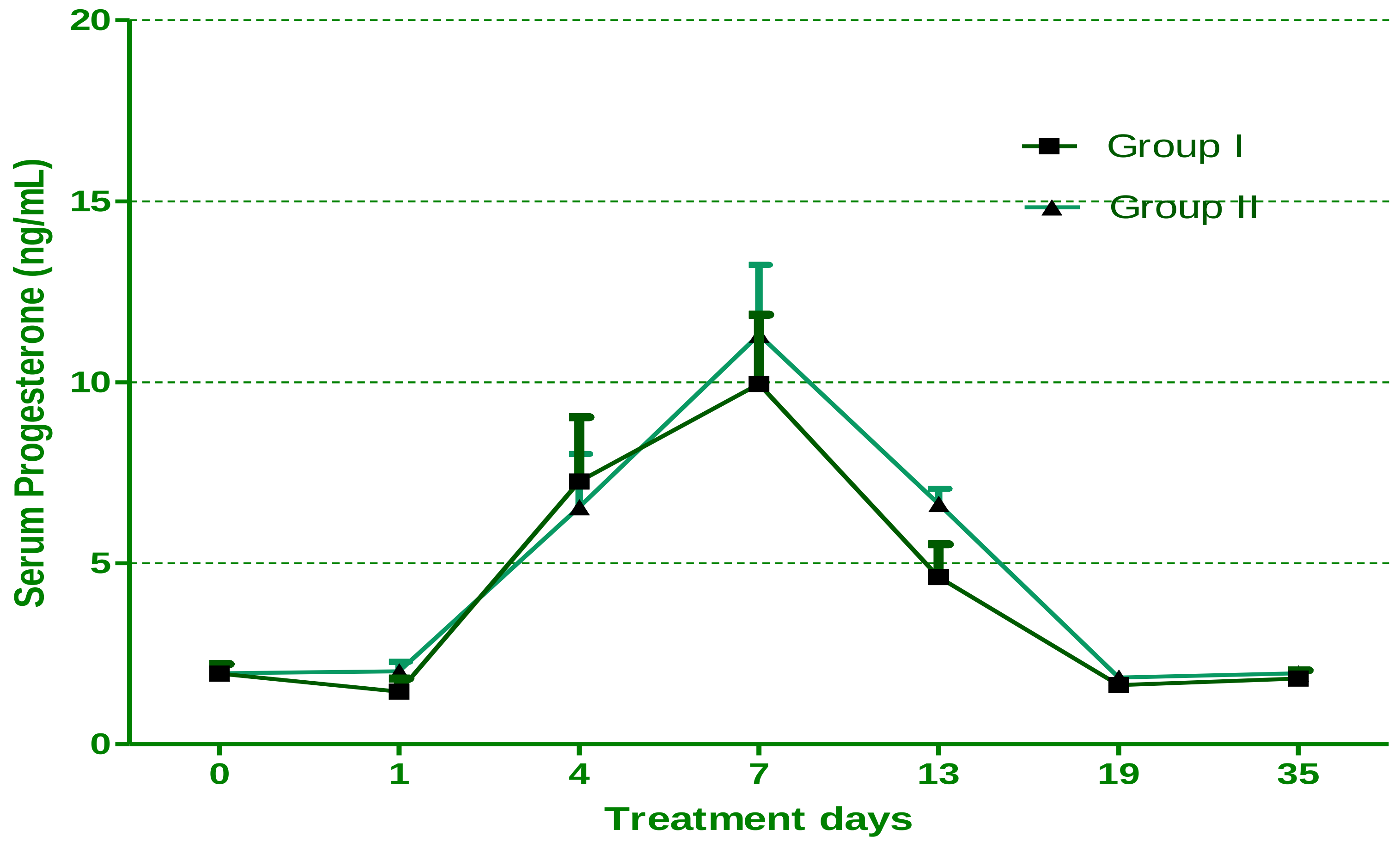


Fig. 4. Mean serum progesterone (ng/mL) concentrations of Group I and Group II on different days of treatment

values and decreased thereafter to 1.82 ± 0.22 ng/mL up to day 16 of milking (day 35 of induction protocol).

In Group II, the mean progesterone concentration on the day 1 of lactation induction protocol before the administration of estradiol – 17 β and progesterone was estimated to be 2.02 ± 0.26 ng/mL. The mean serum progesterone concentration increased significantly ($P < 0.05$) from day 1 values 2.02 ± 0.26 ng/mL to 6.55 ± 1.47 ng/mL on day 4 of lactation induction. The day 7 value of 11.32 ± 1.93 ng/mL was statistically significant ($P < 0.05$) from the values of day 1 and 4 values. After the cession of estradiol – 17 β and progesterone administration (day7), on the day of $PGF_{2\alpha}$ injection and the day before the injection of reserpine and dexamethasone, the mean serum progesterone on day 13 of lactation induction was found to be 6.65 ± 0.42 ng/mL. The decrease in mean serum progesterone concentration continued to day 19 which was recorded to be 1.84 ± 0.16 ng/mL (on first day of milking) and it is then reduced on16th day of milking (day 35) to 1.96 ± 0.14 ng/mL.

4.3.2 Estradiol – 17 β concentration in serum

The mean serum estradiol – 17 β concentration of group I and group II on 0, 1, 4, 7, 13, 19 and 35 days of induction protocol is depicted in table 4 and fig. 5.

In Group I, the mean serum estradiol – 17 β concentration on day 1 of lactation induction protocol was found to be 49.84 ± 8.61 pg/mL. Its concentration increases significantly ($P < 0.05$) from the day 1 values of 49.84 ± 8.61 pg/mL to 2333 ± 257.6 pg/mL on day 4, following the administration of estradiol – 17 β and progesterone. The mean serum estradiol – 17 β concentration further increased significantly ($P < 0.05$) on day 7 from the values of day 1 and 4 to 3021 ± 264 pg/mL. The mean concentration of the hormone declined significantly ($P < 0.05$) after the cession of estradiol – 17 β and progesterone administration (day 7) and the day before the injection of reserpine and dexamethasone, the mean serum estradiol - 17 β concentration on day 13 of induction lactation was 52 ± 11.59 pg/mL which was significantly ($P < 0.05$) decreased from day 4 and day 7 values. The mean serum estradiol – 17 β concentration on the day of milking was 48.25 ± 10.34 pg/mL which non significantly ($P > 0.05$) decreased to 38.69 ± 4.69 pg/mL by day 35 (16^{th} day of milking).

In Group II, the mean serum estradiol – 17 β concentrations on day 1 of lactation induction protocol was found to be 30.58 ± 8.0 pg/mL. Its concentration increased significantly ($P < 0.05$) from the day 1 values of 30.58 ± 8.0 pg/mL to 1933 ± 348.7 pg/mL on day 4, following the administration of estradiol – 17 β and progesterone. The mean serum estradiol - 17β concentration further increases significantly ($P < 0.05$) on day 7 from the values of day 1 and 4 to 3360 ± 374.8 pg/mL. The mean concentration of the hormone declined significantly ($P < 0.05$) after the cession of estradiol 17 - β and progesterone administration (day 7), on the day of $PGF_{2\alpha}$ injection and one day before the injection of reserpine and dexamethasone, the mean serum estradiol – 17 β concentration on day 13 of induction lactation was 63.69 ± 7.47 pg/mL which was statistically significant from day 4 and day 7 values. The mean serum estradiol – 17 β concentration on the day of milking was 31.14 ± 2.41 pg/mL which non significantly ($P > 0.05$) decreased to 26.57 ± 2.17 pg/mL by day 35 (16^{th} day of milking).

Table 4. The mean serum estradiol – 17 β concentration (pg/mL) (Mean ± S.E) in lactation induced groups

Groups	Treatment days						
	0	1	4	7	13	19	35
Group I	58.35 ± 8.47[a]	49.84 ± 8.61[a]	2333 ± 257.6 [b]	3021 ± 264 [c]	52 ± 11.59 [a]	48.25 ± 10.34 [a]	38.69 ± 4.69 [a]
Group II	35.07 ± 7.49 [a]	30.58 ± 8 [a]	1933 ± 348.7 [b]	3360 ± 374.8 [c]	63.69 ± 7.47 [a]	31.14 ± 2.41 [a]	26.57 ± 2.17 [a]

Note: Mean values bearing different superscripts within a row are significantly different ($P < 0.05$).

