THELOSCOPIC AND ULTRASONOGRAPHIC EVALUATION OF UDDER AND TEAT AND MINIMALLY INVASIVE THELOSCOPIC SURGERY WITH BIO-IMPLANT FOR MILK FLOW DISORDERS IN DAIRY COWS AND BUFFALOES

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CERTIFICATE

This is to certify that the thesis entitled **"THELOSCOPIC AND ULTRASONOGRAPHIC EVALUATION OF UDDER AND TEAT AND MINIMALLY INVASIVE THELOSCOPIC SURGERY WITH BIO-IMPLANT FOR MILK FLOW DISORDERS IN DAIRY COWS AND BUFFALOES"** submitted in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY IN VETERINARY SURGERY AND RADIOLOGY** to the Tamilnadu Veterinary and Animal Sciences University, Chennai – 51 is a record of bonafide research work carried out by **H. PUSHKIN RAJ** under my guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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Dedicated

To My

Late Father

&

My Family

&

to Buiatric

Practitioners

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ABSTRACT

Title	**:**	**THELOSCOPIC AND ULTRASONOGRAPHIC EVALUATION OF UDDER AND TEAT AND MINIMALLY INVASIVE THELOSCOPIC SURGERY WITH BIO-IMPLANT FOR MILK FLOW DISORDERS IN DAIRY COWS AND BUFFALOES**
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The study was carried out on the clinical cases of lactating cows and buffaloes, with apparently healthy mammary gland, reported for minor surgical affections and milk flow disorders to the Large Animal Surgical Outpatient Unit of Madras Veterinary College Teaching Hospital, Chennai. A total of 98 cows and 24 buffaloes were reported during the period of study. Based on the parity of third calving with early to mid lactation phase and irrespective of the breed, 36 animals (18 cows and 18 buffaloes) were selected for the study and were divided into groups I and IIA and IIB. In group I six healthy cows and six buffaloes were subjected to normal udder and teat study and in group IIA six cows and six buffaloes were treated for the teat obstructive disorders by lateral theloscopic surgery with implant and in group IIB six cows and six buffaloes were treated by lateral theloscopic surgery without implant.

The incidence, etiology, side, site and type of udder and teat lesions in cows and buffaloes were studied. The incidence was more on the left side and hind teats were found to be more affected with high incidence of single teat and quarter affections were recorded. The internal teat lesions involving the distal part of the teat caused by physical trauma accounted the major cause for obstructive milk flow disorders.

Among the teat lesions in cows and buffaloes, obstructive disorder at the teat canal area was recorded to be the highest incidence. In normal cows and buffaloes the mean

teat tip to floor distance was higher in cows than in buffaloes. The mean fore teat length in cows and the mean hind teat length in buffaloes were found to be higher than their respective mean teat lengths. The mean teat girth and mean teat canal length measured by graduated probe was higher in buffaloes than in cows. The mean milkability by hand was found to be higher in cows than in buffaloes and in both the cows and buffaloes the mean milkability was higher in hind quarters than in fore quarters. The mean electrical conductivity values were found to be higher in buffaloes than in cows and the mean somatic cell count was found to be lower in buffaloes than in cows.

Double contrast radiography enabled better diagnosis than plain, positive and negative contrast techniques.

Ultrasonographic examination of teat was done by water bath technique and gland cistern and udder examination was done by contact with gel application technique. In water bath technique use of triple glass distilled water filled in polyethylene cup were found to be ideal for effective visualisation of teat structures.

Ultrasonographic quantification of teat revealed longer mean teat canal length, increased mean teat wall thickness and cistern width in buffaloes than in cows.

In the diseased cows and buffaloes that were treated with natural teat implant after theloscopic surgery showed higher mean milkability, lowered electrical conductivity and somatic cell count than those that were not treated with implants. Ultrasonographic examination of the diseased teats of cows and buffaloes treated with implants after theloscopic surgery showed normal restoration of mean teat canal length, teat diameters, teat cistern width and teat wall thickness than those animals that were not treated with implants.

Theloscope was useful as an effective and less-invasive diagnostic and therapeutic tool for the treatment of teat lesions and found to have limited application depending on the severity of the pathological lesions of the udder and teat.

Whereas ultrasonography aided in identifying the udder parenchymal diseases early changes in the obstructive disorders of teat and aided to study the healing pattern following removal of the obstructions. However, the diameter of the scope along with the blow pipe used in this study was found to be higher for the Indian cross bred dairy cattle.

Use of natural teat insert implant after theloscopic surgery in group IIA cows and buffaloes restored normal milkability, milk somatic cell count, teat canal length and patency and normal teat tissue structure and function were maintained on long term follow up than those animals that were not treated with implants.

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CHAPTER 1

INTRODUCTION

Dairy industry in India has become an important sector in the agricultural economy and milk is the second largest agricultural commodity contributing to the GDP next to rice. India, the world's largest milk producing country produced about 104.8 million tonnes of milk per annum (2008-09) and contributed about 15 per cent of the world production level through the intensive practice of upgrading the native germplasm of dairy cows and buffaloes.

In dairy cows and buffaloes, the shape, structural and functional integrity of the teat tissues especially the teat canal and the sphincter, anatomical differences such as cisternal size, volume, teat and teat canal length and diameter, and physiological variance such as milk ejection process and time vary with breed, age, nutritional status, phase of lactation and disease condition. These factors are predicted to influence the udder health and productive life of the upgraded dairy cows and buffaloes and play an important role in economic and hygienic milk production (Berry *et al.,* 2005).

Of the various disease conditions of the mammary gland in bovine, milk flow disorders due to traumatic covered teat injuries, faulty milking technique, infection, impaired teat canal status, inflammation in mucosal region, tissue proliferation, foreign bodies, milk stones, congenital disorders in teat cavity and management practices hamper the udder health and milk production (Saifzadeh *et al.,* 2005). This leads to different kinds of mastitis, which consequently leads to reduced milk yield and milk quality (Barlett *et al.,* 1991), detrimental changes in the milk components and raw milk quality (Auldist *et al.,* 1995), increased costs for the treatment of the animal (Berry and Amer, 2005), loss of productive quarters and early involuntary culling of dairy cows, which results in a negative economic impact to the dairy animal owners (Rupp and Boichard, 1999).

The surgical treatment of such obstructive milk flow disorders in bovine remains challenging. A rapid and accurate diagnosis and prognosis is mandatory in patients with udder and teat diseases which requires the use of state of-the-art examination techniques and speedy recovery aiding therapeutic treatments.

For several decades the diagnosis of the udder and teat affections in field conditions are carried out by a good anamnesis with thorough clinical examination involving inspection, palpation and probing techniques. These procedures fail to diagnose and image the lesions in the initial disease process and limit the investigation process of the live nature of the lesion.

Ultrasonography is a practical non-hazardous, non-invasive, radiation free, reliable diagnostic unique imaging modality for soft tissue structures of the body. It helps in early diagnosis to localise and determine the amount of pathologic alteration in mammary tissues and enables us to initiate appropriate treatment without any damage to the udder parenchyma and milk yield. This technique also helps to localise, demarcate and quantify the changes in the teat wall thickness, extent of teat stenosis and other abnormalities (Dinc *et al.,* 2000). Routine and needy applications of this technique enable early diagnosis of the pathological conditions, provide an opportunity to identify the cause, preventive measures and early treatment to be taken and could help the owners to acquire sound productive animals. It is also useful to monitor and document the healing process after surgical removal of proliferative tissue or lesions.

Conventional teat surgery for obstructive milk flow disorders involves blind insertion of the blunt/sharp instruments through the teat canal into the teat cistern and the obstruction is blindly incised or removed (Donawick, 1985; Hull, 1985 and Ducharme *et al.,* 1987). This results in indiscriminate trauma to the delicate mucosal lining of teat cistern and acute to chronic proliferative teat sinusitis with more granulation and fibrous connective tissue formation. As squeal to this, a high rate of complications such as recurrence of obstruction to milk flow, mastitis, decreased milk production, increased milking time are noticed and long-term success rates of these techniques are found to be fair to poor (Witzig *et al.,* 1984).

To overcome these complications teat endoscopy serves as an effective less-invasive diagnostic and minimally invasive therapeutic tool for the treatment of covered teat injuries causing stenosis and obstructive milk flow disorders. This procedure facilitates the direct visualization, precise identification of the lesions and permits to document the diagnosis and treatment of intracisternal teat lesions in cows and buffaloes (Medl *et al.,* 1994; Seeh and Hospes, 1998; Kiossis *et al.,* 2002 and Geishauser *et al.,* 2005). The obstructing tissues are precisely excised with delicate handling and minimal pain without the development of strictures and tissue hyperplasia secondary to scar tissue formation. Thus with the above features, this technique helps to restore an early normal milk flow, milk yield and somatic cell count of the affected quarters with limited hospitalisation, less intensive postoperative care and few recurrent episodes of mastitis in the current and future lactation periods (Hirsbrunner *et al.,* 2001).

To prevent adhesions and to keep the teat canal patent after teat surgery biocompatible teat implants such as silicone rods, free fatty acid rods etc. are currently used. These bio-implant augments the healing pattern of teat canal without stricture formation and thereby normal milk flow through the diseased/injured teat is restored.

Hence, a correlative study on the clinical signs, nature, site and severity of the udder and teat lesion with radiography, ultrasonography and theloscopy and the clinical outcome of treatment of teat lesions using theloscope with or without the use of bioimplants was undertaken with the following objectives.

Objectives :

1. To study the normal and diseased udder and teat in dairy cows and buffaloes with milk flow disorders using theloscope and correlate with the clinical, radiographic and ultrasonographic findings.

2. To study the utility of teat bio-implants for various teat affections in dairy cows and buffaloes.

3. To perform and evaluate minimally invasive theloscopic surgery for milk flow disorders in dairy cows and buffaloes.

CHAPTER 2

REVIEW OF LITERATURE

2.1 FUNCTIONAL ANATOMY AND PHYSIOLOGY OF THE UDDER AND TEAT

2.1.1 Mammary Gland

The mammary gland of bovine is a modified sweat gland located in the inguinal region suspended externally and ventrally on the body wall by ligaments and lamella. The udder of domestic cows and buffaloes is a very large, hemispherical, glandular body consisting of four quarters each with one teat. The size and structure of the gland varies considerably and is dependant on several factors such as age, breed, nutritional status and the phase of lactation. A distinct intermammary groove indicates the borders between the two halves of the udder and is represented as right and left sided glands and a weak demarcation on each side called as udder yoke, represents line of separation of the fore and hind quarters. The rear quarters account for 55 to 60 per cent of the milk produced and 55 to 60 per cent of udder weight. Each quarter is separated from one another with a separate cavity system and glandular lobes. The cranial fore quarters are generally smaller than the caudal or hind quarters. Each gland consists of numerous irregularly round or angular areas with granular yellow appearance structures called as glandular lobules. The lobules are separated by delicate, whitish–yellow interlobular connective tissue networks composed of elastic fibers. The ratio of the glandular tissue to connective tissue varies between individuals. Lactating udder contains less connective tissue than the non-lactating gland. A lactating udder with more supporting connective tissue than glandular parenchyma is defined as “fleshy udder”. Such glands produce little milk and are always felt as firm and hard on palpation even after complete milking. Within the supporting tissues of the udder are the interlobular milk ducts lined by one or two layered columnar epithelium and are accompanied by numerous blood and lymph vessels, nerve fibers and isolated smooth muscle fibers. Each mammary gland lobule (clusters of 150 to 220 alveoli, 7 to 8 mm diameter) is made up of closely placed glandular acini the basic secretory unit of milk of the mammary gland. The acini are separated from one another by thin septa and are linked to the intralobular ducts surrounded by contractile myoepithelial cells, very fine connective tissue fibers and blood capillaries. The acini are one cell layer thick, varies considerably in height from low cuboidal to tall columnar depending on the secretory state and degree of fullness of the udder and are closely linked together at their apex by tight junctions. Tall columnar cells with secretory apices are seen immediately preceding and during the onset of milking. The glandular acini functions as aprocine gland and milk is produced. The intralobular ducts converge and unite with the inter lobular ducts at a fairly acute angle of between 20 and 45 º and forms large milk ducts. In fore quarters they run mainly along the lateral surface and in hind quarters along the caudal surface. The number of ducts varies from quarter to quarter; there are usually between 8 to 12 ducts per quarter and they drain the milk into the glandular cistern. The present day dairy cow has the largest udder and the longest and most branched mammary duct system of all land living mammalian species. At the transition between the teat and glandular parts of the cistern there is usually a distinct constriction caused by the formation of circular fold 2 to 6 mm in thickness and is considered as the border of the base of the teat. It consists of firm connective tissue and circularly arranged veins (Furstenberg's venous ring). This venous ring and the elastic muscular fibers around the teat canal demarcates the teat sinus from the gland sinus and prevents the outflow of milk except during milking and suckling.

The teats of normal individuals are cylindrical, peg-like appendages of the udder with rounded tips and devoid of hair. Average size of the fore teats of cow is about 6.6 cm long and 2.9 cm in diameter and the rear teats is 5.2 cm long and 2.6 cm in diameter. But teats as short as 2.5 cm and as long as 14 cm are also reported. The buffalo teats are larger than the cow teats. The front teats are, on average 5.8 cm to 6.4 cm long and their diameter is approximately 2.5 to 2.6 cm . The respective figures of hind teats are 6.9 cm to 7.8 cm and 2.6 to 2.8 cm (Thomas *et al.,* 2004). The teat consists of the teat wall, apex with the streak canal and teat sinus. Proximally the teat sinus is continuous with the corresponding gland sinus and is usually large and elongated. The total milk storage capacity of the cistern is estimated to be less than 30 per cent in cows and 4.9 per cent in buffaloes. The wall of the teat consists of the following layers: innermost is the teat sinus followed by the submucosa, middle very muscular and vascular connective tissue layer surrounded and externally by cutis. The inner wall consists of thin mucous membrane lined by a two-layered cuboidal epithelium. It is yellow and when empty exhibits a delicate pattern of net like ridges and folds. The middle layer consists of collagenous and elastic connective tissue and contains numerous bundles of smooth muscle with thick – walled veins and form a long-meshed erectile body. These vessels become engorged with blood during milking and suckling processes. This layer also functions as the "haemostatic apparatus" and is important in holding back the milk under certain physiological and psychic influences. Externally the teat is covered by a stratified squamous epithelium devoid of hairs and glands but richly supplied with sensory nerves. The streak canal (teat canal, papillary duct) is lined with a stratified squamous epithelium and keratin. It varies in length from 0.5 to 1.5 cm in cows and 1.9 to 5.5 cm in buffaloes (Thomas *et al.,* 2004). It varies in length between 5 to 10 mm and is located at the apex of the teat. It connects the teat sinus to the outside ending at the teat orifice. The rosette of Furstenberg – where the stratified squamous epithelium of the streak canal meets the two layered cuboidal epithelium of the teat sinus, represents the proximal delineation of the streak canal. The teat sphincter is located beneath the rosette of Furstenberg and consists of circularly oriented bundles of smooth muscle fibers. The teat sphincter and keratin lining of the streak canal are responsible for milk continence and preventing ascending infections. The teat sphincter tonus has been reported to be at least 400 mmHg negative pressure in buffaloes and this is the cause of buffaloes being "hard milkers". During milking the velocity of milk flow from the teat with a teat canal diameter of 2 mm is estimated to be 8.5 m/s and milk flow with a shear force of 1.8×10^{-2} N is estimated to debride the keratin surfaces of the teat canal (Williams and Mein, 1986).