One way Anova – Tukey's Multiple Comparison test

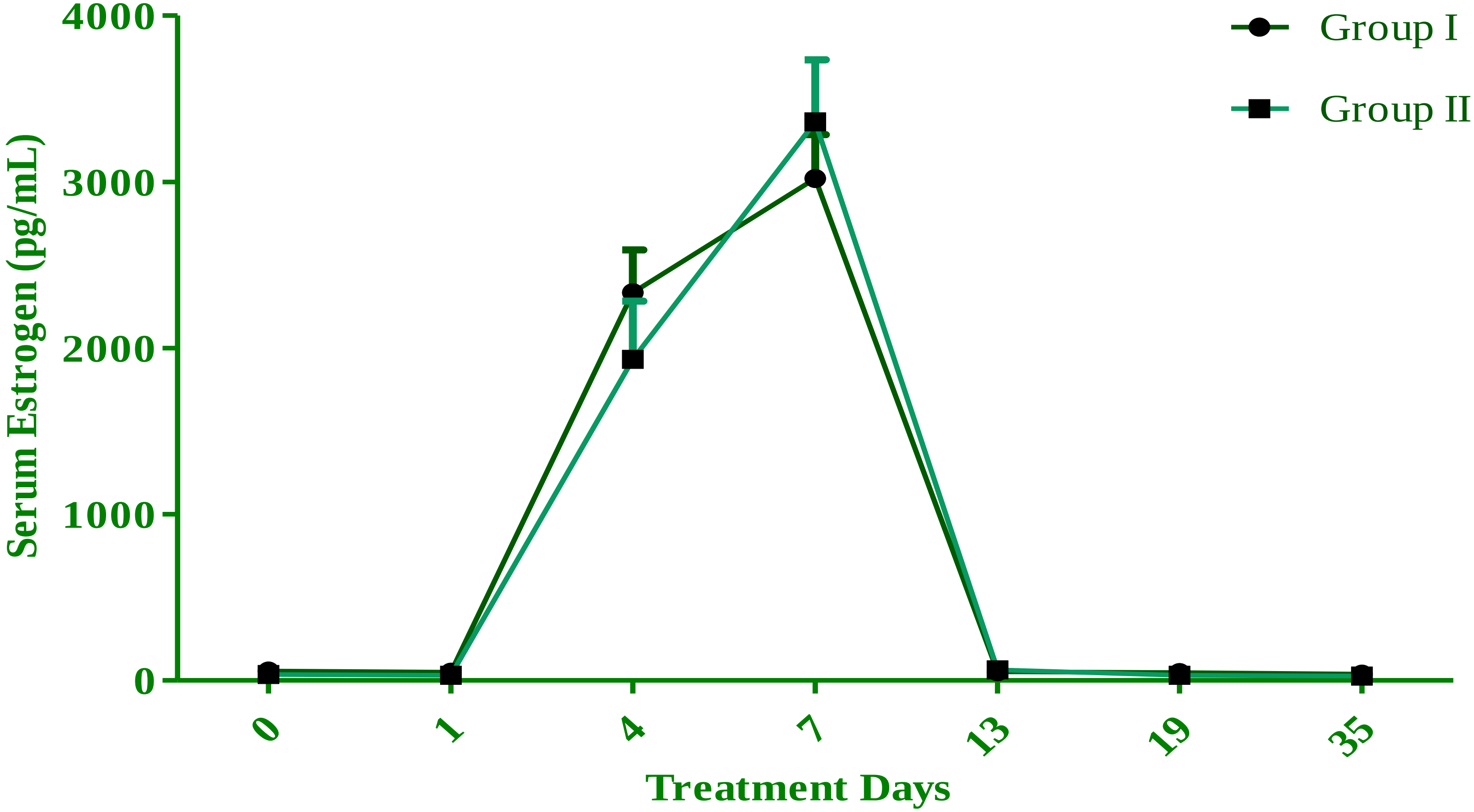


Fig. 5. The mean serum estradiol – 17 β concentrations of Group I and Group II at different days of treatment

Discussion

V. DISCUSSION

5.1 Lactation Induction

The present study was designed with the objective of artificial induction of lactation in non - lactating dairy cows using hormones (estradiol - 17 β at 0.1 mg/kg bw and progesterone at 0.25 mg/kg bw for 1 to 7 days) and drugs (Reserpine, 5 mg/animal, Dexamethasone 20 mg/animal per day for days 14 to 17).

Earlier, the combination of estrogen and progesterone was used to stimulate mammary development that increased the success of re-initiating lactation in cows (Williams and Turner, 1960). Smith and Schanbacher (1973) successfully induced lactation in heifers and multiparous cows and emphasized the efficacy of a combination of estradiol – 17 β at 0.1 mg/kg bw and progesterone at 0.25 mg/kg bw for 7 days. In conformity with the observations of Smith and Schanbacher (1973), the results of the present study concur with their findings.

Mammary tissue from cows successfully induced into lactation undergoing the cellular changes associated with lactogenesis by day 8 and continued through day 17 of treatment period (Collier *et al.,* 1976). Prolactin was considered to be a limiting component of the "lactogenic complex" in cows which failed to lactate following the estrogen and progesterone treatment. Based on these observations, in the present study reserpine and dexamethasone injections (day 14, 15, 16 and 17) were employed (Collier *et al.,* 1977).

Peel *et al.* (1979) suggested release of prolactin from anterior pituitary, decreased variability of the milk yield response and increased milk production by injecting reserpine. This observation clearly reflected in the present study, where in reserpine used in both groups there was 80 per cent response. However, there were few adverse effects like profuse salivation, lethargy and marked increase in respiratory rate during reserpine administration. Hence, caution should be taken while administrating the reserpine for induction of lactation. The adverse effects may possibly be due to inhibitory effect on normal sympathetic activity of the drug on both the central and peripheral nervous system by binding to catecholamine storage vehicles, thereby inhibiting catecholamine synthesis by blocking the uptake of dopamine into storage vehicle (Ellenhron and Barceloux, 1989; Gilman *et al.*, 1990).

The glucocorticoid level increases in the blood at parturition (Collier *et al*., 1977) and dexamethasone is an effective lactogenic stimulator and an increase in percentage area of mammary gland epithelial cells and nuclei/alveolus following dexamethasone injection (Fulkerson and McDowell, 1975). In the present study also dexamethasone injections were included for successful induction of lactation.

Administering glucocorticoids to non-lactating cows with a developed mammary gland aids in the induction of lactation because the increase in glucocorticoids displaces progesterone from mammary cell receptors, thus reducing the progesterone block to prolactin receptor synthesis (Tucker, 1965; Fulkerson, 1975). Milk yields from these induced-lactations can be enhanced with the addition of prolactin, offering additional evidence that the hormones (estrogen, progesterone, prolactin, and glucocorticoids) work synergistically in the onset of lactogenesis (Tucker, 2000; Akers, 2002).

Including injections of dexamethasone in the lactation induction programme was highly beneficial. This agrees with the results of Fulkerson and McDowell (1975) and Chakriyarat *et al*. (1978) and suggests that availability of glucocorticoids might be rate limiting in cows induced into lactation or alternately, that temporal associations among corticoids, other steroids and protein hormones during the induction were less than optimal. The increase in success rate (Chakriyarat *et al*., 1978) and induced lactation milk yields when a corticoid source was included (Fulkerson and McDowell, 1975) was demonstrated.

Growth and enlargement of the mammary glands occurred after experimental day 14 by which time concentrations of plasma steroids already were declining. This increase of the udder size coincided with the increase in plasma concentrations of prolactin as previously observed (Chakriyarat *et al*., 1978). It also corresponded to the period where Smith and Schanbacher (1973) and Chakriyarat *et al.* (1978) observed a rapid accumulation of secretions and Collier *et al*., (1975) and Croom *et al*., (1976) the cellular differentiation of the developing mammary gland.

5.2 Success rate

Out of 10 cows in each Group eight cows in each group were responded for the induction therapy resulting in 80 per cent success rate (≥ 2 litres/day) in both the groups.

One cow in Group I diagnosed with endometritis after the end of steroid hormone therapy. The other animals although apparently healthy did not produce and sustain the required quantity of milk to be called as success.

The success rate observed in the present study was in accordance with Chakriyarat *et al*. (1978), Tracy (2002) and Mohan (2007) who reported the success rate of 82 per cent, 92 per cent and 90 per cent, respectively. While, the success rate in the present study was higher than the Smith and Schanbacher (1973) and Collier *et al.,* 1975 who reported the success rate of 60 per cent and 69 per cent, respectively. Increased success rate in the present study might be due to dose, duration and the type of hormones used for the induction of lactation.

5.4 Milk yield

The artificial induction of lactation is a tool to initiate lactation in non-pregnant, non-lactating dairy cattle. In the present study both the treated groups were successfully induced to lactate. The mean milk yield for the period of 100 days in both Group 1 and Group II cattle were 508.50 ± 44.28 and 493.30 ± 21.60 liters, respectively. The quantity of milk obtained in the present study is in agreement with the reported results of Mohan (2007) whose study was also conducted in same latitude and longitude. However, higher mean milk yield was obtained by Tracy (2002), Freitas *et al*. (2010) and Ramgattie *et al*. (2014). The increased milk yield obtained by the aforementioned authors could be due to breed, managemental practices, superior genetic potential for milk yield (Ramgattie *et al*., 2014).

In the study it was found that there was no significant ($P < 0.05$) difference in the total mean milk yield between Group I and Group II. In Group II a dose $PGF_{2\alpha}$ given on day 13th to remove the progesterone block did not result in the increase in milk yield when compare to the milk yield with Group I whereas it resulted in letdown of milk two days earlier than that of Group I (Tracy, 2002).

The mean milk yield for the period of 100 days is also recorded in weekly intervals for the period of 14 weeks. Highest milk yield was recorded on 8th week in Group I and Group II animals, 5.43 ± 0.55 and 5.14 ± 0.28 litres, respectively. In some of previous lactation-induction studies, peak milk yields were reached 7 to 8 weeks after onset of lactation (Smith and Schanbacher, 1973 and Tervit, 1980). However, Ramgattie *et al*. (2014) reported delayed peak yield in induced cows wherein they obtained the mean daily peak milk yield of 15 litres on 12.7 ± 1.8 weeks. The delayed peak yield in induced cows might be due to the less amount of secretory tissue present at the starting of lactation as compared with non-induced cows, since the mammary development occurs over three week period in the induced lactation and during the last month of gestation (Knight and Wilde, 1993).

Average daily milk yield for a period of 100 days recorded in the present study were found to be 5.04 ± 0.44 and 4.89 ± 0.21 litres, in Group I and Group II cows, respectively. Similar average daily milk yield were reported by Mohan (2007) who followed the same protocols. However, Tracy (2002) and Ramgattie *et al*. (2014) had reported average daily milk yield of 9 litres and 11 litres, respectively which were higher than the results recorded in the present study. The higher average daily milk yield reported by Tracy (2002) and Ramgattie *et al*. (2014) was due to the breed and superior genetic potential of the cow, managemental practices including nutritional status and housing. Further, Tracy (2002) had administered recombinant bovine somatotropin once every two weeks on the day of milk letdown throughout the period of her experiment (21 weeks) which resulted in higher average daily milk yield.

5.4 Serum progesterone concentration

The mean serum progesterone concentration of Group I and Group II on day 0, 1, 4, 7, 13, 19 and 35 of induction of lactation is as shown in table 3 and fig. 4.

The mean serum progesterone concentration on the day 1 of lactation induction just before the administration of estradiol – 17 β and progesterone was estimated to be 1.46 ± 0.36 ng/mL and 2.02 ± 0.26 ng/mL, in Group I and II respectively. Similar mean serum progesterone concentrations of 1.10 ± 0.30 ng/mL, 3.54 ± 1.82 ng/mL and 3.76 ± 0.06 ng/mL have been reported by Diane *et al*. (1981), Fleming *et al*. (1986) and Mohan (2007), respectively in their lactation induction protocols. The values of progesterone on this day in the present study and that of others correspond with the normal luteal phase progesterone values of estrous cycle.

The mean concentration of serum progesterone increased significantly ($P < 0.05$) in both the groups from day 4 (7.26 ± 1.78 ng/mL and 6.55 ± 1.47 ng/mL) and maintained its elevated concentration till day 7 (9.95 ± 1.91 ng/mL and 11.32 ± 1.93) of induction of lactation when compared with day 1(1.46 ± 0.36 ng/mL and 2.02 ± 0.26 ng/mL) (Table 3 Fig. 4).

The pattern of increase in mean serum progesterone concentration during the exogenous administration of estradiol – 17 β and progesterone for 7 days similar to this study was also reported by several authors (Davis *et al*., 1983; Fleming *et al*., 1986 and Mohan, 2007). The increase in the progesterone values during the 7 day period may be attributed to the exogenous administration of estradiol – 17 β and progesterone.

The mean serum progesterone concentration decreased significantly after the cession of estradiol – 17 β and progesterone administration after day 7 and continued to decrease till day of milking (day 19) in both the groups. Similar trend of gradual decline in mean progesterone concentration was observed by Mohan (2007). The decreased values of progesterone are because of withdrawal of administration of exogenous progesterone and estradiol – 17 β.

In this study while mean serum progesterone concentration on the day of milking was found to be 1.64 ± 0.21 ng/mL and 1.84 ± 0.16 ng/mL in Group I and Group II, respectively, a basal level of serum progesterone concentration has been observed by Erb *et al*. (1976), Diane *et al*. (1981) and Tracy (2002), on the day of milking 1.4 ± 0.30 ng/mL, 0.60 ± 0.10 ng/mL and 0.15 ± 0.03 ng/mL, respectively. Whereas the progesterone values of 1.60 ± 0.08 ng/mL observed by Mohan (2007) is in accordance with the study. The increased mean serum progesterone concentration above the basal level observed in this study (> 1ng/mL) could be due to the endogenous production of progesterone by the corpus luteum that could have developed subsequent to withdrawal of exogenous estradiol – 17 β and progesterone administration, leading to new follicular wave.

The mean serum progesterone concentrations on the first day of lactation in the present study was estimated to be 1.64 ± 0.21 and 1.84 ± 0.16 ng/mL in Group I and Group II, respectively. while this study records slightly higher values of progesterone on first day of lactation, other researchers have observed 0.70 ± 0.10 ng/mL, 0.15 ± 0.02 ng/mL and 1.77 ± 0.13 ng/mL by Erb *et al*. (1976), Tracy (2002) and Mohan (2007), respectively.

On the first day of lactation the serum progesterone values in the induced lactating cows of this study was higher than the values (0.8 ± 0.10 ng/mL) of normally calved cows as reported by Erb *et al*. (1976). Similar mean serum progesterone concentration observed in the hormonally induced lactating cattle in present study. However, Tracy (2002) and Mohan (2007) reported much lower mean serum progesterone concentration of 0.15 ± 0.02 ng/mL and 0.62 ± 0.03 ng/mL, respectively in normally calved cows on the first day of lactation.

5.5 Serum estradiol – 17 β concentration

The mean serum estradiol – 17 β concentration of group I and group II on 0, 1, 4, 7, 13, 19 and 35 days of induction protocol is as shown in table 4 and fig. 5.

The mean serum estradiol – 17 β concentrations on the day 1 of lactation induction just before the administration of estradiol – 17 β and progesterone was estimated to be 49.84 ± 8.61 pg/mL and 30.58 ± 8.0 pg/mL in Group I and II, respectively. The estrogen values obtained in this study are similar to that of the values of estrogen in normal estrous cycle. However, lower mean serum estradiol – 17 β concentrations have also been reported by Diane *et al*. (1981) as 21 ± 6 pg/mL. So also a higher mean serum estradiol – 17 β concentrations have been reported Fleming *et al*. (1986) and Mohan (2007) as 336.2 pg/mL and 232 ± 21.24 ng/mL, respectively on this day.

The mean concentration of serum estradiol – 17 β increased significantly ($P < 0.05$) in both the groups from day 4 (2333 ± 257.60 pg/mL and 1933 ± 348.80 pg/mL) and maintained its elevated concentration till day 7 (3021 ± 264 pg/mL to 3360 ± 374.80 pg/mL) of induction of lactation when compared day with 1 (49.84 ± 8.61 pg/mL and 30.58 ± 8 pg/mL) (Table 3 Fig. 4). This pattern of increase in serum estradiol – 17 β concentration during the administration of estradiol 17 – β and progesterone for 7 days was in accordance with many authors (Fleming *et al*., 1986; Davis *et al*., 1983; DeLouis *et al*., 1978 and Mohan, 2007). The increase in the estradiol – 17 β values during the 7 day period might be attributed to the exogenous administration of estradiol – 17 β and progesterone.