2.1.2 Arterial System

The arterial blood supply to the bovine udder is mainly derived from the external pudental artery and from a few smaller branches of the internal pudental artery. External pudental artery approaches the base of the udder makes sigmoid bend and divides above the posterior teat into cranial and caudal mammary arteries and they branch further as it descends down into the gland. Many possible deviations of arterial blood supply to the gland have been reported. Cranial mammary artery runs in a cranioventral direction within the mammary parenchyma and vascularises the cranial part of the hind quarter. Caudal mammary artery runs caudoventrally in the hind quarter. The papillary artery is the only artery supplying the teat and may originate from various parent vessels. In the teat wall it takes a lateral, caudal or medial course depending from which mammary artery or from which the artery stems. It runs from the base to the tip of the teat near the inner surface of the wall and passes between large and greatly branched veins.

There is essentially no cross over of blood supply between left and right udder halves (although there sometimes are a few minor exceptions) but it is reported that the mammary vessels of the left side are usually thicker than those of the right. About 400 to 500 litres of blood must flow through the udder to facilitate exchange of nutrients to produce one liter of milk.

2.1.3 Venous System

A continuous rich blood supply is essential for the normal physiological function of the lactating gland. The capacity of the udder veins is about fifty times greater than that of the arteries. From the erectile body of the teat blood passes to the Furstenberg's venous ring and they reach the cranial, caudal and mammary veins at the base of the udder. By cross anastomoses these veins finally terminate into the following veins. External pudental vein passes through the inguinal space into the pudento-epigastric vein, dorsal labial and mammary vein runs caudodorsally over the ischiatic notch into the internal pudental vein and voluminous milk vein (Subcutaneous abdominal vein) carries the blood in a cranial direction and enters the body cavity at the xiphoid process via "milk wells", which eventually empties into the vena cava.

2.1.4 Nervous System

The udder is well supplied by the sensory and autonomic nerves. Sensory innervation is mainly through the following spinal nerves: iliohypogastric and ilioinguinal nerve which innervates the skin of the forequarters and the cranial part of the base of the udder, genitofemoral nerve innervates the skin, teats and the glandular tissue except the caudal regions of the hindquarters and pudental nerve innervates the caudal regions of the hindquarters. Autonomic innervation is mainly by the sympathetic fibers of the caudal mesenteric ganglion. It courses along with the genitofemoral nerve to the udder and innervates the myoepithelial cells, the smooth muscle fibers and the blood vessels, not the acini.

2.1.5 Lymph and Lymphatics

In the udder, all the lymph flows through the supramammary lymph nodes. There are usually one or two per udder half, but sometimes up to 7 nodes per udder half. Frequently there are accessory lymph nodes inside the gland and often there are superficial lymph nodes just beneath skin. The lymph vessel leaves the mammary gland via the inguinal canal (similar to the blood vessels) (Schumer *et al.,* 1981).

2.2 PATHOPHYSIOLOGY OF MILK OUTFLOW DISORDERS OF TEAT

The teat canal functions as a strong and important primary barrier against invasion of mastitis pathogens into the udder (Hamann, 1987). The sphincter muscle surrounding the teat duct is tightly closed between milkings and impedes bacterial intrusion from the teat opening into the interior of the gland (Nickerson, 1994).

The keratin in the teat canal can trap invading bacteria and contains antimicrobial agents (Hibbit *et al.,* 1969 and Capuco *et al.,* 1992). An essential mechanism of the ability of limiting bacterial growth is the desquamation of mature keratin largely by the speed or changing speed of milk flow that removes keratin and adhering bacteria. Production and loss of keratin by the teat canal should be in equilibrium. It is estimated that 40 per cent of the keratin should be washed out during milking to maintain a fresh and healthy layer in the teat canal (Woolford, 1997).

Cows with higher milk yield often exhibit higher peak flow rates (Miller *et al.,* 1993; Wellnitz *et al.,* 1999; Wagner and Ruegg, 2002 and Inderwies *et al.,* 2003). In high yielding cows intramammary pressure is higher, probably due to larger vertical milk columns in the udder cisterns.

A relationship between peak flow rate and intramammary infection has been found in several studies (Van de Geer *et al.,* 1988; Grindal *et al.,* 1991; Grindal and Hillerton, 1991; Duda, 1995; Lacy-Hulbert and Hillerton, 1995; Dodenhof *et al.,* 1999 and Rupp and Boichard, 1999). Quarter peak flow rate of >1.6 kg/min (compared to < 0.8 kg/min) was associated with a 12 fold increase in intramammary infection (Grindal and Hillerton, 1991) and udder peak flow rate as well as mean flow rate of 3.5-4.0 kg/min was associated with the lowest intramammary infection rate (Roth *et al.,*1998). The shear forces of high peak flow rate (Williams and Mein, 1986) may remove keratin above the physiologically preferred amounts.

Higher flow rates with shorter teat canals (Naumann and Fahr, 2000) and higher milk yield are associated with higher stretchability of the teat canal (Rathore and Sheldrake, 1977) and are caused by a combination of thinner teat walls and the fact that the teat canal is surrounded by a small volume of elastic tissue rear versus front quarters (Ledu *et al.,* 1994).

Hamann and Mein (1988) documented the recovery of the teat tissues in cows after suckling by calves. The authors observed a recovery of ± 2 per cent of pre-milking thickness of the teat tip within 15 to 30 minutes.

Inflammation to the teat can be caused by many types of injury including infectious agents and their toxins, physical trauma or chemical irritants. Changes in the pliability of teat tissue caused by congestion or edema may change the resistance of the teat canal to bacterial invasion (O'Shea, 1987). Changes of the teat wall such as increased congestion and edema results in slower closure of the teat canal and/or hypoxia in teat tissues (Hamann *et al.,* 1994). Zecconi *et al.* (1992 and 1996) showed that an increase of teat thickness of over five per cent after milking is associated with an increased incidence of IMI (intramammary infection).

In the dairy cow, mastitis is nearly always caused by microorganisms, usually bacteria, that invade the udder, multiply in the milk-producing tissues and produce toxins that are the immediate cause of injury. The development of the disease starts with penetration of the teat canal by pathogens. When pathogens enter the udder, a non-specific (innate) cellular defence and a specific immunity response of polymorphonuclear leucocytes may be initiated. This constitutes the second line of defence. In the udder, the first response to pathogens is phagocytosis and bactericidal activities of primarily neutrophils and macrophages; these responses may be followed rapidly by a massive influx of polymorphonuclear leucocytes (Sordillo *et al.,* 1997 and 2003). The rate of IMI is established by a combination of exposure of the teat-end to pathogens and the effectiveness of the defence mechanisms of the cow.

2.3 SURGICAL AFFECTIONS OF UDDER AND TEAT

2.3.1 Incidence

Agger and Willeberg (1986) documented the site of teat lesions in 154 cows; 12 per cent involved the superior half of the teat, 29 per cent the inferior half, 52 per cent the inferior extremity of the teat and seven per cent the whole teat. Of the 52 per cent located at the distal extremity, 36 per cent were noticed in the papillary canal.

Ducharme *et al.* (1987) reported the incidence of teat affections in 60 cows. With regard to etiology, physical trauma 23 per cent, severe mastitis eight per cent and unknown reasons 69 per cent was recorded. Among the quarters affected left and right fore quarters each 31 per cent, left hind quarter 23 per cent, right hind quarter 15 per cent were found to be affected.

Hofmeyer (1987) reported that physical injury to the teat might result from chronic mastitis, traumatic hand milking, calves sucking each other and tick bites.

Zecconi *et al.* (1992) and Hamann *et al.* (1994) stated that a decreased blood flow period after milking was associated with an increase in teat thickness and greater microbial colonization in the teat canal. The authors also observed an increase in teat thickness more than five per cent (caliper measurement).

Querengasser *et al.* (2001) revealed that teat canal stenoses accounted for 80 per cent of all the teat stenotic lesions. The authors examined the diseased teats of slaughtered cows and stated that 70 per cent of all teat alterations were located in the teat canal and Furstenberg rosette area.

Bleul *et al.* (2005) recorded the incidence of teat affections with obstructive disorders in 52 cows. Twenty six per cent of the cows were found to be affected in the first lactation and 30 per cent, 14 per cent, six per cent and two per cent were recorded during second, third, fourth and fifth lactation periods respectively. Of the teat affections 76.9 per cent was noticed in the hind teat with the highest incidence in the left hind teat 46.2 per cent, followed by right hind teat 30.8 per cent, 13.5 in the left front teat and 9.6 per cent in the right front teat respectively.

Rambabu (2007) studied the incidence of udder and teat affections in buffaloes. The udder and teat lesions were recorded as 4.2 and 2.51 per cent respectively. Among the udder affections udder oedema was recorded as the highest incidence 40 per cent followed by mammitis 34.60 per cent, atrophy/fibrosis 16 per cent, abscess four per cent and haematoma and vericosity each 2.66 per cent respectively. Intraluminal obstructions of teat 28.80 per cent was recorded as the highest percentage among the teat affections followed by polythelia 26.66 per cent, oligothelia, teat lacerations/ fistulas and intraluminal foreign bodies each 8.88 per cent, allergic mastitis and papilloma/neoplasms 6.66 per cent, fused teats and pox lesions 2.22 per cent respectively.

2.3.2 Gross and Microscopic Alteration in Obstructive Disorders

Kubieck (1975) and Seeh *et al.* (1998) reported that 70 to 90 per cent of the teat injuries were covered in nature without the involvement of the skin. The injuries were reported to be caused by contusions of the tip of the teat or the distal teat wall. In 80 to 90 per cent of such injuries teat stenosis was caused by mucosal detachment in the region of the streak canal or Furstenberg rosette. The free end of the detached tissue projected into the papillary sinus and obstructed the streak canal in a valve-like fashion during milking.

Ducharme *et al.* (1987) studied the histopathology of the lesions excised in 13 teats out of 52 teats subjected to thelotomy. The authors identified fibrous tissue in eight cases, normal mammary tissue in three cases, fibropapilloma, mammary polyps and inflammed mucosa each in one case respectively.

Saifzadeh *et al.* (2005) studied the histological changes of healing at anastomosed teat tip with supernumerary teat graft in cattle. The principal changes at the anastamosed sites noticed were mucosal alterations and granulation tissue. Mucosal changes included papilloid hyperplasia, squamous metaplasia, hyperkeratosis and acanthosis. Variable amounts of granulation tissue were observed in the submucosal layer. All the teats had mild submucosal infiltration of lymphocytes and a few neutrophils at the anatomotic region. Submucosal fibrosis was noticed to a lesser extent however its contribution to luminal compromise was difficult to assess and was not quantified.

2.4 CLINICAL STUDY

2.4.1 Clinical Evaluation

2.4.1.1 Normal udder and teat

Boge (1965) reported that the highest incidence of mastitis was in cows with plate-shaped teat-ends (43 per cent) and those with funnel-shaped teat canals (56 per cent), compared with a 30 per cent incidence in round-shaped teat-ends.

The total length of the teat canal in cows was reported to be about 10 cm with variations between 3 and 18 mm (McDonald, 1973; Hamann, 1987 ; Geishauser and Querengasser, 2000 and Paulrud and Rasmussen, 2004) and was reported to be dependant on breed, parity and stage of lactation (McDonald, 1973). Rear teat canals were claimed to be 5 to 10 per cent longer than front teat canals (Paulrud and Rasmussen, 2004).

Alieve (1970) and Lind *et al.* (1997) stated that in buffaloes because of the smaller cisternal volume, no cisternal milk was obtainable after cannulation and the udder on palpation indicated the absence of mammary cistern.

Jankus and Baumann (1986) examined the blood flow through the distal parts of the teat and found that the blood flow through the teat canal epithelium and the papillated portion of the stratum papillare was four times higher than that of equivalent structures of the mucosa of Furstenberg's rosette.

Gourreau (1995) quantified and declared that the length of the papillary canal varied between 5 and 13 mm with an average of approximately 8.5 mm. The diameter in the distal part was 0.45 mm and 0.94 mm in the proximal part. The author also observed 30 per cent increase in the diameter of the papillary canal in the infected quarters than in noninfected quarters.

Kuczaj (2003) studied population of 100 high-yielding Holstein- Friesian cows. The authors indicated increase in the thickness of fore and rear teats, depth of fore udders and rear teats span volume of udders associated with increased number of somatic cells in cm^3 of milk. The author also observed that the increased values of zoometric measurements, distance from fore and rear teats from the floor and size span resulted in reduced number of somatic cells in milk.

Sastry *et al.* (1988), Singh and Singh (1994) and Uppal *et al.* (1994) stated that the longer teat canals and a stronger teat sphincter were the important reasons for a lower incidence of mastitis in buffaloes than in cows.

Agger and Willeberg (1986), Burmeister *et al.* (1998) and Larsen *et al*. (2000) opined that rough teat skin and wounds on skin were the reservoir for *S. aureus* and even superficial lesions of the teat skin increased the risk of subclinical and clinical mastitis.

2.4.1.2 Normal milk parameters study

Kitchen *et al.* (1980) stated that the normal electrical conductivity of cow milk varied between 4.0 and 5.5 mS/cm at 25ºC. The authors also stated that during infections with mastitis the concentrations of sodium and chloride were found to be increased.

Silva and Silva (1994) reported that total somatic cell count in normal non-infected buffalo varied from 0.5 X 10^5 to 3.75 X 10^5 cells per ml.