The mean serum estradiol – 17 β concentrations on the day of milking was found to be 48.25 ± 10.34 pg/mL and 31.14 ± 2.41 pg/mL, respectively in Group I and Group II. The results were in accordance with Tracy (2002) and Erb *et al*. (1976) where in the mean serum estradiol – 17 β concentration was 45 ± 45 pg/mL and 109 ± 18 pg/mL, respectively.

The higher results were obtained by Mohan *et al.* (2007) and Diane *et al*. (1981) where in the mean serum estradiol – 17 β concentration were 533 ± 29.96 pg/mL and 569 ± 107 pg/mL, respectively. The estradiol – 17 β values obtained in the present study are in the similar range to the estradiol values in the normal lactating animals.

The concentrations of estradiol – 17 β and progesterone in blood serum is in accordance with Mohan (2007), but higher than the concentrations reported by Tracy (2002) and Sawyer *et al*. (1986) but lower than reported by Fleming *et al*. (1986). Such results are not unexpected because the quantities of exogenous steroids used in the present study were higher than used by Sawyer *et al.* (1986). When compared to the results of the present experiment, higher serum concentration of estradiol – 17 β and progesterone observed by Fleming *et al*. (1986) might be due to the continuous subcutaneous injections of estradiol – 17 β and progesterone for 21 days.

The variations in responses of hormone concentration in serum could be due attributed to difference in the duration of treatment for induction of lactation, the carrier for the hormone (alcohol *vs.* oil), the form of hormone (estradiol – 17 β *vs.* estradiol benzoate) and the intervals of injection (Harness *et al*., 1978 and Mohan, 2007). The other possibility may be contributed to variability are the rate of absorption of hormone from the injection site and existing functional status of endocrine glands, pituitary and adrenals during and following treatment (Smith and Schanbacher, 1973 and Mohan, 2007). The variations in the serum estradiol – 17 β and progesterone concentrations in the present study in both the groups might be attributed to the above said factors.

Summary

VI. SUMMARY

The artificial induction of lactation is a valuable technique to induce lactation in non-pregnant and non-lactating apparently healthy dairy cattle. This was clearly demonstrated in the present study wherein both the treated groups were successfully induced to lactation. The present study was conducted with the objective to induce lactation in unproductive dairy cattle by using two different protocols and to study the estrogen and progesterone concentrations in lactation induced animals. Induction of lactation was done hormonally in infertile dairy animals with combination of hormones and drugs. The present study demonstrated that lactation could successfully be induced in Group I and Group II cattle using combination of hormones (estradiol - 17β at 0.1mg/kg bw and progesterone at 0.25 mg/kg bw for 1 to 7 days) and drugs (Reserpine, 5 mg/animal, Dexamethasone 20 mg/animal per day for day 14 to 17).

The relative efficacy of different treatment protocols employed in the present study was noticed to be 80 per cent efficacious in both Group I and Group II. The observation clearly indicates that use of reserpine and dexamethasone improves the number of animals responding to the induction of lactation along with estradiol – 17 β and progesterone in both the groups.

The mean daily milk yield for the period of 100 days in both Group I and Group II were 5.04 ± 0.44 and 4.85 ± 0.21 litres, respectively. The mean total milk yield for the period of 100 days in Group I and Group II were 508.5 ± 44.28 and 493.3 ± 21.6 litres, respectively. The highest milk yield in 100 days of period was noticed on 8^{th} week in Group I and Group II which was 5.43 ± 0.55 and 5.14 ± 0.28 litres, respectively. The

quantity of mean milk recorded for the period of 100 days can be variable with the nutritional status of the animals and managemental practices.

The mean serum progesterone concentration on the first day of milking (day 19) was 1.64 ± 0.21 ng/mL and 1.84 ± 0.16 ng/mL in Group I and Group II, respectively. Similar mean serum progesterone values are reported in normally calved cows on first day of milking. On 16^{th} day of lactation (day 35) the mean serum progesterone concentration in lactation induced cows was increased to 1.82 ± 0.22 ng/mL and 1.96 ± 0.14 ng/mL in Group I and Group II, respectively which could be due to endogenous production of progesterone from the corpus luteum.

The mean serum estradiol – 17 β concentrations on first day of milking in lactation induced cows was 48.25 ± 10.34 pg/mL and 31.14 ± 2.41 pg/mL in Group I and Group II, respectively. The estradiol – 17 β values obtained in the present study are in similar to the estradiol values in the normally calved cows on first day of lactation.

This study shows that, lactation may be induced successfully in unproductive dry cattle by the use of estradiol - 17β, progesterone, reserpine, dexamethasone and $PGF_{2\alpha}$ which will reduce the economic burden on the farmer and improves the reproductive status of the animal.

Current results suggest that synchronizing estrous cycles of cows prior to initiation of lactation-induction protocol with two injections of $PGF_{2\alpha}$ 10 days apart, improves success rates of induced lactations and eliminate the variability in milk yields between cows, but this method of induction of lactation was not successful in increasing milk yields. Further research in the area of artificial induction of lactation with various programs for estrous synchronization and/or using other modifications to this protocol may increase milk yields of cows induced into lactation.

Conclusions from the present study;

1. Induction of lactation in unproductive dairy cattle can be induced successfully after overcoming the ethical issues.
2. The mean serum progesterone concentrations in induced cows can be comparable with mean serum progesterone concentrations of normal lactating cows.
3. The mean serum estradiol – 17 β concentrations in induced cows can be comparable with mean serum estradiol – 17 β concentrations of normal lactating cows
4. Lactation in induced cows can be maintained with good nutrition and managemental practices.
5. Current results suggest that synchronizing estrous cycles of cows prior to initiation of lactation-induction protocol with two injections of PGF2α 10 days apart, improves success rates of induced lactations, but this method of induction of lactation was not successful in increasing milk yields.
6. Further research in the area of artificial induction of lactation with various programs for estrous synchronization and/or using other modifications to this protocol may increase milk yields of cows induced into lactation.

Bibliography

VII. BIBLIOGRAPHY

ACCORSI, P. A., PACIONI, B., PEZZI, C. and FORNI. M., 2002. Role of prolactin, growth hormone and insulin-like growth factor 1 in mammary gland involution in the dairy cow. *J. Dairy Sci*., **85**: 507–513

AGARWAL, S., LAKHCHAURA, B. D., ATHEYA, U. K., DABAS, Y. P. S., SUD, S. C., and MISHRA, D. P., 1993. Levels of estrogen and progesterone in milk of animals induced into lactation by reproductive steroids and thyrotropic releasing hormone. *Indian J. Biol.,* **3**: 554-556

AKERS, R. M. and CLEALE, R. M., 1990. Effect of somatotropin during the dry period on subsequent milk production and induced secretion of somatotropin, prolactin and insulin pre and postpartum. *J. Dairy Sci*., **73**: 154

AKERS, R. M., 2002. Lactation and the mammary Gland. Iowa State Press, Iowa.

AKERS, R. M., BAUMAN, D. E., CAPUCO, A. V., GOODMAN, G. T. and TUCKER, H. A., 1981. Prolactin regulation of milk secretion and biochemical differentiation of mammary epithelial cells in periparturient cows. *Endocrinol.,* **109**: 23–30

AMENOMORI, Y., CHEN, C. L and MEITES, J., 1970. Serum prolactin levels in rats during different reproductive states. *Endocrinol.*, **86**: 506–510

ASIMOV, G. J. and KROUZE. N. K., 1937. The lactogenic preparations from the anterior pituitary and the increase of milk yield in cows. *J. Dairy Sci*., **20**: 289–306

BALL, S., POLSON, K., EMENY, J., EYESTONE, W. and AKERS, R. M., 2000. Induced Lactation in Prepubertal Holstein Heifers. *J. Dairy Sci.,* **83**: 2459–2463

BAUMAN, D. E. and VERNON. R. G., 1993. Effects of exogenous bovine somatotropin on lactation. *Annu. Rev. Nutr*., **13**: 437– 461

BAUMAN, D. E., 1991. Bovine somatotropin: review of an emerging animal technology. *J. Dairy Sci.*, **74**: 3913–3932

BAUMAN, D. E., COLLIER, R. J. and TUCKER, H. A., 1977. Effect of reserpine on serum prolactin, growth hormone, and glucocorticoids in dairy cows. *Proc. Soc. Exp. Biol. Med*., **155**: 189

BAUMAN, D. E., EPPARD, P. J., DEGEETER, M. J. and LANZA, G. M., 1985. Responses of high producing dairy cows to long-term treatment with pituitary somatotropin and recombinant somatotropin. *J. Dairy Sci*., **68**: 1352

BAUMAN, D. E., EVERETT, R. W., WEILAND, W. H. and COLLIER, R. J., 1999. Production responses to bovine somatotrophin in North-east dairy herds. *J. Dairy Sci.*, **82**: 2564–2573

BENSON, G. K., COWIE, A. T. and TINDAL. J. S., 1958. The pituitary and the maintenance of milk secretion. *Proc. Royal Soc*., **149**: 330–336

BLAXTER, K. L., REINEKE, E. P., CRAMPTON, E. W. and PETERSEN, W. E., 1949. The role of thyroidal materials and of synthetic goitrogens in animal production and an appraisal of their practical use. *J. Anim. Sci.*, **8**: 307–352

BRAUN, R. K., BERGMAN, E. N. and ALBERT. T. F., 1970. Effects of various synthetic glucocorticoids on milk production and blood glucose and ketone body concentrations in normal and ketotic cows. *J. Am. Vet. Med. Assoc*., **157**: 941–946

BRESCIANI, F., 1968. Topography of DNA synthesis in the mammary gland of the C3H mouse and its control by ovarian hormones: an auto radiographic study. *Cell Tissue Kinet*., **1**: 51-63

BRISKEN, C. 2002. Hormonal control of alveolar development and its implications for breast carcinogenesis. *J. Mammary Gland Biol. Neoplasia*., **7**: 39- 48

BRISKEN, C., KAUER, S., CHAVARRIA, T. E., BINHART, N., SUTHERLAND, R. L., WEINBERG, R. A., KELLY, P. A. and ORMANDY, C. J., 1999. Prolactin controls mammary gland development via direct and indirect mechanisms. *Dev. Biol.*, **210**: 96-106

BYATT, J. C., EPPARD, P. J., VEENHUIZEN, J. J., SORBET, R. H., BUONOMO, F. C., CUMAN, D. F. and COLLIER, R. J., 1992. Serum half-life and *in vivo* actions of recombinant bovine placental lactogen in the dairy cow. *J. Endocrinol.,* **132**: 185–193

CAMPBELL, P. G., SKAAR, T. C., VEGA, J. R. and C. R. BAUMRUCKER., 1991. Secretion of insulin-like growth factor-I and IGF-binding proteins from bovine mammary tissue *in vitro*. *J. Endocrinol.,* **128:** 219–228

CHAKRIYARAT, S., HEAD, H. H., THATCHER, W. W., NEAL, F. C. and WILCOX. C. J., 1978. Induction of lactation: Lactational, Physiological, and Hormonal Responses in the bovine. *J. Dairy Sci*., **61**: 1715

COLLIER, R. J. and TUCKER, H. A., 1978. Regulation of cortisol uptake in mammary tissue of cows. *J. Dairy Sci.,* **61:** 1709–1714

COLLIER, R. J., BAUMAN, D. E. and HAYS, R. L. 1977. Effect of reserpine on milk production and serum prolactin of cows hormonally induced into lactation. *J. Dairy Sci.* **60:** 896-901

COLLIER, R. J., BAUMAN, D. E. and HAYS, R. L., 1975. Milk production and reproductive performance of cows hormonally induced into lactation. *J. Dairy Sci*., **58**: 1524

COLLIER, R. J., CROOM, W. J., BAUMAN, D. E., HAYS, R. L. and NELSON, D. R., 1976. Cellular studies of mammary tissue from cows hormonally induced into lactation: lactose and fatty acid synthesis. *J. Dairy Sci*., **59**: 1226

CONVEY, E. M., 1974. Serum hormone concentrations in ruminants during mammary growth, lactogenesis, and lactation - A review. *J. Dairy Sci*., **57**: 905

COWIE, A. T. and FOLLEY, S. J., 1961. The mammary gland and lactation. In: Sex and Internal Secretions, 3rd Edn., Vol. I. W.C. YOUNG, Edn., The Williams & Wilkins Co., Baltimore, MD., *pp* 590–642

COWIE, A. T., FORSYTH, I. A. and HART, I.C., 1980. Hormonal control of lactation. Springer-Verlag, Berlin

CROOM, W. J., COLLIER, R. J. BAUMAN, D. E. and HAYS. R. L., 1976. Cellular studies of mammary tissue from cows hormonally induced into lactation: Histology and ultrastructure. *J. Dairy Sci.*, **59**: 1232

DABAS, Y. P. S. and SUD, S. C., 1989. Successive induction of lactation in cattle. *Asian Australas. J. Anim. Sci.,* **2 (4)**: 571-574

DAVIS, S. R., WELCH, R. A. S. PEARCE, M. G. and PETERSON, A. J., 1983. Induction of Lactation in Nonpregnant Cows by Estradiol-17β and Progesterone from an Intravaginal Sponge. *J. Dairy Sci*., **66**: 450-457

DELOUIS, C., DJIANE, J., KANN, G., TERGUI, M. and HEAD. H. H., 1978. Induced lactation in cows and heifers by short-term treatment with steroid hormones. *Ann. Biol. Anim. Bioch. Biophys.*, **18**: 721

DEMBINSKI, T. C. and SHIU, R. P. C., 1987. Growth factors in mammary gland development and function. In: The Mammary Gland Development, Regulation and Function. NEVILLE, M. C. and DANIEL, C. W. Edn. Plenum Press, New York, *pp* 355–381

DESHMUKH, B. T., JOSHI, V. G., KATKAM, R. R. and PURI, C. P., 1993. Hormonal induction of lactation in dairy cattle: Major milk constituents and oestradiol and progesterone levels in serum and milk. *Indian J. Anim. Sci.,* **63**: 611-617

DIANE L. J., ERB, R. E., CALLAHAK, C. J., MALVEN, P. V. and. VEENHUIZEN, E. L., 1981. Artificial Induction of Lactation In Cattle: Effect Of Modified Treatments On Milk Yield, Fertility, And Hormones In Blood Plasma And Milk. *Theriogenol*., **16**: 315-327

DJIANE, J. and DURAND, P., 1977. Prolactin-progesterone antagonism in self-regulation of prolactin receptors in the mammary gland. *Nature*., **266**: 641–643

EDGERTON, L. A. and HAFS. H. D., 1973. Serum luteinizing hormone, prolactin, glucocorticoid and progestin in dairy cows from calving to gestation. *J. Dairy Sci*., **56**: 451–458

EDMONSON, A. J., LEAN, I. J., WEAVER, L. D., FARVER, T. and WEBSTER, G., 1989. A body condition scoring chart for Holstein dairy cows. *J. Dairy Sci*., **72**: 68–78

ELLENHORN, M. J. and BARCELOUX, D. G., 1989. Medical toxicology – Diagnosis and treatment of human poisoning. New York. Elsevier, *pp* 295-299

ERB, R. E., MALVEN, P. V., MONK, E. L., MOLLETT, T. A., SMITH, K. L., SCHANBACHER, F. L. and WILLETT, L. B., 1976. Hormone induced lactation in the cow. *In*: Relationships between lactational performance and hormone concentrations on blood plasma. *J. Dairy Sci*., **59**: 1420

ERB, R. E., MONK, E. L., MOLLETT, T. A. MALVEN, P. V. and CALLAHAN. C. J., 1976a. Estrogen, progesterone, prolactin, and other changes associated with bovine lactation induced with estradiol-17 β and progesterone. *J. Anim. Sci*., **42**: 644

FLEMING, J. R., HEAD, H. H., BACHMAN, K. C., BECKER, H. N. and WILCOX. C. J., 1986. Induction of lactation: histological and biochemical development of mammary tissue and milk yields of cows injected with estradiol-17β and progesterone for 21 days. *J. Dairy Sci*., **69**: 3008