Greut *et al.* (2001) stated that milk free from intra mammary infections contained relatively few cells; averaging 50,000 cells per milliliter. The predominant cell types noticed in normal milk as reported by the authors were macrophages (35 to 79 per cent), B and T lymphocytes (10 to 24 per cent), polymorphonuclear neutrophil leucocytes (PMN, 3 to 26 per cent) and epithelial cells (2 to 15 per cent) respectively.

Ludwiczuk *et al.* (2001) documented lowest somatic cell count in milk of primiparous cows (276 X 10^3 cm^{-3}) and highest in the seventh and higher lactations (710 X 10^3 cm^{-3}).

Mahendra and Dang (2001) recommended that if somatic cell count in buffalo milk was less than 1 X 10^5 cells per ml per udder , the udder was considered to be healthy and while the somatic cell count was 2.5 X 10^5 cells per ml and above the udder can be declared as infected in condition.

Ormian *et al.* (2001) observed a two fold increase of somatic cell number in the milk of fourth lactation cows in relation to first lactation cows; the lowest somatic cell count was recorded in the milk samples of second lactation cows (415 X 10^3 cm^{-3}).

Kuczaj (2003) observed that the young cows with udders suspended high above the floor more than 45 cm and rear teats $\leq$ 5.0 cm and fore teats $\leq$ 6.1 cm with an average diameter of about 3.0 cm produced superior quality of milk. Whereas, the old cows with droopy udders, less than 45 cm, long rear teats >5.1 cm and fore teats > 6.2 cm with diameter of above 3.2 cm, were more vulnerable to inflammatory states of udders indicated by increased number of somatic cells in milk.

Ruegg (2003) described the relationship between California Mastitis Test (CMT) and somatic cell count (SCC). CMT score approximated to corresponding SCC values:

negative, 0–200000 cells/ml; CMT traces, 150000– 500000 cells/ml; CMT 1, 400000–1500000 cells/ml; CMT 2, 800000–5000000 cells/ml; and CMT 3, above 5000000 cells/ml.

Dhakal (2006) stated that the average somatic cell count of right front and right hind quarters were significantly higher than left front and left hind quarters and nearly 94 per cent of California mastitis test negative quarters had somatic cell count greater than or equal to 2,00,000 cells/ml.

Kavitha *et al.* (2009) stated that the mean normal somatic cell count of buffalo's milk was 1,60,536 cells/ml of milk.`

Syed *et al.* (2009) revealed that the somatic cell in buffalo milk with count less than 250,000 cells/ml of milk was considered as insignificant for the presence of mastitis.

2.4.1.3 Diseased udder and teat conditions

Dohoo and Meek (1982) reported that the most important cause of increased somatic cell count was bacterial infection of the mammary gland and the major pathogens that caused greatest increase in somatic cell count included were *Staphylococcus* sp., *Coliform* and *Streptococcus* sp.

Heuwieser *et al.* (1985), Bristol (1989) and Hassig *et al.*(1990) reported that the obstructions of the teat and mammary gland cisternae developed as complications following injury to the mucosa or submucosa or through chronic mechanical or microbial irritation of the teat cistern.

Reneau (1986) stated that the presence of somatic cells greater than 283,000 cells per ml of milk was considered to be an indicative of presence of mastitis condition.

Ducharme *et al.* (1987) classified the teat lesions into five categories based on surgical findings in 52 teats as follows: Type I – focal teat cistern obstructions involving less than 30 per cent of the mucosal surface of the teat cistern; Type II - diffuse teat cistern obstructions involving greater than 30 per cent of the teat cistern mucosa; Type III – a membranous or fibrous structure separating either the gland cistern from the teat cistern or the lactiferous ducts from the gland cistern; Type IV – stenosis or obliteration of the gland and the teat cisterns and Type V – teat fistula , webbed teats or lacerations leading to fistulae.

Hassig *et al.* (1990) and Trent *et al.* (1990) stated that in pluriparous cows, teat obstructions were mostly due to chronic, proliferative mastitis or blunt trauma to the teat. In traumatic injury excess granulation tissue originating from the submucosa was documented by the authors as the primary cause of obstruction.

Grindal and Hillerton (1991) stated that any teat injury such as teat canal protrusion greatly increased the degree of exposure of the mammary gland to pathogens and also predisposed the udder to milk leakage and negatively influenced the hygiene and milking efficiency.

Dhakal and Kapur (1992) reported that somatic cell count and bacteriological examination of milk samples were more effective method in diagnosis of subclinical mastitis in buffaloes.

Pednekar *et al.* (1992) observed that somatic cell count was 69 per cent specific and 73.13 per cent sensitive in the detection of mastitis in cows.

Medl *et al.* (1994) and Dinc (1995) opined that inspection, palpation, catheterization (probing) and checking the milk flow from the teat would help in tentative diagnosis of teat stenosis and its fibrotic proliferative lesions.

Slettbakk *et al.* (1995) reported that the reduction in teat-end-to-floor space was associated with increased somatic cell count and cows with increased milk leakage, increased milk flow, asymmetric udders and flat/wide teat-ends were considered to be more susceptible to clinical mastitis.

Geishauser and Querengasser (2000) examined 133 cows presented with papillary canal dysfunctional problems. The results indicated that the length of the affected papillary canal was found to be increased by at least 2 mm compared with the contra lateral papillary canal.

Chrystal *et al.* (2001) revealed that most mastitis-causing pathogens gained entry to the mammary gland through the streak canal; the presence and shape of teat-end lesions played an important role in the pathogenesis of the disease. Teat ends that were plate-or inverted funnel-shaped were associated with higher incidence of clinical and subclinical mastitis.

Fremont *et al.* (2002) classified and documented the incidence of teat lesions in dairy cows as functional, organic and unrelated lesions. The organic lesions recorded were acquired 98.7 per cent and congenital 1.3 per cent. The acquired lesions consisted of nontraumatic conditions (foreign body, acute cisternitis, chronic proliferation and disease complication) 6.3 per cent and traumatic 92.4 per cent. Traumatic lesions were classified as uncovered lesion 1.3 per cent and covered lesion 91.1 percent. The covered lesions included sphincter and Furstenberg's rosette were 89.3 per cent (torn rosette 86.2% and normal 3.1%) and cisternal lesions 1.8 per cent respectively.

Querengasser *et al.* (2002) investigated 100 cows with hard milking conditions. The authors recorded the following observations: The affections were more in pluriparous (55%) than in primiparous (45%) animals. The highest incidence was noticed in the left hind teat 42 per cent, followed by right hind teat 29 per cent, right fore teat 17 per cent and left fore teat 12 per cent. During endoscopic examination the rupture of teat canal area with dislocation was noticed in 50 per cent, without dislocation in 46 per cent and other conditions related to obstruction in four per cent of the animals. Cisternitis was observed in 64 per cent of the animals. The pathological condition of the teat sphincter was recorded as no valve like action in 54 per cent, partial valve action in 15 per cent and complete valve action in 31 per cent of the animals. 23 per cent of the animals were reported for the treatment of the condition.

Pyorala (2003) stated that the in cows the quarters infected with *Staphylococcus aureus* and *Streptococcus agalactiae* had lower electrical conductivity values than quarters infected with environmental *Streptococci.* The author also stated that during mastitis the chloride concentration in the milk was increased.

Waller *et al.* (2003) and Rovai *et al.* (2007) reported that the leakage of milk in cows with incontinentia lactis condition was noticeable both in recumbent and standing position and more leakage was observed in recumbent position from the rear quarters than from the front quarters.

Steiner (2004) stated that the obstruction in the area of the rosette of Furstenberg as the most common cause of reduced milk flow in dairy cows.

Geishauser *et al.* (2005) studied the incidence of teat affections in 244 Braunvieh cows using theloscopy. The authors stated that hind teats were found to be predominantly affected by acute milk flow disorder. In 96 per cent of the affected teats a rupture in the area of the teat canal with tissue dislocation was diagnosed in 49 per cent and without tissue dislocation in 47 per cent, four per cent had other diagnosis such as ruptures in the teat cistern or papilloma. In 64 per cent of the affected teats inflammation of the teat lining (cisternitis) was diagnosed. Subclinical mastitis was presented in 52 per cent of the affected quarters.

Khan and Muhammad (2005) studied and reported the frequency of bacterial isolates in the milk samples of cross bred cows and buffalo. The authors opined that *Staphylococcus aureus* showed the highest (45%) followed by *Streptococcus agalactiae*

(23%), *E.coli* (18%) and *Bacillus sps.* (14%) respectively. In cross bred cows, *Staphylococcus aureus, Streptococcus agalactiae, E.coli and Bacillus sps.* were isolated from 48, 30, 13 and 8 per cent of milk samples respectively.

Flock and Winter (2006) correlated the ultrasonographic findings of udder oedema with presence of bacteria in the milk. The authors opined that *A.pyogenes* caused oedema in 62.5 per cent of the cases, *E.coli* in 41.7 per cent, *Streptococci* in 37.5 per cent and *S.aureus* in 25 per cent.

Rovai *et al.* (2007) opined that the etiology of involuntary milk leakage through the teat canal was not related to milk production, age, or stage of lactation. The continuous leakage of milk was due to the yield of large amounts of cisternal milk compared to the cisternal volume creating an increased intramammary pressure. This condition along with shorter teat canals eventually predisposed the teat for continuous leakage of milk.

2.4.1.4 Abnormal milk parameters in obstructive disorders

Querengasser *et al.* (2002) detected the following pathogenic organisms in the milk of 100 cows reported with hard milking condition. *Streptococcus agalactiae* (1%), *Streptococcus* esc. positive (33%), *Streptococcus* esc. negative (22%), *Staphylococcus* species (14%), *Staphylococcus aureus* (9%), *Arcanobacterium pyogenes* (3%) and coliforms (6%). In 94 per cent of animals mastitis condition was detected with the somatic cell count of >1,00,000 cells per ml in the milk.

Piepers (2007) stated that *Staphylococcus* spp., *Streptococcus* spp. and *Corynebacterium* spp, were the microorganisms responsible for the largest number of cases of intramammary infections.

Bengtsson *et al.* (2009) revealed that few isolates of *E.coli* were resistant to more than one antimicrobial and more than half of 20 isolates were resistant to streptomycin, sulphonamide and ampicillin. *Klebsiella spp.* was identified to be resistant to enrofloxacin. Among *Staphylococci* and *Streptococci* spp. resistance to more than one microbial was reported to be rare.

2.4.2 Radiographic Evaluation of Normal and Diseased Udder and Teat

2.4.2.1 Plain and contrast radiography

McDonald (1968), McDonald (1973) and Witzig *et al.* (1984) performed contrast radiography of the teat to study certain aspects such as evolution of the papillary canal's diameter during first lactation and to measure its length to analyse the importance of the diameter in relation to protection from infection.

McDonald (1975) performed contrast radiography of teat using barium sulphate and documented that the length of the teat canal in bovines varied between 5 and 13 millimeter with an average of approximately 8.5 millimeter. The diameter at the distal part was 0.45 millimeter and in the proximal part was 0.94 millimeter. The author also opined that the teats of infected quarters had an increase of 30 per cent of the diameter of the papillary canal than from the non infected quarters.

Witzig *et al.* (1984) performed contrast radiography to study stenosis of the papillary gland and that of teat's cistern. The authors also studied the radiographic image of the lactiferous sinus contrast study and revealed that the image was greatly dependant on the production status of the gland such as dry, stage of lactation and time after milking. The authors also found that the papillary sinus diameter was greater immediately after milking than four or six hours later.

Ducharme *et al. (*1987) performed contrast radiographic examination of teat cistern in 53 of 61 cases. The authors used 10 milliliter of iodine based contrast material through the teat canal into the teat cistern and lateral or xeroradiograph was taken. Forty six cases were subjected to thelotomy. The authors also opined that the radiographic interpretation when compared with the surgical findings was found to be accurate in 63 of the cases.

Couture and Mulon (2005) opined that contrast radiographic study provided better diagnostic details for lesions located at the base of the teat than the lesions involved in the gland and teat cistern.

2.4.3 Ultrasonographic Examination

2.4.3.1 Instrumentation and techniques

Cartee *et al.* (1986) examined the cadaveric and clinical cases of udder and teat of lactating cows with B-mode ultrasonography using a 5-MHz linear array transducer and a 5-MHz or 10-MHz mechanical sector transducer. The mammary glands were scanned within the water bath in sagittal and transverse planes and the details of the images were studied.

Bruckmaier and Blum (1992) conducted B-mode ultrasonography of teat and cisternal parts of the mammary glands of dairy cows, goats and sheep with 5-MHz linear array transducer in a water bath during α- and β-adrenergic agonist and oxytocin administration. The authors concluded that water bath technique was an excellent method for continuous observation of mammary cistern cavities in these species and the cross sectional images of the cisternal areas revealed excellent information about the form and volume of these cavities.

Dinc *et al.* (2000) reported the advantages and disadvantages of B-mode ultrasonographic examination of teats performed by direct contact and water bath methods. Direct contact technique was easy to perform but the details of the teat wall and Furstenberg rosette near the probe region could not be visualized completely. In water bath technique the lesions involving the base of the teat were difficult to visualize and direct contact method enabled easy examination of these areas and proximal to it.

Franz *et al.* (2001) examined the teat canal with 8.5 MHz linear transducer in vertical and horizontal planes using contact gel method and water bath method. The teat was dipped in a water filled plastic cup and B-mode ultrasonography was performed.

Twardon *et al.* (2001) diagnosed teat disorders by ultrasonography using 5 to 10 MHz linear or sector probes. The authors opined that the teat to be examined should be scanned with transducers in horizontal and vertical planes and the teat should be coated with gel or dipped in liquid medium.

Santos *et al.* (2004) evaluated direct contact, direct contact with standoff water bath and liquid pressure methods before and after milking in cows using 7.5-MHz linear transducer. The results revealed that liquid pressure technique was effective for identifying polyps and calculi. Both direct contact with standoff techniques were more useful in clinical cases. The water bath technique was more ideal for identifying the teat anatomy.

Nak *et al.* (2005) diagnosed various teat lesions in dairy cows by ultrasonography. The authors scanned the teats collected from the abattoir by placing the probe closer to the teat within the water bath and in live animals the teat was dipped in a 8cm diameter polyethylene cup filled with water and the probe was placed on outer surface of the cup smeared with coupling gel.