FLINT, D. J., 1995. Hormonal regulation of uptake and metabolism of milk precursors in normal lactating mammary gland. *J. Anim. Sci*., **73 (2)**: 61–71

FORSYTH, I. A., 1994. Comparative aspects of placental lactogens: structure and function. *Exp. Clin. Endocrinol.*, **102**: 244–251

FORSYTH, I. A., 1996. The insulin-like growth factor and epidermal growth factor families in mammary cell growth in ruminants: action and interaction with hormones. *J. Dairy Sci*., **79**: 1085–1096

FREITAS, P. R. C., COELHO, S. G., RABELO, E., LANA, A. M. Q. and ARTUNDUAGA M. A. T., 2010. Artificial induction of lactation in cattle. *J. Dairy Sci*., **86**: 23–24

FULKERSON, W. J., 1979. Hormonal control of lactation. *In*: Annual Research Reviews. Edt. HORROBIN. D. F., Eden Press, Montreal Canada

FULKERSON, W.J. and MCDOWELL, G.H., 1975. Artificial induction of lactation in cattle by use of dexamethasone trimethylacetate. *Aust. J. Biol. Sci*., **28**: 183-188

GALA, R. R. and WESTPHAL, U., 1965. Corticosterone-binding globulin in the rat: possible role in the initiation of lactation. *Endocrinol.,* **76**: 1079–1088

GARG, S. K. and NANGIA, O. P., 1972. Artificial induction of lactation in sterile cattle-A preliminary study. *Haryana Vet.,* **11**: 8-10

GILMAN, A. F., RALL, W. T., NIES, A. D. and TAYLOR, P., 1990. Goodman and Gilman's; *The Pharmacologic Basis of Therapeutics.* 8th Edn. Pergamon Press. New York. *pp* 795

GLIMM, D. R., BARACOS, V. E. and KENNELLY, J. J., 1990. Molecular evidence for the presence of growth hormone receptors in the bovine mammary gland. *J. Endocrinol.*, **126**: R5–R8

GOREWIT, R. C. and TUCKER, H. A., 1976. Glucocorticoid binding in mammary slices of cattle in various reproductive states. *J. Dairy Sci.*, **59**: 1890–1896

GRIINARI, J. M., MCGUIRE, M. A., DWYER, D. A., BAUMAN, D. E., BARBANO, D. M. and HOUSE, W. A., 1997. The role of insulin in the regulation of milk protein synthesis in dairy cows. *J. Dairy Sci.*, **80**: 2361–2371

GUPTA, A. P. and GUPTA, P. K., 1985. Metoclopramide as a lactogogue. *Clin. Pediatr.*, **24**: 269-273

HARNESS, J. R., ANDERSON, R. R., THOMPSON, L. J., EARLY, D. M. and YOUNIS, A. K., 1978. Induction of Lactation by Two Techniques: Success Rate, Milk Composition, Estrogen and Progesterone in Serum and Milk, and Ovarian Effects. J. Dairy Sci., **61**: 1725-1735

HARTMANN, P. E., TREVETHAN, P. and SHELTON, J. N., 1973. Progesterone and oestrogen and the initiation of lactation in ewes. *J. Endocrinol.*, **59**: 249-59

HASLAM, S. Z. and SHYAMALA, G., 1979a. Effect of oestradiol on progesterone receptors in normal mammary glands and its relationship with lactation. *Biochem. J.*, **182**: 127–131

HASLAM, S. Z. and SHYAMALA, G., 1979b. Progesterone receptors in normal mammary glands of mice: characterization and relationship to development. *Endocrinol.*, **105**: 786–795

HEAD, H. H., CHAKRIYARAT, S., THATCHER, W. W., WILCOX, C. J. and BECKER, H. N., 1982. Induction of Lactation: Comparison of Injections of Estradiol-173 and Progesterone for 7 or 21 Days on Prolactin Response to Thyrotropin Releasing Hormone and Milk Yield in Dairy Cattle. *J. Dairy Sci.*, **65**: 927-936

HEALD, C. W., 1974. Hormonal effects on mammary cytology. *J. Dairy Sci*., **57**: 917

HERRENKOHL, L. R., 1972. Effects on lactation of progesterone injections administered after parturition in the rat. *Proc. Soc. Exp. Biol. Med.*, **140**: 1356–1359

HOLLY, J. M. P. and WASS, J. A. H., 1989. Insulin-like growth factors: autocrine, paracrine or endocrine? New perspectives of the somatomedin hypothesis in the light of recent developments. *J. Endocrinol.*, **122**: 611–618

HOVEY, R. C., MCFADDEN, T. B. and AKERS, R. M., 1999. Regulation of mammary gland growth and morphogenesis by the mammary fat pad: a species comparison. *J. Mammary Gland Biol. Neoplasia.*, **4**: 53–68

HOVEY, R. C., TROTT, J. F. and VONDERHAAR., K., 2002. Establishing a framework for the functional mammary gland: from endocrionology to morphology. *J. Mammary Gland Biol. Neoplasia*., **7**: 17–38

IMAGAWA, W., YANG, J., GUZMAN, R. and NANDI, S., 1994. Control of mammary gland development. *In:* The Physiology of Reproduction. Edt. KNOBIL, E. and NEILL, J. D., 2nd Edn. Raven Press, Ltd., New York, *pp* 1033–1063

INGALLS, W. G., CONVEY, E. M., and HAFS. H. D., 1973. Bovine serum LH, GH, and prolactin during late pregnancy, parturition and early lactation. *Proc. Soc. Exp. Biol. Med*., **143**: 161

JACK, L. J. W., KAHL, S., ST. GERMAIN, D. L. and CAPUCO. A. V., 1994. Tissue distribution and regulation of 5′-deiodinase processes in lactating rats. *J. Endocrinol.*, **142**: 205–215

JOSIMOVICH, J. B. and MACLAREN. J. A., 1962. Presence in the human placenta and term serum of a highly lactogenic substance immunologically related to pituitary growth hormone. *Endocrinol.*, **71**: 209–220

JUERGENS, W. G., STOCKDALE, F. E., TOPPER, Y. J. and ELIAS, J. J., 1965. Hormone-dependent differentiation of mammary gland *in vitro*. *Proc. Natl. Acad. Sci.,* **54**: 629–634

KAHL, S., BITMAN, J., CAPUCO, A. V., and KEYS, J. E., 1991. Effect of lactational intensity on extrathyroidal 5′-deiodinase activity in rats. *J. Dairy Sci.,* **74**: 811–818

KANEKO, J. J., HARVEY, J. W and BRUSS, M. L., 2008. Clinical biochemistry of the domestic animals (Sixth Edition). *Clin. Reprod. Endocrinol*., *pp*, 639-640

KASUGA, M., VAN OBERGHEN, E., NISSLEY, S. P. and RECHLER. M. M. 1981. Demonstration of insulin-like growth factor receptors by affinity cross-linking. *J. Biol. Chem.,* **256**: 5305–5308

KEHRLI, M. E., BURTON, J. L., NONNECKE, B. J. and LEE, E. K., 1999. Effects of stress on leukocyte trafficking and immune responses: implications for vaccination. *in* Advances in Veterinary Medicine 41: Veterinary Vaccines and Diagnostics. R. D. Schultz, ed. Acad. Press, New York, *pp* 61–81

KENSINGER, R. S., 2000. Induced lactation: physiology, perception, profitability and propriety. *J. Dairy Sci.,* **83**: 23–24

KEYS, J. E. and DJIANE. J., 1988. Prolactin and growth hormone binding in mammary and liver tissue of lactating cows. *J. Receptor Res.,* **8**: 731–750

KNIGHT, C. H. and WILDE, C. J., (1993). Mammary cell changes during pregnancy and lactation. *Liv. Prod. Sci.,* **35**: 3-19

KNIGHT, C. H., 2001. Overview of prolactin’s role in farm animal lactation. *Livest. Prod. Sci.,* **70**: 87–93

KOPROWSKI, J. A. and TUCKER, H. A., 1973. Serum prolactin during various physiological states and its relationship to milk production in the bovine. *Endocrinol*., **92**: 1480–1487

KUHN, N. J., 1969. Progesterone withdrawal as the lactogenic trigger in the rat. *J. Endocrinol.*, **44:** 39-54