Flock and Winter (2006) diagnosed diseases of the mammary gland in cows by ultrasonography. The udder was scanned with 3.5 and 5.0 MHz convex and 7.5 and 13.0 MHz linear transducers by using coupling gel.

Rambabu *et al.* (2008) studied the udder and teat lesions in buffaloes using 7.5 MHz linear array transducer by direct contact, gel application, water bath and stand off methods in clinical cases. The authors observed and concluded that gel application and water bath methods were superior to other techniques to visualize the anatomical structures of udder and teat.

2.4.3.2 Normal udder and teat study

Many authors had described the normal echogenic pattern of the udder and teat tissues by ultrasound examination (Cartee *et al.,* 1986; Takeda, 1989; Stocker *et al.,* 1989; Trostle and O'Brien, 1998; Nudda *et al.,* 2000 and Franz *et al.,* 2001). From external to internal to the teat tissues the echogenic pattern were as follows; skin appeared as hyperechoic, muscular layers – longitudinal and circular and conjunctive tissue layers - medium echoic, blood vessels - hyperechoic, submucosa - medium echoic, Furstenberg rosette - hyperechoic and papillary duct - hyperechoic as thin, bright white line delineated on each side by parallel thick, dark, grey black bands. The glandular parenchyma of udder appeared as homogenous, hyperechoic with anechoic alveoli. The gland sinus appeared as homogenous anechoic area with mixed hyper-hypoechoic folds. The lactiferous ducts appeared as anechoic areas within the hypoechoic matrix of the fold.

Thomas *et al.* (2004) studied and quantified the mammary gland anatomy of lactating murrah buffaloes by B-mode ultrasonography with 6-MHz linear array rectal probe. The teat length and girth before and after milk ejection was found to be increased by 12 to 13 per cent in the fore teats and 10 to 12 per cent in the hind teats. The authors found that the teat canal length ranged from 1.9 to 4.3 cm with an overall mean teat canal length of 3.1±0.1 cm. The mean length of the teat canal was greater in the hind teats 3.7±0.2 cm than the fore teats 3.0±0.1 cm. The cisternal areas were found to be small with the mean teat and gland cisternal areas irrespective of quarters were 10.7± 1.9 and 13.1±1.1 cm^2 respectively and differed significantly at different stages of lactation. The cisternal milk yield was low (0.17±0.01 kg) and cisternal fraction of milk was only 4.9 per cent of the total milk secreted per milking. The latency period of induced milk ejection noticed was 25±1seconds.

Klein *et al.* (2005) studied the influence of teat canal length and diameter on the occurrence of mastitis in bovines by ultrasonographic procedure. The authors found that the teat canals of healthy udders tended to be longer (17.4 mm) and narrower (1.8 mm) than teat canals of infected udders (15.8 mm and 2.1 mm).

Flock and Winter (2006) reported the echogenic pattern of the udder in cows using a 3.5 and 5-MHZ convex transducer as well as a 7.5 and a 13 MHz linear array transducer. The bovine mammary gland was imaged as uniformly echoic with a granular structure and only minimal difference was noticed before and after milking. The authors stated that the image was due to the even distribution of connective tissue with a higher echoic density and gland parenchyma with less echoic density. The anechoic antrums corresponded to blood vessels or lactiferous ducts. The entries of the large lactiferous ducts into the gland cistern were clearly visible and the echogenicity were dependant upon the fill volume. The gland cistern was depicted as an anechoic antrum and the milk in the gland cistern was presented as anechoic with possible presence of echoic particles.

Rambabu (2007) quantified the teat morphology in lactating and nonlactating buffaloes using 7.5 MHz linear array transducer. Teat wall thickness was 10.33±0.42 mm and 8.00±0.25 mm, teat cistern diameter 16.16±0.30 mm and 11.83±0.30 mm, teat canal length 4.83±0.16 mm and 2.66±0.21 mm respectively in lactating and non lactating buffaloes.

2.4.3.3 Diagnosis of diseased udder and teat conditions

Jeninger (1989) performed ultrasonography of bovine udder and teat and reported physiological and pathological findings with a 5MHz linear transducer. With this technique the author diagnosed and documented teat obstruction, teat stenosis, abscesses, haematoma, gangrenous lesion and secretory disorders.

Twardon *et al.* (2001) diagnosed the teat disorders by use of ultrasonography with 5 to 10 MHz linear/sector probes. The authors concluded that the technique was atraumatic and non invasive to visualize morphological abnormalities within canals, sinuses and glandular tissue of the mammary gland.

Flock *et al.* (2006) performed diagnostic ultrasonography in cattle with teat disorders. The normal ultrasonographic appearance of the teat was compared with pathological findings including inflammation of the teat canal, mucosa of Furstenberg's rosette, teat wall, stenosis of the annular ring located between the teat sinus and gland sinus and fistulas.

Hoque *et al.* (2004) evaluated and documented the udder and teat lesions in bovine with 6-MHz linear array and 5-MHz sector transducers. The ultrasonographic image of thelitis appeared as thick hyperechoic teat lining replacing typical central hypoechoic images of the teat canal, intraluminal teat obstructions as hyperechoic shadow located adjacent to the mucosa, intraluminal foreign body as hyperechoic line in teat canal, traumatic tract/fistula as hypoechoic tubular appearance, atresia and fibrosis of the udder and teat as hyperechoic appearance and loss of typical echopattern of udder and teat and abscess, necrosis, gangrene of udder as hypoechoic space occupying lesions.

Nak *et al.* (2005) diagnosed and described various teat lesions in dairy cows by ultrasonography. Teat stenosis and obstruction appeared as hyperechoic or sometimes hypoechoic. In thelitis all layers of the teat wall appeared as hyperechoic, cyst in the teat wall appeared as spherical anechoic rounded in shape.

Rambabu (2007) evaluated the sonographic images of the following pathological udder and teat lesions in buffaloes: The udder lesions were mammitis, oedema, varicosity, haematoma, abscess, atrophy and fibrosis. Mammitis and oedema appeared as hyperechoic parenchyma. Udder abscess, haematoma, necrosis and gangrene appeared as hypoechoic space occupying images within the homogenous hyperechoic udder parenchyma. Atresia and fibrosis of udder was visualized as hyperechoic appearance and loss of typical echopattern of udder. Vericosity of the udder was represented by and anechoic elongated vein with homogenous hyperechoic udder parenchyma on sagittal section and numerous anechoic spaces representing the tortuous course of the vein within the homogenous hyperechoic udder parenchyma on transverse section. The teat lesions were thelitis, intracisternal obstructions, intracisternal foreign bodies, lacerations, fistula, atresia and fibrosis of the teat canal. Thelitis was visualized as thick hyperechoic teat lining with loss of typical central hypoechoic images of the teat canal with proportionate variation of echointensity and degree of thickness of the teat lining to the severity of the lesion. Intraluminal teat obstructions were appeared as hyperechoic shadow located adjacent to the teat mucosa. Intraluminal foreign body appeared as hyperechoic line in teat canal. The extent of traumatic tract was assessed by its hypoechoic tubular appearance. Atresia and fibrosis of the teat was detected as hyperechoic appearance and loss of typical echogenic pattern of the teat. Teat stenotic lesions appeared as hyperechoic or sometimes hypoechoic. In total obstruction of the teat canal absence of the teat intima condition was noticed. The characteristic pathological echogenic patterns of the diseased udder in bovines were documented by several authors. Udder oedema was noticed as image resembling the appearance of skin of an onion (Stocker and Rusch, 1997), milk with increased somatic cell count due to mastitis appeared as mixed heterogenous echogenicity pattern (Trostle and O'Brien, 1998), acute mastitis as non-homogeneous primarily hypoechoic pattern and increased echogenicity due to fibrosing chronic mastitis (Stocker and Rusch, 1997; Banting, 1998 and Trostle and O'Brien, 1998), gangrenous mastitis as increased echongenicity (Jenninger, 1989), *Streptococci* sp. and *Staphylococcus aureus* infections as non-homogenous hyperechoic as well as hypoechoic pattern, udder abscess as varying degrees of echogenicity and homogenicity of the abscess content with a generally wide and hypoechoic capsule, udder haematomas as large anechoic or hypoechoic spaces with thin echoic free-floating septae, mastitis due to Gram-negative organisms as non-homogeneity with hyperechoic spots or with bands casting dirty shadows due to gas formation in the parenchyma and mastitis with *A.pyogenes* as numerous hyperechoic round spots of 1 cm with a small hyperechoic centre (Flock and Winter, 2006).

2.4.4 Theloscopic Examination (Teat endoscopy)

2.4.4.1 Instrumentation and techniques

Tulleners and Hamir (1990) described for the first time the use of teat endoscopy in bovines. The authors inserted a 4-mm OD, 30º arthroscope with 5-mm OD lubricated arthrosopic sleeve through the teat canal and performed teatoscopy of the teat cistern and teatoscopy, mural biopsy through lateral stab incision with Ferris-Smith rongeurs in the proximal and distal parts of the teat cistern.

Medl and Querengasser (1994) performed teat endoscopy by lateral perforation of the teat walls and concluded that this lateral port technique enabled an easy examination of the whole teat lumen, mainly the Furstenberg rosette region of the teat and via the teat canal precise treatment of the obstructive lesion was possible under endoscopic imaging control units.

Geishauser and Guerengasser (2001) used wireless 0º, 15 cm long and 2.7 mm diameter rigid telescope enclosed in a blow pipe sleeve with 3mm outer diameter (Eickemeyer, Tuttlingen, Germany) attached to an inbuilt battery operated light source and pump for theloscopic examination procedures. The authors described two theloscopic techniques : *axial theloscopy* – scope was introduced through the teat canal, and *lateral theloscopy* –through the lateral teat wall scope was introduced into the teat cistern and the tissue excising instruments were introduced through the teat canal.

Bleul *et al.* (2005) used 0º, 19.7 long and 1.9 mm diameter rigid telescope with a coagulation electrode enclosed in a 3.63 mm sleeve for theloscopic procedure. The teat was insufflated with air and through the teat canal the theloscopy procedure and excision of the obstructive lesions were performed.

2.4.4.2 Normal teat structure study

Geishausser *et al.* (2005) demonstrated the endoscopic appearance of a normal teat through the teat canal and lateral wall of the teat cistern. Through the teat canal the teat canal was viewed with longitudinal folds, and the teat cistern via teat canal appeared with circular folds. Through the lateral teat wall theloscopy the inner opening of the teat wall could be examined and the Furstenberg rosette appeared with radial folds of the mucosa.

2.4.4.3 Diagnosis of diseased teat conditions

Teat endoscopy procedure was described as an effective and less-invasive diagnostic and therapeutic tool for the treatment of teat lesions. This method was reported to allow a relatively accurate diagnosis and prognosis of teat diseases and occasionally allowed treatment of specific lesions such as stenosis (Medl *et al.,* 1994; Seeh and Hospes, 1998; Kiossis *et al.,* 2002 and Geishauser *et al.,* 2005).

Geishauser *et al.* (2005) opined that theloscopy was a useful technique for diagnosis and therapy of covered teat injuries. The authors also stated that the minimally invasive theloscopic surgery might help to restore milk flow, milk yield and somatic cell count of the affected quarter with no significant change of infection with pathogens.

2.5 TEAT SURGICAL PROCEDURES

2.5.1 Conventional Teat Surgery

2.5.1.1 Technique

Witzig *et al.* (1984) revealed that the conservative techniques by providing rest to the affected gland and draining the milk passively every three days to allow the acute inflammation to subside subsequently or surgical treatment via blind resection of obstructing tissue through the streak canal resulted in unsatisfactory udder health and milk flow.

Donawick (1985), Hull (1985) and Ducharme *et al.* (1987) stated that the insertion of blunt-tipped, sharp bladed instruments such as Hug tumor extractor or Cornell teat curette etc., through the teat canal into the teat cistern and blind incision or excision of the obstruction resulted in indiscriminate trauma to the delicate teat cistern mucosal lining. The authors also attributed that this traditional method of treatment resulted in more granulation and fibrous connective tissue formation and might terminate to more severe obstructive form of milk flow disorders.

2.5.1.2 Clinical outcome – recovery, recurrence and complications

Witzig *et al.* (1984) opined that the treatment of covered teat injuries via thelotomy was a time consuming procedure with a moderate outcome of better milk flow and yield without any improvement of udder health. However, the authors documented a success rate of 92 per cent following thelotomy for the treatment of prolapsed mucous membrane of the proximal teat canal.

Ducharme *et al.* (1987) performed thelotomy on 52 teats with implantation of prosthesis in 27 teats for the treatment of obstructive disorders of teat. Short term success of 77 per cent was reported by the authors with short term complications such as intraoperative bleeding in 6 cases, milk leakage through the incision in four cases and failure to milk by machine milking in 26 cases respectively. The authors concluded that this technique resulted in more granulation and fibrous connective tissue formation and might terminate to more severe obstructive form of milk flow disorders.

Johnson (1988) and Bristol (1989b) stated that the prognosis for function after teat tissue obstruction depended on the extent, chronicity, orientation, involvement of the teat canal sphincter. Involvement of the teat sphincter decreased the prognosis for future function because the teat sphincter might become enlarged or stenotic and increased the risk of mastitis.

Hull (1995) and Steiner (2004) stated that in bovines with obstructive milk flow disorders of teat involving the gland sinus, teat canal or teat sphincters, the anatomical repair of these tissues by conventional surgical procedure was always difficult and a poor prognosis for restoration of milk flow and yield was noticed.

Bristol (1989a) reported that the treatment of teat obstructions with milk flow disorders by conventional surgical techniques carried a poor prognosis. Following conventional treatment the author observed a success rate of 5.6 per cent.

Steiner (2004) reported that milk incontinence, recurrence of hard milking and episodes of acute mastitis were the complications of conventional teat surgery following the treatment of tight streak canal condition. For a favourable long-term prognosis the author suggested to avoid cutting of tissues before regression of inflammation and the usage of stiff teat dilators before and after surgery.

2.5.2 Theloscopic surgery

2.5.2.1 Technique

John *et al.* (1998) performed theloscopic surgery in three lactating dairy cows with stenotic fibrotic lesion of 8 to 10 mm thick in the proximal region of a teat using 0 º and 30 º, 8mm diameter urological resectoscope (Karl Storz, GmbH, Tuttlingen, Germany) attached with an electrocautery unit. The scope was introduced into the teat cistern by a small incision made on the lateral wall of the teat and the blood clots and tissue debris were removed through a cannula inserted into the teat canal.

Hirsbrunner and Steiner (1999) performed theloscopy using 0º, 14 mm length, 3 mm diameter rigid telescope with halogen light source and air insufflation (Dr. Fritz). The author performed theloscopy by triangulation port technique. A perforating stab incision was made using a sharp trocar through the lateral teat wall approximately 10 mm distal to the base of the teat and the endoscope along with the sleeve was inserted into the teat cistern. An instrumental portal was created by making a stab incision approximately 5 mm proximal to the opening of the streak canal and the obstructing tissue was cut out with a pair of endoscopic scissors.

Geishauser *et al.* (2005) demonstrated the axial and lateral theloscopic techniques using 3 mm OD, 0º and 12 mm rigid scope (Eickemeyer, Tuttlingen, Germany) in cows with obstructive milk flow disorders. Axial theloscopy was performed through the teat canal and lateral theloscopy was performed through the lateral wall of the teat.

2.5.2.2 Postoperative management

Hirsbrunner and Steiner (1999) inserted wax bougie into the teat canal following theloscopy. Postoperatively the authors suggested to examine the affected quarters daily for the following nine days and on day 3, 6 and 9 the milk from the quarter had to be drained passively with sterile cannula. The sutures if any placed on the teat could be removed on day 9 and routine milking had to be resumed from day 10 onwards.

Geishauser *et al.* (2005) demonstrated the postoperative procedure after the theloscopic procedure. The authors suggested to insert silicone or natural teat wax bougie into the teat canal to prevent the postoperative stenosis of the teat canal. Then the teat with implant had to be covered with long lasting elastic bandage in U shaped fashion and then allowed to rest for 3 X 3 days and commence milking on day 10. In case of mastitis the authors suggested to milk the mastitic quarters twice daily without any trauma to the teat canal.

2.5.2.3 Clinical outcome – recovery, recurrence and complications

Hospes and Seeh (1998) also documented that the cows treated after theloresectoscopy had a success rate of 59.4 per cent following six weeks after surgery.

Bleul *et al.* (1999) revealed that the development of tissue hyperplasia and stricture composed of scar tissue was minimal with the use of wire loop cautery and theloresectoscopy when compared with the use of cutter in conventional endoscopy.

Hirsbrunner *et al.* (2001) treated the internal lesions of teat by theloscopic triangulation technique and the long term follow up revealed the success rate of 75.9 per cent and 18 per cent of the cows were culled during the follow up period. Similarly a culling rate of 64 per cent was also reported by Kiossis *et al.* (2002).

Zulaul and Steiner (2001) reported that 14 out of 15 (93.3 per cent) teats treated by theloresectoscopy had normal milk flow in short-term and long term evaluation.

Steiner (2004) stated that the outcome and prognosis of the theloscopic surgery for the removal of obstructing teat tissue in the area of the rosette of Furstenberg was superior to thelotomy. The author also opined that cutting the teat sphincter along with the obstructing tissue removal during theloscopy avoided the recurrence and incidence of mastitis.

Bleul *et al.* (2005) reported the success rate of 60 per cent out of 52 cases that were treated for theloresectoscopy technique and 40.8 per cent (20 cases) were culled before the end of the next lactation. The authors also opined that the cows that were treated by theleresectoscopy with positive results of California Mastitis Test and microbial cultures resulted in frequent episode of mastitis and overall a negative outcome after surgery.

Geishauser *et al.* (2005) reported the outcome of theloscopic surgery in 100 cows treated for milk flow disorders. The authors documented that the peak milk flow before surgery from the teat with obstructive disorder was on average 24 per cent and one and six months later to surgery the flow was around 73 and 82 per cent respectively.

2.5.2.4 Limitations

Bleul *et al.* (2005) reported the limiting factors that influenced the favourable long term outcome after endoscopic procedure. High somatic cell count and clinical mastitis due to inappropriate treatment attempt of the teat prior to endoscopic surgery, impaired defense status and mechanical function of teat canal and teat sphincter were the factors described by the authors that influenced the outcome after surgery.

2.6 TEAT IMPLANTS

Seeh *et al.* (1997) experimented the use of natural teat insert implant in healthy cows. The authors observed that natural teat implant retained the normal appearance of the teat canal lining without any change in the somatic cell count and electrical conductivity of the milk and ultimately on long term follow up the udder health was not altered.

Bleul *et al.* (2000) reported that the blind resection of obstructive lesions in conventional techniques followed by the use of teat dilators or plastic in dwelling cathethers resulted in teat sinusitis.

Geishauser *et al.* (1999) stated the use of teat dilators after teat endoscopic procedures injured the teat canal and teat cistern epithelium, increased the somatic cell count and the risk of presence of bacteria in the milk. The authors advocated to use silicone implants or natural teat inserts rather than dilators to prevent adhesions in the teat canal following teat injury.

Querengasser *et al.* (2002) studied the utility of natural teat insert implant in cows with obstructive milk flow disorders subjected to theloscopic surgery. The implants were observed to disintegrate after its use and the milk from the natural teat implant treated teats had less frequent incidence of the mastitis, lowered isolation of pathogenic organsisms, decreased somatic cell count with increased milk flow and yield. The authors concluded that usage of this implant in cows with milk flow disorders maintained the teat canal patent for long term after surgery without any risk of removal from the herd.

CHAPTER 3

MATERIALS AND METHODS

3.1 STUDY MATERIALS

A total of 122 clinical cases of lactating cows (n=98) and she buffaloes (n=24) reported during the period from 01.05.2007 to 30.05.2010 to the Large Animal Surgery Unit of Madras Veterinary College Teaching Hospital, Chennai with the anamnesis of mammary gland affections and milk flow disorders were taken up as investigation materials for this study.

Three cadaveric mammary glands of cows and she buffaloes collected during post-mortem were utilised as study materials and the techniques of ultrasonographic and theloscopic procedures were initially studied and standardised.

3.2 SELECTION OF CASES

Based on the parity of the third calving with early to mid lactation phase and irrespective of the breeds, 18 lactating cows and 18 lactating she buffaloes were selected for the clinical study.

3.3 GROUPING

3.3.1 Normal Udder and Teat (Group I)

In this group, the normal udder and teat condition was studied without any therapeutic procedures and twelve animals of (six cows and six she buffaloes) of third parity and early to mid lactation stages irrespective of breed with minor surgical affections related to udder and teat were taken up for the study. A prior informed consent was obtained from the owners of the selected cases and investigation was carried out and documented.

3.3.2 Diseased Udder and Teat (Group II)

Twenty-four diseased animals (12 cows and 12 she buffaloes) of third parity and early to mid lactation stages and irrespective of breed with obstructive milk flow disorders were selected for the study and were classified as group II. This group was randomly divided into two subgroups, each consisting of twelve animals (six cows and six she buffaloes) as groups IIA and IIB. Based on the owner's compliance the cases were selected and were subjected to the investigation and treatment procedures.

3.3.2.1 With bio-implants

The twelve animals (six cows and six she buffaloes) in group IIA were subjected to investigation procedures and were treated by minimally invasive theloscopic surgery with natural teat insert implant (NIT).

3.3.2.2 Without bio-implants

The twelve animals (six cows and six she buffaloes) animals in group IIB were subjected to investigation procedures and were treated by minimally invasive theloscopic surgery without bio-implants.

3.4 CLINICAL DESIGN

The clinical design of the study is as follows :

Table 1 : Design of the study

<table>
<tr><th colspan="2">Group</th><th>I</th><th colspan="2">II</th></tr>
<tr><td colspan="2" rowspan="3">Clinical condition studied</td><td rowspan="3">Normal
Mammary gland</td><td colspan="2">Mammary gland with obstructive milk flow disorder of teat</td></tr>
<tr><td colspan="2">Sub Groups</td></tr>
<tr><td>A</td><td>B</td></tr>
<tr><td colspan="2">Surgical procedure</td><td>-</td><td>Theloscopic surgery</td><td>Theloscopic surgery</td></tr>
<tr><td colspan="2">Bio-implant usage</td><td>-</td><td>with bio-implant (NIT)</td><td>without bio-implant (NIT)</td></tr>
<tr><td rowspan="3">No. of cases studied</td><td>Cow</td><td>6 cases</td><td>6 cases</td><td>6 cases</td></tr>
<tr><td>She buffalo</td><td>6 cases</td><td>6 cases</td><td>6 cases</td></tr>
<tr><td>Total</td><td>12 cases</td><td>12 cases</td><td>12 cases</td></tr>
</table>

3.5 PARAMETERS STUDIED

3.5.1 Incidence

The incidence of udder and teat affections related to milk flow disorders in cows and buffaloes reported to the Large Animal Surgical Outpatient Unit, Madras Veterinary College, Chennai from 01.05.2007 to 30.05.2010 were recorded. The details of the incidence with regard to etiology, type of lesion, side/quarter affected and the site and type of lesion in cows and buffaloes were recorded.

3.5.2 Clinical Evaluation

3.5.2.1 Udder and teat parameters

The following clinical examination parameters of udder and teat as defined by Rosenberger (1979), Houe *et al,* (2002) and Klass *et al.* (2004) were studied in group I and group IIA and IIB cows and buffaloes.

The following parameters I to IV were studied in group I cows and buffaloes on the day 0 of examination and in group IIA and IIB cows and buffaloes on 0, 30 and long term follow up days and the observations were recorded. In all the groups the mean long term follow up day varied from two months to four months after the theloscopic procedure with a mean duration of 3.7 months.

I. Teat tip to floor distance

The teat to be examined was measured from its tip to the floor/ground surface using a calibrated plastic tape in centimeters and the readings were recorded in centimeters (Plate 1).

II. Teat length

Teat length was measured from the base of the teat to the teat tip using a cotton thread (2-3mm diameter). The length was marked in a paper and was measured with a scale. The reading was recorded in centimeters (Plate 2).

III. Teat girth

Teat girth was measured in centimeters approximately at the base of the teat using a calibrated tape in centimeters and the reading was recorded as for the teat length (Plate 3).

IV. Teat canal length (Medl's test)

A sterile metal probe (Thelometer, Eickemeyer, Germany) was inserted into the teat canal and the corresponding length was measured in centimeters using a scale (Querengasser and Geishauser, 1999) (Plate 4).

The parameters V to XIV were studied on day 0 of examination in cows and buffaloes in group IIA and IIB and the observations were recorded.

V. Udder shape

1 - normal; 2 - small; 3 - long abdominal; 4 - backward bulging; all four teats present between hind legs; 5 - slanting; 6 - deep and 7 - deep and slanting.

VI. Udder tissue condition

1- soft; easy palpation of the deep parts of gland with fingers, 3 – generally firm; can be palpable only 3 to 5 cm into the tissue and 5- hard, cannot palpate the udder tissues with fingers.

VII. Assymetry between fore/between hind quarters

1- slight (just noticeable), 3 - pronounced, 5 - complete atrophy of one quarter

VIII. Udder oedema

1- slight, 2 - large area of the udder, 3 - most of the udder

IX. Teat cleanliness score

0 - clean teat, 1 - almost clean, 2 - slightly dirty, 3 - dirty and 4 - extremely dirty (Hovine *et al.,* 2005).

X. Teat shape

1- normal, 2 – short (<5cm), 3 - conical, 4 - fleshy and 5 - others

XI. Teat wound

Based on the presence or absence : Yes or No

XII. Scar tissue in the teat canal

Based on the presence or absence : Yes or No

XIII. Skin quality of teats

1 - smooth like silk, 2 - smooth, 3 - intact and moderately smooth, 4 - rough and 5 - very rough.

XIV. Teat palpation score

0 - normal, 1- mild thickening, 2 - moderate thickening and 3 - severe thickening.

3.5.2.2 Milk parameters

The following parameters of milk were studied in group I animals on day 0 of examination and in group IIA and IIB on day 0, 3, 9, 30 and long term follow up days and the observations were documented.

I. Total milk yield of the quarter

The total milk yield of the quarter in millilitres after a milking interval of minimum 8 to 12 hours was measured after prestimulation and hand milking.

II. Electrical conductivity

The electrical conductivity of the milk from the quarter to be investigated was measured using a hand held conductivity meters and the reading was recorded in mS/cm (Plate 5).

III. Somatic cell count

The somatic cell in milk was estimated as per the technique described by Schalm *et al.,* 1971. The milk sample was thoroughly mixed and 10µl of milk was drawn and placed on a predrawn one sq.cm area marked over a grease free clean micro-slide and was uniformly smeared within that area with a fine sterile glass rod. The smears were dried and examined after staining with Giemsa or modified Newman's stain. The counting of cells in 30 different fields was carried under oil immersion lens (100X) and the counting was repeated thrice per smear to an average number of somatic cells in 30 fields. The total number of cells /ml of milk were estimated by multiplying total number of cells in thirty fields to working factor of microscope and expressed as cells per ml of milk sample.

$$\text{Somatic Cell Count} = \frac{\text{N X Area of smear (100mm}^2\text{)}}{\text{Area of one field (3.14 x r}^2\text{)}} \text{ X 100}$$

$$= \text{N X 50 X 100 X 100 cells per ml}$$

Where N = average number of leucocyte and r = radius of microscope oil immersion field in mm. The radius of the oil immersion field is usually about 0.08 mm, so that the area of the field will be 0.02 mm^2.

IV. California mastitis test score

California mastitis reagent was mixed with the milk collected from the affected quarter and was mixed gently in clockwise and anticlockwise direction for 15 seconds. The degree of precipitation and gel formation in the milk was evaluated, scored and the equivalent somatic cell count per ml was interpreted as defined by Radostits *et al.* (2000) and Jackson and Cockcroft (2002) was recorded and interpreted as follows:

CMT Score	Interpretation	Visible reaction	Equivalent somatic cell count per ml
0	Negative	No reaction	0-2,00,000 (0-25% neutrophils)
T	Trace	Slight precipitation	1,50,000-5,00,000 (30-40% neutrophils)
1	Weak Positive	Distinct precipitation but no gel formation	4,00,000-15,00,000 (40-60% neutrophils)
2	Distinct Positive	Mixture thickness with gel formation	8,00,000-50,00,000 (60-70% neutrophils)
3	Strong Positive	Viscosity greatly increased. Strong gel that was cohesive with a convex surface	>50,00,000 (70-80% neutrophils)

V. Bacterial isolation and identification

The milk samples of cows and buffaloes in group IIA and IIB were subjected to bacterial isolation by direct plating on Muller Hinton agar, blood agar and nutrient agar media. After incubation at 37ºC for 24 hours colony morphology was studied and the isolated organisms were identified as Gram positive or Gram negative bacteria based on the Gram staining technique. Then the colonies of the bacteria were subcultured in a selective media such as Mannitol Salt agar for S*taphylococcus* sp., Edwards medium for *Streptococcus* sp., MacConkey agar for *Enterobactor* sp. etc., lifted on blood agar slants and were identified based on the shape, surface, size, consistency, pigmentation, opacity of the colonies and media changes. Further confirmation was done by microscopic, cultural and biochemical characteristics (Cruickshank, 1960 and Cruick, 1989).