LAARVELD, B., CHAPLIN, R. K. and BROCKMAN, R. P., 1985. Effects of insulin on the metabolism of acetate, *β*-hydroxybutyrate and triglycerides by the bovine mammary gland. *Comp. Biochem. Physiol*., **82**: 265–267

LAMOTE, I., MEYER, E., MASSART-LE¨EN, A. M. and BURVENICH, C., 2004. Sex steroids and growth factors in the regulation of mammary gland proliferation, differentiation, and involution. *Steroids*., **69**: 145–59

LEMBOWICZ, K., RABEK, A., and SKREZECZKOWSKI, L., 1978. Hormonal induction of lactation in the cow. *Br. Vet. J*., **138**: 203

LORSCHEIDER, F. and REINEKE, E. P., 1971. The influence of lactational intensity and exogenous prolactin on serum thyroxine levels in the rat. *Proc. Soc. Exp. Biol. Med.*, **138**: 1116–1118

LYONS, W. R., 1958. Hormonal synergism in mammary gland growth. *Proc. Royal Soc.* **149**: 303–325

MACRINA, A. L., KAUF, A. C. W., PAPE-ZAMBITO, D. A. and KENSINGER, R. S., 2014. Induced lactation in pubertal heifers: Effect of dexamethasone and age at induction on milk yield and composition. *J. Dairy Sci.*, **97**: 1446-1453

MACRINA, A. L., TOZER, P. R. and KENSINGER, R. S., 2011. Induced lactation in pubertal heifers: Efficacy, response to bovine somatotropin, and profitability. *J. Dairy Sci.*, **94**: 1355–1364

MAGLIARO, A. L., KENSINGER, R. S., FORD, S. A., O'CONNOR, M. L., MULLER, L. D. and GRABOSKI, R., 2004. Induced Lactation in Nonpregnant Cows: Profitability and Response to Bovine Somatotropin. *J. Dairy Sci.*, **87**: 3290–3297

MARROW, D. A., 1980. *Current Therapy in Theriogenology*. WB Saunders Co., USA

MCFADDEN, T. B., AKERS, R. M. and KAZMER, G. W., 1987. Alpha-lactalbumin in bovine Serum: relationships with udder development and function. *J. Dairy Sci.*, **70**: 259

MCGRATH, C. M., 1983. Augmentation of the response of normal mammary epithelial cells to estradiol by mammary stroma. *Cancer Res*., **43**: 1355–1360

MEITES, J., 1950. Milk: the mammary gland and its secretions. Academic Press, New York. **1**: 321

MELLADO, M., ANTONIO-CHIRINO, E., MEZA-HERRERA, C., VELIZ, F. G., AREVALO, J. R., MELLADO, J. and DE SANTIAGO, A., 2011. Effect of lactation number, year, and season of initiation of lactation on milk yield of cows hormonally induced into lactation and treated with recombinant bovine somatotropin. *J. Dairy Sci.*, **94**: 4524–4530

MELLADO, M., NAZARRE, E. and OLIVARES, L., 2006. Milk production and reproductive performance of cows induced into lactation and treated with bovine somatotropin. *Animal Sci.*, **82**: 555-559

MOHAN, K., 2007. Pharmacological induction of lactation in repeat breeders. M.V.Sc thesis submitted to the KVAFSU, Bidar

NAGASAWA, H., CHEN, C. L. and MEITES. J., 1969. Effects of estrogen implant in median eminence on serum and pituitary prolactin levels in the rat. *Proc. Soc. Exp. Biol. Med*., **132**: 859–861

NARAYAN, D. and BHALERAO, V. R., 1947. Initiation of lactation in heifers and cows. *Curr. Sci.*, **16**: 29

NARENDARAN, R., HACKER, R. R., SMITH, V. G., and LUT, A., 1979. Hormonal induction of lactation oestrogen and progesterone in milk. *J. Dairy Sci.*, **62**: 1069-1075

NARENDRAN, R. and HACKER, R. R., 1974. Hormonal induction of lactation in heifers and cows. *J. Dairy Sci.,* **57**: 635

NEERU. and SINGH, M., 1997. Plasma progesterone levels in hormonally induced lactation in buffaloes. *Indian Vet. J.,* **74**: 652-654

NEVILLE, M. C., MCFADDEN, T. and FORSYTH, I., 2002., Hormonal regulation of mammary differentiation and milk secretion. *J. Mammary Gland Biol. Neoplasia*., **7**: 49-66

NEVILLE, M. C., MEDINA, D., MONKS, J. and HOVEY. R. C., 1998. The mammary fat pad. *J. Mammary Gland Biol. Neoplasia*., **3**: 109-116

OXENDER, W. D., HAFS, H. D. and Edgerton, L. A., 1972. Serum growth hormone, LH and prolactin in the pregnant cow. *J. Anim. Sci*., **35**: 51–55

PAAPE, M. J., and GUIDRY, A. J., 1973. Preliminary observations following hormonally induced lactation. *J. Dairy Sci.,* **56**: 657

PEEL, C. J., TAYLOR, J. W., ROBINSON, I. B. and HOOLEY, R. D., 1979. The use of oestrogen, progesterone and reserpine in the artificial induction of lactation in cattle. *Aust. J. Biol. Sci.,* ***32:*** 251-260

PEEL, C. J., TAYLOR, J. W., ROBINSON, I. B., MCGOWAN, A. A., HOOLEY, R. D. and FINDLAY, J. K., 1978. The importance of prolactin and the milking stimulus in the artificial induction of lactation in cows. *Aust. J. Biol. Sci.,* ***31***(2): 187-196

PUCA, G. A. and BRESCIANI, F., 1969. Interactions of 6,7-3H-17β estradiol with mammary gland and other organs of the C3H mouse *in vivo*. *Endocrinol*., **85**: 1–10

RADCLIFF, R. P., VANDEHAAR, M. J., SKIDMORE, A. L., CHAPIN, L. T., RADKE, B. R., LLOYD, J. W., STANISIEWSKI, E. P. and TUCKER, H. A., 1997. Effects of diet and bovine somatotropin on heifer growth and mammary development. *J. Dairy Sci*., **80**: 1996–2003

RAMGATTIE, R., SIEW, N., DIPTEE, M., STOUTE, V. and KNIGHTS, M., 2014. Effect of mammary stimulation on dairy cows and heifers exposed to a lactation induction protocol. *Open J. Animal Sci.,* **4:** 1-12

RANDEL, R. D. and ERB, R. E., 1971. Reproductive steroids in the bovine. VI. Changes and interrelationships from 0 to 260 days of pregnancy. *J. Anim. Sci*., **33**: 115–123

RAY, D. B., JANSEN, R. W., HORST, I. A., MILLS, N. C. and KOWAL. J., 1986. A complex noncoordinate regulation of α-lactalbumin and 25 K β-casein by corticosterone, prolactin, and insulin in long term cultures of normal rat mammary cells. *Endocrinol*., **118**: 393-407

REECE, R. P, 1943. Initiation and Maintenance of Lactation in Dairy Heifers by Hormone Administration. *J. Dairy Science*., 145

RIVERA, E. M. 1964. Maintenance and development of whole mammary glands of mice in organ culture. *J. Endocrinol*., **30**: 33–39

ROBINSON, G. W., 2007. Cooperation of signalling pathways in embryonic mammary gland development. **8**: 963-973

ROSE, M. T. and OBARA, Y., 2000. The manipulation of milk secretion in lactating dairy cows: review. *Asian-Aus. J. Anim. Sci*., **13**: 236–43

SAWYER, G. J., FULKERSON, W. J., MARTIN, G. B. and GOW, C., 1986. Artificial Induction of Lactation in Cattle: Initiation of Lactation and Estrogen and Progesterone Concentrations in Milk. *J. Dairy Sci.,* **69**: 1536-44

SHETH, N. A., TIKEKAR, S. S., RANADIVE, K. J. and SHETH, A. R, 1978. Influence of bromoergocryptine on estrogen-modulated prolactin receptors of mouse mammary gland. *Mol. Cell. Endocrinol*., **12**: 167–176

SHRIDHAR, N. B., and NARAYANA, K., 2006. Pharmacological induction of lactation in heifers. *Indian Vet. J.,* **83**: 582-583

SILBERSTEIN, G. B. and DANIEL, C. W., 1982. Elvax 40P implants, sustained local release of bioactive molecules influencing mammary ductal development. *Dev. Biol*., **93**: 272–278

SIRBASKU, D. A., 1978. Estrogen induction of growth factors specific for hormone-responsive mammary, pituitary, and kidney tumor cells. *Proc. Natl. Acad. Sci.*, **75**: 3786–3790

SJAASTAD, O. V., HOVE, K. and SAND, O., 2003. Physiology of domestic animals. Scandinavian Veterinary Press

SMITH, B. B. and WAGNER, W. C., 1980. Lactation physiology of the pig. 9^{th} International Congress on Animal Reproduction and Artificial Insemination, Held between 16th-20th June 1980. III. Symposia (free communications). Editorial Garsi.