VI. Antibiotic sensitivity test

The antibiotic sensitivity test was conducted by Bauer method (Bauer *et al.*, 1966). Milk sample was inoculated into nutrient broth and incubated at 37ºC for 24 hours. A sterile swab dipped in broth culture was evenly smeared on 5 per cent bovine blood agar plate. Then antibiotic discs were placed on the inoculated surface. The different antibiotics used for the purpose were enrofloxacin, ciprofloxacin, gentamicin, ceftrioxone, ampicillin, amoxicillin, gatifloxacin. After incubation at 37ºC for 24 hours the zone of growth inhibition was recorded as sensitive and lack of growth inhibition as resistant. The antibiotic to which the organisms were most sensitive was selected for the treatment.

3.5.3 Radiographic Examination of Normal and Diseased Teat

3.5.3.1 Preparation and positioning of the animal

All the animals in group I, IIA and IIB were subjected to plain, positive and double contrast radiographic studies. In this study Siemens 500 mA, 3 phase, 6 pulse x-ray generator was used for the radiographic investiagation. The udder and teat to be investigated was cleaned, scrubbed, dried and finally degreased with 70 per cent surgical spirit. The animal's temperament was judged and in needy cases epidural analgesia with 2 per cent lidocaine hydrochloride was administered at the sacrococcygeal space. The animal was positioned laterally with the mammary gland to be investigated facing towards the X-ray machine and the leg was kept extended caudally to avoid super imposition of the limb structures. The cassette was placed at the inter-mammary groove and behind the affected parts of the mammary gland (Plate 6).

3.5.3.2 Plain radiography

On day 0, plain radiograph of lateral view was taken in all the groups and the details were documented and studied.

3.5.3.3 Contrast radiography

Positive, negative and double contrast radiographic procedures were also carried out in all the groups on day 0 of investigation. A sterile teat cannula was inserted and the milk from the teat was drained out. In conditions necessitating the teat alone to be examined a rubber ring was applied at the base of the teat and the diffusion of contrast agent into the secretory structures of the gland was avoided.

Negative contrast radiography was done by infusing atmospheric air as contrast medium into the teat. 18 G blunt needle attached to a rubber tube was inserted into the teat canal. The air was infused using a 50 ml empty syringe. After sufficient inflation of the udder and teat as noticed by the distension and backpressure to the syringe, the needle was withdrawn and lateral radiographs were taken. The results were interpreted and documented.

Positive contrast radiography was done using the ionic water soluble positive contrast agent, Iohexol 350 mgI/ml (Omnipaque, GE laboratories, India) (Plate 7). Depending upon the disease status the agent was diluted with normal saline and was injected into the teat at the rate of 20 to 30 ml per quarter or to an infusible volume of 4-10 ml into the teat cistern. The teat/quarter was tumbled and massaged and the contrast agent was uniformly distributed. During this procedure, care was taken to avoid spillage or contact over the external surface of the teat to avoid false details in the radiographs. Lateral radiographs were taken and the results were interpreted and documented. After the completion of this procedure the excess contrast agent was milked out.

Double contrast radiography was done as a combined technique of positive and negative contrast radiographic procedure. First positive contrast agent as mentioned above was injected into the teat and the excess quantity was milked out. Then air was injected to the volume of resistance of leakage through the teat canal and lateral radiograph was taken and the details were analysed and documented (Coutre and Mulon, 2005).

3.5.3.4 Limitations

The limitations encountered during the investigation of udder and teat by plain and contrast radiographic procedures were documented and studied.

3.5.4 Ultrasonographic Examination

In this study ultrasound machines (ALOKHA, Prosound 3500 SSV, Japan) (Plate 8) with 5-MHz convex and 7.5 to 10 MHz (Plate 9) linear array rectal transducers were used and two dimensional B-mode ultrasonographic pictures of the udder and teat in vertical and horizontal planes were investigated. The structure to be quantified was freezed and was measured by two-distance ellipse method. The freezed images were either recorded or a print out was taken on polaroid paper with thermal printer.

3.5.4.1 Anaesthesia, restraint and positioning

In case of painful udder and teat conditions epidural analgesia using two per cent lidocaine solution was administered at the sacro-coccygeal region. The hair over the udder was shaved. The udder and teats were washed to ensure free of debris, dried with clean cloth, degreased with 70 per cent surgical spirit and prepared for the ultrasonographic examination. For an easy comfortable dexterity of ultrasound scanning of udder and teat by the examiner and to overcome the hindrance of the side bars of the trevis at the udder level, the animals were scanned mostly in standing posture and trevis restraint was avoided. Before scanning the animal was positioned laterally on the side to be investigated by the examiner and the monitor was kept faced to the side view of the examiner. The head was secured manually and the hind limbs were secured with milk man’s rope as figure of eight twitch over the hock joint. During the procedure care was taken to avoid any positional change and trauma by the animal.

3.5.4.2 Procedure

Contact with gel application method

This technique was followed to examine the udder and the gland cistern area. Over the dried surface of the udder and gland cistern acoustic coupling gel was applied. In this study convex transducers of either 5-MHz or linear transducers 7.5 or 8-MHz were used for examination of the udder and gland cistern area. The transducers were placed directly on the gel smeared skin surface of udder and teat and the normal and pathological conditions in vertical and horizontal planes were documented and studied (Flock and Winter, 2006) (Plate 10).

Water bath method

This technique was employed mainly to examine the teat to the level of proximal cisternal area without any direct contact. The teat to be investigated was degreased with 70 per cent surgical spirit and dried with a clean cloth. In this technique, the teat was dipped in a triple glass distilled water (30º to 35ºC) filled disposable polyethylene cup and acoustic coupling gel was applied over the cup. Linear transducers of 7.5-MHz to 10.0 MHz were used in this study and was placed in vertical/horizontal planes of the outer wall of the gel smeared polyethylene cup and the ultrasonographic images were recorded (Bruckmaier and Blum, 1992; Santos *et al.* 2004; and Nak *et al.* 2005) (Plate 11).

3.5.4.3 Normal udder and teat

B-mode ultrasonograms of the normal udder and teat was studied in group I animals. The structures were quantified, documented and analysed.

3.5.4.4 Diseased udder and teat

In group IIA and IIB B-mode ultrasonograms of the animals with diseased udder and teat subjected to theloscopic surgery was studied preoperatively on day 0 and postoperatively on 9, 30 and long term follow up day. The details were documented and analysed.

3.5.4.5 Teat quantification of normal and abnormal teat

In all the groups the following structures of the teat were measured just before milking with a milking interval of (8-12 hours).

i. Teat canal length (TCL) - from Furstenberg's rosette to the teat tip,
ii. Teat diameter (TD1) - on the level of the Furstenberg's rosette
iii. Teat diameter (TD2) - 1.5 cm proximal to the Furstenberg's rosette
iv. Teat wall thickness (TWT) - 1.5 cm proximal to the Furstenberg's rosette
v. Teat cistern width (TCW) - 1.5 cm proximal to the Furstenberg's rosette

The details were recorded and documented

3.5.4.6 Limitations

The limitations encountered during ultrasonographic investigation of udder and teat in group I, IIA, IIB was documented and analysed.

3.5.5 Theloscopic Examination

The animals subjected to theloscopic examination on day 0 in group I, IIA and IIB and on 30th day in group IIA and IIB cows and buffaloes. In this study, wireless battery operated theloscope (0º, 2.7 mm OD, 12 cm length, Eickemeyer, Germany) with inbuilt halogen light source and air pump (Plate 12) enclosed in a blow pipe (3 mm OD, 12 cm length, Eickemeyer, Germany) secured with LUER-lock adaptor was used as sleeve (Plate 13). The theloscope and surgical instruments were sterilized by cold sterilization using activated dialdehyde two per cent solution (Cidex ®) soaked for a minimum of 10 minutes (Yovich and McIlwraith, 1986) (Plate 15). The endovision TELECAM-DX video camera with a focal length of 30 mm was attached to the eyepiece of the theloscope and connected with camera control unit (CCU) through a 180 cm long cable. A 4.8 mm diameter, 250 cm long fibreoptic cable carrying the cold xenon light fountain from XENON 300 or halogen miniature light source 150 Watt-481C (Karl Storz Endoscopy India Pvt. Ltd.) was fixed to the theloscope by an adaptor and an air pump calibrated to generate a maximum pressure of 200 millibar was attached to the blow pipe by a rubber tube. Air was blown into the teat and the teat cistern was dilated. The light source was switched on and the scope was progressed. The endoscopic process was displayed as continuous motion picture with magnification in a colour monitor. The displayed picture was recorded and the details were interpreted and studied (Plate 16).

3.5.5.1 Preparation, anaesthesia and positioning of the animal

Prior to examination the animal was restrained in a special trevis and hind legs were secured with milk man's rope as figure of eight twitch over the hock joint. Epidural analgesia with two per cent lidocaine hydrochloride solution was administered at the sacro-coccygeal region. The milk from the cisternal cavity of the teat to be investigated was drained out by either by hand milking or by using a sterile cannula. The udder and teat was thoroughly washed with soap and dried with a clean cloth. The teats were degreased with 70 per cent surgical spirit and dried out (Plate 17). Then oxytocin was administered intravenously @ 10 to 20 i.u. per animal. After two minutes a sterile cannula (3 mm OD, 10 cm length, Thelkal, Eickemeyer, Tuttlingen, Germany) (Plate 18) was inserted and the secreted milk was drained out. With the help of an applicator a rubber ring was applied at the base of the teat and the entry of the milk into the teat cistern and blood flow to the distal part of the teat was prevented (Plate 19). The teat was anesthetized by either a ring block at the base of the teat (before application of the rubber ring) or regional intravenous analgesia of teat (puncturing an engorged vein in the teat wall with 26G hypodermic needle and 4 to 8 ml of two per cent lidocaine solution was injected) (Plate 20). Prior to the theloscopic procedure the teat cistern was again lavaged with normal saline using a sterile milking tube. In few cases depending on the severity of the intra cisternal lesions, 5 to 10 ml of two per cent lidocaine solution was infused into the teat cistern and intracisternal analgesia was attempted (Plate 21). Depending on the temperament of the animal, the animal to be examined was subjected to fasting for 24 hours and the animal was sedated with intramuscular administration of xylazine @ 0.05 mg/kg body weight. The animal was positioned mostly in standing posture in the special trevis or secured in lateral recumbency with the udder and teat to be examined facing upward on a trolley.

3.5.5.2 Procedure

Axial theloscopy - *insight via the teat canal:* In this technique, the theloscope along with blow pipe was directed upwards into the teat cistern via the teat canal and was progressed towards the proximal part of the teat cistern to the level of the gland cistern. This procedure was mainly useful to visualize and document the structure of obstructive intracisternal lesions of the teat above the level of Furstenberg rosette and during withdrawal of the scope and blowpipe the structure and lesions of the teat canal was also visualized (Geihauser *et al.* 2005) (Plate 22).

Lateral theloscopy and theloscopic surgery - *insight via the lateral teat wall* – This technique was used to visualize and document the excision/removal of the obstructive lesions noticed at the inner walls of the teat cistern, Furstenberg rosette and inner opening of the teat canal areas in downward direction.

In this technique a sterile obturator of 3 mm OD, 10 cm length (Eickemeyer, Germany) (Plate 23) was inserted through the teat canal into the teat cistern and was positioned to a level of 1 to 2 cm below the base of the teat. With the sharp tip of the obturator, the lateral teat wall opposite to the site of lesion was punctured and the obturator tip was exposed to the outer surface of the teat skin (Plate 24). Through the punctured teat wall and exposed obturator tip a sterile slide pipe was inserted from outside into the teat cistern along with the obturator. The obturator was removed and the scope and blow pipe were inserted via slide pipe into the teat cistern and were held in fixed position by an assistant (Plate 25). The endocamera, light source and air pump were connected to the scope and the teat cistern was inflated with air. The scope was then directed downwards and sterile accessory instruments were inserted through the teat canal and the obstructing tissue/mass in the teat cistern and teal canal was excised and removed (Geihauser *et al.* 2005).

During this procedure, depending upon the type and location of the obstructing tissue/mass to be excised and removed the following instruments were used.

- Teat punch (Thelotom, Eickemeyer, Germany) - to remove the ruptured tissues in everted or inverted conditions at Furstenberg rosette and free floating or partially peeled out tissues from the walls of the teat cistern (Plate 14).
- Teat foreign body forceps (Thelab, Eickemeyer, Germany) - to remove the foreign bodies, papilloma, milk or blood clots (Plate 14).
- Hug's Lancet (Eickemeyer, Germany) - to widen the narrowed teat canal lumen incisions were made at 0º and 180º or at 0º, 120º and 240º in the proximal/inner opening of the teat canal (Plate 14).

Post operative care

At the end of theloscopic surgical procedure, the outer surface of the teat was cleaned with normal saline. The punctured skin wound in the lateral teat wall (artificial opening) was closed with a cross mattress suture using 2-0 braided silk (Plate 26) and a medicated water proof plaster was applied. The rubber ring applied as tourniquet at the base of the teat was cut with scissors. The teat was massaged gently for few minutes to restore the texture, circulation and withdraw the passive congestion. The residual milk from the quarter was removed by hand milking and the milk flow pattern and teat patency was evaluated. The teat cistern was lavaged with normal saline using an extra wide milking tube and intramammary antibiotic was instilled. The outer surface of the teat was again cleaned with normal saline, dried and degreased (except at the suture site) with 70 per cent surgical spirit and dried again with a sterile mop. To prevent postoperative teat canal stenosis, a sterile natural teat insert-waxy teat implant (NIT, www.profs-products.com, Germany) bio-implant was inserted into the teat canal (Plate 27) and was retained by bandaging the teat with elastic water proof long lasting adhesive tape applied in 'U' shaped longitudinal strip overlaid with a circular strip pattern (Plate 28). After theloscopic surgery, antibiotics and analgesics were administered and the teat was rested depending on the production of abnormal milk, type and severity of the obstructive disorder. In case of normal milk production, the teat was rested with parentral administration of antibiotics for a minimum period of two days. In mastitis condition, for the first two days, the milk from the operated teat was passively drained and flushed with normal saline at least four times a day and the bio-implant was inserted intermittently into the teat canal during the milking intervals.

3.5.5.3 Normal and abnormal teats

The morphology of the internal structures of the normal and diseased udder and teat during the theloscopic procedure was investigated and documented.

3.5.5.4 Limitations

The limitations encountered during the theloscopic investigation procedures of the udder and teat was documented and discussed.

3.5.6 Evaluation of Teat Surgical Procedures

3.5.6.1 Theloscopic surgery – utility, limitations and complications

The utilities of the minimally invasive theloscopic surgery with regard to the treatment of obstructive teat lesions with and without the usage of the bio-implants, limitations and the intra and postoperative complications were documented and analysed.

3.5.7 Evaluation of Wound Healing with Ultrasound and Theloscopy

3.5.7.1 Group IIA cows and buffaloes

Postoperatively, the efficacy of minimally invasive theloscopic surgery with the usage of bio-implants was studied in subgroup IIA cases on 30th day using ultrasonography and theloscopy and the results were documented and discussed.

3.5.7.2 Group IIB cows and buffaloes

The postoperative efficacy of minimally invasive theloscopic surgery without the usage of bio-implants was studied in subgroup IIB cases on 30th day using ultrasonography and theloscopy and the results were documented and analysed.

3.5.8 Evaluation of Utility of Teat Bio-implants

3.5.8.1 Group IIA cows and buffaloes

The cases in group IIA that were subjected to minimally invasive theloscopic procedure and with usage of bio-implant (NIT) were evaluated in terms of restoration and maintenance of teat patency was evaluated on 30th day and long term followup day and the observations were documented and discussed.

3.6 STATISTICAL ANALYSIS

The data collected in group I were analysed by unpaired ‘t’ test and in group IIA and IIB by one way analysis of variance (ANOVA) as per the procedure described by Snedecor and Cochran (1994) and the results were discussed.

CHAPTER 4

RESULTS

4.1 INCIDENCE OF UDDER AND TEAT AFFECTIONS WITH OBSTRUCTIVE MILK FLOW DISORDERS

During the period of study, incidence of obstructive milk flow disorders was recorded in 98 cows and 24 buffaloes. The incidence of teat affections in cows (78.57 %) and buffaloes (92.31%) was found to be highest when compared with affections of quarter alone in cows (18.37 %) and quarter and teat in cows (3.06%) and in buffaloes (7.69 %). The incidence of single teat affections was more in cows followed by double triple and all the four accounting a total of 98 number of teat affections. Both in cows and buffaloes single teat affections were found to be higher. Among the quarter affections in cows single, double and triple quarter affections were recorded as 83.33 per cent, 11.11 per cent and 5.56 per cent respectively and a total of 22 quarters were found to be affected. In buffaloes no such incidence of quarter affection was recorded. The percentage incidence of single, double, triple and quadrant affections of teat in cows were 84.42, 7.80, 3.89 and 3.89 respectively and a total of 98 numbers of teat affections were recorded. In buffaloes single and double teat affections were 91.67 and 8.33 per cent and a total of 24 numbers were recorded. A percentage of 100.00 was recorded for a single whole quarter and teat affections in three cows and two buffaloes (Table 2 and Fig.1).

The etiology of obstructive milk flow disorders in cows and buffaloes recorded in this study were physical trauma 46.94 and 66.67 per cent, as a squeal to mastitis 31.63 and 25.00 per cent and other or unknown reasons in 18.37 and 8.33 per cent respectively and congenital affections 3.06 per cent in cows. In cows and buffaloes the lesions causing milk flow disorders were classified and recorded as external in 31.63 and 33.33 per cent and internal in 68.37 and 66.67 per cent respectively revealing higher percentage of obstructive disorders due to physical trauma followed by secondary to mastitis.(Table 3 and Fig.2 and 3).

In both cows and buffaloes hind teats were found to be more affected (59.18 % and 54.17 %) than the fore teats (40.82 % and 45.83 %) and left sided affections were more common (56.12 and 83.33 %) than the right sided (43.88 and 16.67 %) respectively. In cows the highest incidence was recorded in left hind (36.73 %) followed by right hind

Table 2 : Incidence of udder and teat affections in cows and buffaloes

Animal		Cow			Buffalo	
Total no.		98			24	
Part involved		Quarter	Teat	Quarter and teat	Teat	Quarter and teat
		Per cent	Per cent	Per cent	Per cent	Per cent
No. of animals affected	Total	18.37 (n=18)	78.57 (n=77)	3.06 (n=3)	92.31 (n=22)	7.69 (n=2)
	Single	83.33 (n=15)	84.42 (n=65)	100.00 (n=3)	91.67 (n=20)	7.69
	Two	11.11 (n=2)	7.80 (n=6)	- -	8.33 (n=2)	-
	Three	5.56 (n=1)	3.89 (n=3)	- -	-	-
	Four	-	3.89 (n=3)	- -	-	-
Total no. of affections		22	98	3	24	2

Table 3 : Incidence of udder and teat affections based on etiology and type of lesion in cows and buffaloes

Etiology	Cow (n=98)	Buffalo (n=24)
Physical trauma (%)	46.94 (n = 46)	66.67 (n = 16)
Secondary to mastitis (%)	31.63 (n = 31)	25.00 (n = 6)
Congenital (%)	3.06 (n = 3)	-
Others/unknown (%)	18.37 (n = 18)	8.33 (n = 2)
Type of lesion		

External (%)	31.63 (n = 31)	33.33 (n = 8)
Internal (%)	68.37 (n = 67)	66.67 (n = 16)

(22.45 %), right fore (21.43 %) and left fore (19.39 %). In buffaloes left hind (50 %) followed by left fore (33.33 %), right fore (12.50 %) and right hind (4.17 %) (Table 4 and Fig.4).

In cows fore quarters (68 %) were found to be more affected than the hind quarters (32 %) and left sided udder was found to be more affected (52 %) than the right sided udder (48 %). In buffaloes an equal incidence (50 %) was noticed with regard to fore/hind and left/right udder affections (Table 5).

Among the teat lesions in cows, obstructive disorder at the teat canal area was recorded to be the highest incidence (15.30 %) followed by fibrosis condition of the teat canal (13.30 %), hard milker condition (11.22 %), fibrosis condition of the whole teat (8.16 %), obstructive disorder at the distal part of the teat cistern (6.12 %), equal incidence of teat wall wound, teat laceration and teat cistern haematoma (5.10 %), teat fistula, teat tip injury and leaky teat (4.08 %), obstructive disorder at the teat base, obstructive disorder at the mid teat cistern, obstructive disorder at the Furstenberg rosette, teat canal agenesis and thelitis (3.06 %) and obstructive disorder at the proximal part of teat cistern (2.04 %) were noticed (Table 6).

In buffaloes obstructive disorder at the teat canal area was recorded to be the highest incidence (33.33 %) followed by the obstructive disorder at the mid teat cistern (16.66 %), teat laceration (12.50 %), teat tip injury and hard milker (each 8.33 %) and equal incidence of fibrosis of the whole teat, teat cistern haematoma, teat wall wound, leaky teat and thelitis (4.17 %) were noticed (Table 7).

4.2 PARAMETERS STUDIED

4.2.1 Clinical Evaluation

4.2.1.1 Udder and teat parameters

I. Udder shape

In group IIA two cows (33.33 %) on the day 0 of examination had normal udder shape and four cows (66.67 %) had long abdominal type of udder and in group IIB two cows (33.33 %) had normal udders, three cows (50 %) had long abdominal type and one cow (16.67 %) with backward bulging of the quarters. In group IIA buffaloes three

Table 4 : Incidence of the teat obstructive disorders in cows and buffaloes

Disease	**Teat obstructive disorders**							
Animal	**Cow (n=98)**				**Buffalo (n=24)**			
Quarter	**Fore**		**Hind**		**Fore**		**Hind**	
Side	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**
No.	21	19	22	36	3	8	1	12
Per cent	21.43	19.39	22.45	36.73	12.50	33.33	4.17	50.00
Total Per cent	40.82		59.18		45.83		54.17	

Table 5 : Incidence of the whole quarter and teat involvement in cows and buffaloes

Part involved	**Udder**							
Animal	**Cow (n=25)**				**Buffalo (n=2)**			
Quarter	**Fore**		**Hind**		**Fore**		**Hind**	
Side	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**
Whole quarter and teat	-	1	-	2	1	-	-	1
Whole quarter	8	8	4	2	-	-	-	-
Total	8	9	4	4	1	-	-	1
Per cent	32.00	36.00	16.00	16.00	50.00	-	-	50.00
Total Per cent	68.00		32.00		50.00		50.00	

Table 6 : Incidence of teat affections in cows

<table>
<tr><th rowspan="2">Condition</th><th colspan="2" rowspan="2">Site of lesion</th><th colspan="2">Fore</th><th colspan="2">Hind</th><th rowspan="2">Total</th><th rowspan="2">Per cent</th></tr>
<tr><th>Right</th><th>Left</th><th>Right</th><th>Left</th></tr>
<tr><td rowspan="6">Obstructive disorder</td><td colspan="2">Teat base</td><td>1</td><td>-</td><td>-</td><td>2</td><td>3</td><td>3.06</td></tr>
<tr><td rowspan="3">Teat cistern</td><td>Proximal</td><td>1</td><td>-</td><td>-</td><td>1</td><td>2</td><td>2.04</td></tr>
<tr><td>Middle</td><td>-</td><td>-</td><td>2</td><td>1</td><td>3</td><td>3.06</td></tr>
<tr><td>Distal</td><td>-</td><td>1</td><td>2</td><td>3</td><td>6</td><td>6.12</td></tr>
<tr><td colspan="2">Furstenberg rosette</td><td>-</td><td>1</td><td>1</td><td>2</td><td>4</td><td>4.08</td></tr>
<tr><td colspan="2">Teat canal</td><td>3</td><td>3</td><td>6</td><td>3</td><td>15</td><td>15.30</td></tr>
<tr><td rowspan="2">Fibrosis</td><td colspan="2">Whole teat</td><td>2</td><td>2</td><td>3</td><td>1</td><td>8</td><td>8.16</td></tr>
<tr><td colspan="2">Teat canal</td><td>3</td><td>3</td><td>5</td><td>2</td><td>13</td><td>13.30</td></tr>
<tr><td colspan="3">Teat cistern haematoma</td><td>-</td><td>2</td><td>-</td><td>3</td><td>5</td><td>5.10</td></tr>
<tr><td colspan="3">Teat laceration</td><td>1</td><td>2</td><td>1</td><td>1</td><td>5</td><td>5.10</td></tr>
<tr><td colspan="3">Teat fistula</td><td>1</td><td>-</td><td>-</td><td>3</td><td>4</td><td>4.08</td></tr>
<tr><td colspan="3">Teat wall wound</td><td>3</td><td>2</td><td>-</td><td>-</td><td>5</td><td>5.10</td></tr>
<tr><td colspan="3">Teat tip injury</td><td>1</td><td>2</td><td>-</td><td>1</td><td>4</td><td>4.08</td></tr>
<tr><td colspan="3">Hard Milker</td><td>2</td><td>-</td><td>1</td><td>8</td><td>11</td><td>11.22</td></tr>
<tr><td colspan="3">Leaky teat</td><td>1</td><td>-</td><td>1</td><td>2</td><td>4</td><td>4.08</td></tr>
<tr><td colspan="3">Teat canal agenesis</td><td>1</td><td>-</td><td>-</td><td>2</td><td>3</td><td>3.06</td></tr>
<tr><td colspan="3">Thelitis</td><td>2</td><td>1</td><td>-</td><td>-</td><td>3</td><td>3.06</td></tr>
</table>

Total	21	19	22	36	98	100
Per cent	21.43	19.39	22.45	36.73	100	
Total Per cent	40.82		59.18			

Table 7 : Incidence of teat affections in buffaloes

Condition	Site of lesion	Fore		Hind		Total	Per cent
		Right	Left	Right	Left		
Obstructive disorder	Mid Teat cistern	-	-	1	3	4	16.66
	Teat canal	2	5	-	1	8	33.33
Fibrosis	Whole teat	-	-	-	1	1	4.17
Teat cistern haematoma		-	-	-	1	1	4.17
Teat laceration		-	1	-	2	3	12.50
Teat wall wound		-	-	-	1	1	4.17
Teat tip injury		-	1	-	1	2	8.33
Hard Milker		-	1	-	1	2	8.33
Leaky teat		-	-	-	1	1	4.17
Thelitis		1	-	-		1	4.17
Total		3	8	1	12	24	100
Per cent		12.50	33.33	4.17	50.00	100	
Total Per cent		45.83		54.17			

Fig. 1 : Incidence of udder and teat affections in cows and buffaloes

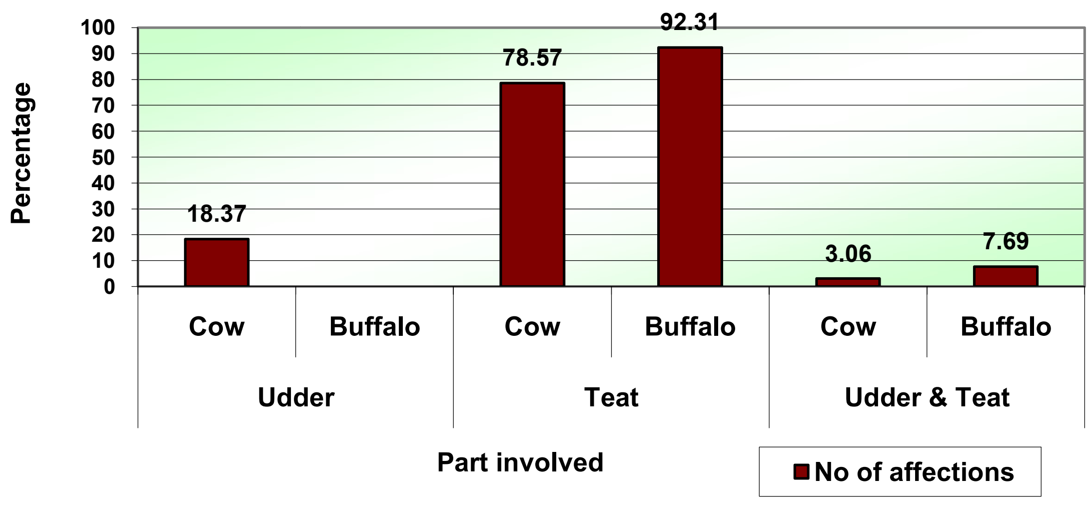


Fig. 2 : Etiology of udder and teat affections in cows

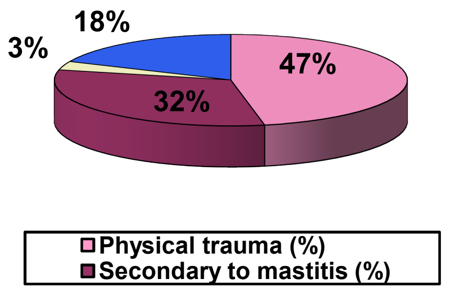
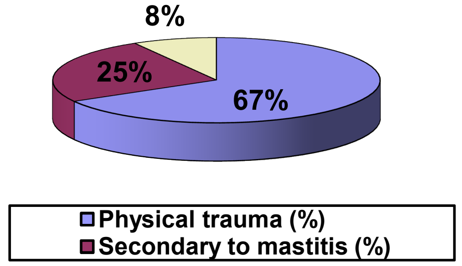