SMITH, K. L. and SCHANBACHER, F. L., 1973. Hormone induced lactation in the bovine. I. Lactational performance following injections of 17-*β* estradiol and progesterone. *J. Dairy Sci*., **56**: 738–743

SMITH, K. L. and SCHANBACHER, F. L., 1974. Hormone induced lactation in the bovine. II. Response of nulligravida heifers to modified estrogen-progesterone treatment. *J. Dairy Sci.,* **57**: 296

SMITH, K. L., 1971. Role of estrogen in selective transport of IgG_1, into the mammary gland. *J. Dairy Sci.*, **54**: 296-303

SMITH, V. G., EDGERTON, L. A., HAFS, H. D. and Convey, E. M., 1973. Bovine serum estrogens, progestins and glucocorticoids during late pregnancy, parturition and early lactation. *J. Anim. Sci.*, **36**: 391–396

SORENSSON, A. and KNIGHT, C. H., 2002. Endocrine profiles of cows undergoing extended lactation in relation to the control of lactation persistency. *Domest. Anim. Endocrinol.*, **23**: 111–23

SPEICHER, J. A., TUCKER, H. A., ASHLEY, R. W., ATANISIEWSKI, E. P., BOUCHER, J. F. and SNIFFEN, J., 1994. Production responses of cows to recombinantly derived bovine somatotropin and to frequency of milking. *J. Dairy Sci.*, **77**: 2509–17

STEEL, R. G. D. and TORRIE, J. 1981. Principles and procedures of statistics: A biometric approach. 2nd Edn. McGraw Hill International Book Agency, Singapore

SUD, S. C., 1972. Induction of lactation in intact and ovariectomized heifers with 9 fluroprednisolone acetate. *Indian J. Exp. Biol.*, **10**: 265-267

SUD, S. C., TUCKER, H. A. and MEITES, J., 1968. Estrogen-progesterone requirements for udder development in ovariectomized heifers. *J. Dairy Sci.*, **51**: 210–214

TERVIT, H. R., FAIRCLOUGH, R. J., MCGOWEN, L. T., MACKEN- ZIE, D. S., MACMILLAN, K. L. and PETERSON, A. J., 1980. Induction of lactation in dry cattle. *New Zealand Vet. J.*, **28**: 15

THATCHER, W. W. and TUCKER, H. A., 1970. Lactational performance of rats injected with oxytocin, cortisol-21-acetate, prolactin and growth hormone during prolonged lactation. *Endocrinol*., **86**: 237–240

TOPPER, Y. J. and FREEMAN, C. S., 1980. Multiple hormone interactions in the developmental biology of the mammary gland. *Physiol. Rev*., **60**: 1049–1056

TRACY., 2002. Artificial induction of lactation in nonbreeder dairy cows. M.Sc. Thesis submitted to Virginia Polytechnic Institute and State University

TUCKER, H. A. 2000. Hormones, mammary growth, and lactation: a 41-year perspective. *J. Dairy Sci*., **83**: 874

TUCKER, H. A. and MEITES, J., 1965. Induction of lactation in pregnant heifers with 9-fluoroprednisolone acetate. *J. Dairy Sci*., **48**: 403–405

TUCKER, H. A., 1981. Physiological control of mammary growth, lactogenesis, and lactation. *J. Dairy Sci.,* **64**(6): 1403-1421

TURNER, C.W., YAMAMOTO, H. and RUPPERT, H. L. J. R., 1956. The experimental induction of growth of the cow's udder and the initiation of milk secretion. *J Diary Sci.* **39**: 1717

TYLER, H. D. and ENSMINGER, M. E., 2006. Dairy Cattle Science. Pearson Prentice Hall

VANHOUTEN, J., DANN, P., MCGEOCH, G., BROWN., E. M., KRAPCHO, K. and NEVILLE, M., 2004. The calcium-sensing receptor regulates mammary gland parathyroid hormone-related protein production and calcium transport. *J. Clin. Invest*., **113**: 598–608

VERMA, H. K., KUMAR, S., JINDAL, R., SINDHU, S. S. and PANHAONKAR, G. R., 2004. Artificial lactogenesis in bovines – A review. *J. Res. Punjab Agri. Univ.,* **41**(2): 266-283

WALLACE, C. R. 1993. Concentration of bovine placental lactogen in dairy and beef cows across gestation. *Domest. Anim. Endocrinol.,* **10**: 67–70

WILFET, L. B., LARRY, B. K., SMITH, F., SCHANBACHER, L., ERB, E., and MALVER, P. V., 1976. Hormonal induced lactation in the bovines: Dynamics of injected and endogenous hormones. *J. Dairy Sci.,* **59**: 504-514

WILLIAMS, R. and TURNER, C. W., 1960. Effect of increased levels of ovarian hormones and duration of treatment on the experimental induction of growth of the cow udder. *J. Dairy Sci.,* **44**: 524

WILLIAMS, R., CHILDS, O. A., SMITH, D. and TURNER, C. W., 1955. The Effects of the Hormones Progesterone and Estrogen in Initiating Lactation in Dairy Cows. *J. Dairy Sci*., **38**: 609

WOODWARD, T. L., BEAL, W. E. and AKERS, R. M., 1993. Cell interactions in initiation of mammary epithelial proliferation by estradiol and progesterone in prepubertal heifers. *J. Endocrinol*., **136**: 149–157

YAMAMOTO, H. and TURNER, C. W., 1955. Experimental udder growth and lactation in infertile dairy heifers. *J. Dairy Sci.,* **38**: 629-634

YANG, J., RICHARDS, J., GUZMAN, R., IMAGAWA, W. and NANDI, S., 1980. Sustained growth in primary culture of normal mammary epithelial cells embedded in collagen gels. *Proc. Natl. Acad. Sci*., **77**: 2088–2092

YOUNG, F. G., 1947. Experimental stimulation (galactopoiesis) of lactation. *Br. Med. Bull.* **5**: 155

Abstract

VIII. ABSTRACT

The present study was conducted with the objective to induce lactation in unproductive dairy cattle by using two different protocols and to study the estrogen and progesterone concentrations in lactation induced animals. Induction of lactation was done hormonally in infertile dairy animals with combination of hormones and drugs. Two groups of cattle (Groups I and II, n=10) were treated with 0.1 mg/kg estradiol – 17 β and 0.25 mg/kg progesterone S/C for seven consecutive days. Both groups of animals were administered with dexamethasone (20 mg/day) and reserpine (5 mg TD/day) IM on days 14 to 17. Both the groups of animals were synchronised by double $PGF_{2\alpha}$ protocol in 10 days apart prior to initiation of hormone therapy. Group II cows received two doses of 0.25 mg $PGF_{2\alpha}$, IM on day 13 of hormone therapy. Results revealed that the 100-day mean milk production in Group I and Group II was 508.50 ± 44.28 litres and 493.30 ± 21.60 litres, respectively. It is suggested that administration of progesterone and estradiol – 17 β with $PGF_{2\alpha}$, reserpine and dexamethasone is a viable method of inducing lactation in unproductive dairy cattle. Induction of lactation in unproductive dairy cattle can be induced successfully after overcoming the ethical issues. Synchronizing estrous cycles of cows prior to initiation of lactation-induction protocol with two injections of $PGF_{2\alpha}$ 10 days apart, improves success rates of induced lactations, but this method of induction of lactation was not successful in increasing milk yields. Lactation in induced cows can be maintained with good nutrition and managemental practices. Further research in the area of artificial induction of lactation with various programs for estrous synchronization and/or using other modifications to this protocol may increase milk yields of cows induced into lactation.