Fig. 3 : Etiology of udder and teat affections in buffaloes



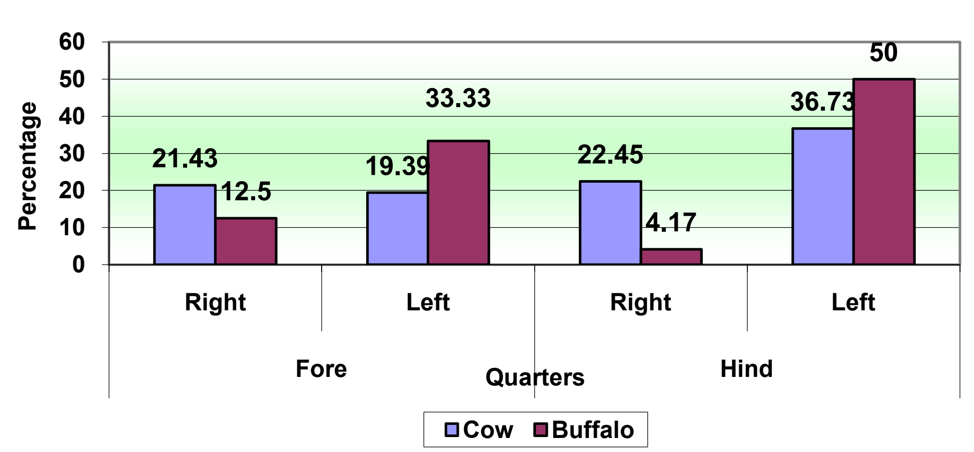


Fig. 4 : Incidence of the teat obstructive disorders in cows and buffaloes

animals (50 %) were examined to have normal udders and two animals (33.33 %) with long abdominal and one animal (16.67 %) had small udder and in group IIB two animals (33.33 %) with normal udder, one animal (16.67 %) with small size and three animals (50 %) with long abdominal type of udders (Table 8).

II. Udder tissue condition

In group IIA cows four animals (66.67 %) on the day 0 of examination had firm udder tissue condition score of 3 and two animals (33.33 %) with normal udder tissue score of one and in IIB three cows (50 %) each were examined with scores of one and three. In group IIA and IIB buffaloes three animals (50 %) in each group had an equal score of one and three on the day of examination (Table 8).

III. Assymetry between fore/between hind quarters

In group IIA cows three animals each (50 %) had slight asymmetry with score of one and pronounced condition with score of three on the day 0 of examination and in group IIB one animal (16.67 %) had the score of one and five animals (83.33 %) had the score of three. In group IIA buffaloes, four animals (66.67 %) with a score of one and two animals (33.33 %) with score of three and in group IIB three animals (each 50 %) with the score of one and three were recorded (Table 8).

IV. Udder oedema

In group IIA cows on the day 0 of examination two animals each (33.33 %) had the score of one, two and three for slight, large and whole area involvement of udder. In group IIB cows, one animal was recorded (16.67 %) with score of one and three animals (50 %) with score of two and two animals (33.33 %) with score of three. In group IIA buffaloes, three animals each (50 %) had a score of one and two (33.33 %) and in group IIB four animals (66.67 %) had a score one and two animals (33.33 %) had score of two (Table 8).

V. Teat cleanliness score

In group IIA cows on the day 0 of examination two animals each (33.33 %) were recorded with teat cleanliness score of two for slight dirty condition and score of three and four for dirty and extremely dirty condition. In group IIB cows two animals (33.33 %) with score two, three animals (50 %) with score three and one animal (16.67 %) with score of four were recorded. In group IIA buffaloes four animals (66.67 %) with score of two and two animals (33.33 %) with score of three and in group IIB buffaloes five animals (83.33 %) with score of five and one animal (16.67 %) with score of three were recorded (Table 8).

VI. Teat shape

In group IIA cows, on the day 0 of examination one animal (16.67 %) had normal teat shape with score of one, two animals (33.33 %) with score of three for conical type of teat, two animals (33.33 %) were recorded with score of four for fleshy condition and one animal (16.67 %) with score of five for other types of teat shape and in group IIB three cows (50 %) with score of one, one cow each (16.67 %) for scores three four and five. In group IIA buffaloes, three animals (50 %) with score one, one animal (16.67 %) with score three and two animals (33.33 %) with score four and in group IIB one animal each (16.67 %) with score of one and three and two animals each (33.33 %) with score of four and five were recorded (Table 8).

VII. Teat wound

In group IIA and IIB cows, three cases (50 %) and two cases (33.33 %) were recorded with presence of wound on the external surface of the teat and in group IIA and IIB buffaloes one (16.67 %) and two cases (33.33 %) were recorded on the day of examination (Table 8).

VIII. Scar tissue in the teat canal

In group IIA cows on day 0 of examination one animal (16.67 %) and IIB two cows (33.33 %) and in group IIA buffaloes two animals (33.33 %) and in group IIB buffaloes one animal (16.67 %) was recorded with the presence of scar tissues in the teat canal (Table 8).

IX. Skin quality of teats

In group IIA cows on the day 0 of examination three animals (50 %) were recorded with the teat skin quality score of three for smooth quality, two cows (33.33 %) for intact and moderately smooth and one cow (16.67 %) for rough type of skin quality and in group IIB cows score of two, three and four were recorded in one (16.67 %), three (50 %) and two (33.33 %) cases. In group IIA buffaloes, score of two (33.33 %), three (50 %) and four in one (16.67 %), three (50 %) and two cases (33.33 %) and in group IIB score of two in five (83.33 %) and three in one case (16.67 %) was recorded (Table 8).

X. Teat palpation score

In group IIA cows, score of one for mild thickening of teat on palpation was recorded in two cases (33.33 %) and score of two for moderate thickening of teat in four cases (66.67 %). In group IIB one cow with score of one (16.67 %) and five cows (83.33 %) with score of two were recorded. In group IIA buffaloes, five animals (83.33 %) with score of two and one animal (16.67 %) with score of three for severe thickening and in group IIB three animals (each 50 %) with score of one and two were recorded (Table 8).

XI. Teat tip to floor distance

Normal teat condition

The mean ± S.E. values of teat tip to floor distance (in cm) of normal left fore, left hind, right fore and right hind teats in group I cows on the day of examination were 51.13 ± 0.9, 50.07 ± 0.60, 50.17 ± 0.49 and 49.65 ± 0.51 and in buffaloes were 48.43 ± 0.36, 47.50 ± 0.33, 48.32 ± 0.37 and 47.15 ± 0.42 respectively.

In cows and buffaloes the mean length of teat tip to floor distance showed statistically significant difference ($P < 0.05$) in the lengths of left and right fore teats and highly significant difference ($P < 0.01$) in the lengths of left hind and right hind teats (Table 9).

Diseased teat condition

The mean ± S.E. values of teat tip to floor distance (in cm) of diseased teat in cows with obstructive milk flow disorder on 0 and 30^{th} day in group IIA were 45.83 ± 1.19 and 48.67 ± 0.42 and in group IIB were 47.67 ± 1.02 and 48.00 ± 0.63 respectively.

Table 8 : Percentage of animals with score values of udder shape, udder tissue condition, asymmetry between fore/hind quarters, udder oedema, teat cleanliness score, teat shape, teat wound, scar tissue in the teat canal, skin quality of teat and teat palpation in group IIA and group IIB cows and buffaloes with milk flow disorders

Clinical Parameter	Score Values	Group IIA		Group IIB	
		Cow	Buffalo	Cow	Buffalo
Udder shape	1	33.33 (n=2)	50.00 (n=3)	33.33 (n=2)	33.33 (n=2)
	2	-	16.67 (n=1)	-	16.67 (n=1)
	3	66.67 (n=4)	33.33 (n=2)	50.00 (n=3)	50.00 (n=3)
	4	-	-	16.67 (n=1)	-
Udder tissue condition	1	33.33(n=2)	50.00 (n=3)	50.00 (n=3)	33.33 (n=2)
	3	66.67 (n=4)	50.00 (n=3)	50.00 (n=3)	66.67 (n=4)
Asymmetry between fore/hind quarters	1	50.00 (n=3)	66.67 (n=4)	16.67 (n=1)	50.00 (n=3)
	3	50.00 (n=3)	33.33 (n=2)	83.33 (n=5)	50.00 (n=3)
Udder oedema	1	33.33 (n=2)	50.00 (n=3)	16.67 (n=1)	66.67 (n=4)
	2	33.33 (n=2)	50.00 (n=3)	50.00 (n=3)	33.33 (n=2)
	3	33.33 (n=2)	-	33.33 (n=2)	-
Teat cleanliness score	2	33.33 (n=2)	66.67 (n=4)	33.33(n=2)	83.33 (n=5)
	3	33.33 (n=2)	33.33 (n=2)	50.00 (n=3)	16.67 (n=1)
	4	33.33 (n=2)	-	16.67 (n=1)	-
Teat shape	1	16.67 (n=1)	50.00 (n=3)	50.00 (n=3)	16.67 (n=1)
	3	33.33 (n=2)	16.67 (n=1)	16.67 (n=1)	16.67 (n=1)
	4	33.33 (n=2)	33.33 (n=2)	16.67 (n=1)	33.33 (n=2)
	5	16.67 (n=1)	-	16.67 (n=1)	33.33 (n=2)
Teat wound	Yes	50.00 (n=3)	16.67 (n=1)	33.33 (n=2)	33.33 (n=2)
	No	50.00 (n=3)	83.33 (n=5)	66.67 (n=4)	66.67 (n=4)
Scar tissue in the teat canal	Yes	16.67 (n=1)	33.33 (n=2)	33.33 (n=2)	16.67 (n=1)
	No	83.33 (n=5)	66.67 (n=4)	66.67 (n=4)	83.33 (n=5)
Skin quality of teat	2	50.00 (n=3)	83.33 (n=5)	16.67 (n=1)	83.33 (n=5)
	3	33.33 (n=2)	16.67 (n=1)	50.00 (n=3)	16.67 (n=1)
	4	16.67 (n=1)	-	33.33 (n=2)	-
Teat palpation	1	33.33 (n=2)	-	16.67 (n=1)	50.00 (n=3)
	2	66.67 (n=4)	83.33 (n=5)	83.33 (n=5)	50.00 (n=3)

The mean values showed no statistically significant difference ($P<0.05$) between the two groups but in each group the values were found to be increased from 0 day to 30th day (Table 10).

In buffaloes the mean ± S.E. values on 0 and 30th day in group IIA were 46.72 ± 0.62 and 48.92 ± 0.99 and in group IIB were 49.28 ± 0.66 and 50.63 ± 0.62 respectively and the mean values of 0 and 30th day showed a statistically significant difference ($P<0.05$) between the two groups (Table 11).

XII. Teat length

Normal teat condition

The mean ± S.E. values of teat length (in cm) of normal left fore, left hind, right fore and right hind teats in group I cows on the day of examination were 7.77 ± 0.12, 7.37 ± 0.15, 7.72 ± 0.09 and 7.32 ± 0.21 and in buffaloes were 8.05 ± 0.13, 8.38 ± 0.22, 7.85 ± 0.10 and 8.22 ± 0.12 respectively.

In cows and buffaloes the mean values were found to be non significant for the left and right fore teats and highly significant difference ($P<0.01$) for the left and right hind teats (Table 9).

Diseased teat condition

The mean ± S.E. values of teat length (in cm) of diseased teat in cows with obstructive milk flow disorder on 0, 30 and long term follow up days in group IIA were 10.32 ± 0.27, 7.78 ± 0.14 and 7.67 ± 0.19 and in group IIB were 9.95 ± 0.38, 8.40 ± 0.12 and 7.90 ± 0.21 respectively. The mean values between the treatment days and groups were found to be statistically highly significant ($P<0.01$) (Table 10).

In buffaloes the mean ± S.E. values on 0, 30 and long term follow up days in group IIA were 9.48 ± 0.22, 8.75 ± 0.24 and 8.05 ± 0.25 and in group IIB were 9.20 ± 0.19, 8.62 ± 0.17 and 8.65 ± 0.15 respectively.

The mean values of group IIA between 0, 30 and long term follow up days and in group IIB between 0 and 30th days were found to be statistically highly significant ($P<0.01$) (Table 11).

XIII. Teat girth

Normal teat condition

The mean ± S.E. values of teat girth (in cm) of normal left fore, left hind, right fore and right hind teats in group I cows on the day of examination were 9.27 ± 0.32, 9.58 ± 0.28, 9.03 ± 0.25 and 9.73 ± 0.24 and in buffaloes were 10.18 ± 0.26, 10.62 ± 0.21, 9.97 ± 0.21 and 10.52 ± 0.21 respectively. The mean values showed a statistically significant difference (P<0.05) between cows and buffaloes (Table 9).

Diseased teat condition

The mean ± S.E. values of teat length (in cm) of diseased teat in cows with obstructive milk flow disorder on 0, 30 and long term follow up days in group IIA were 10.28 ± 0.35, 8.12 ± 0.18 and 8.07 ± 0.19 and in group IIB were 10.93 ± 0.27, 9.03 ± 0.17 and 8.62 ± 0.13 respectively. In group IIA and group IIB the mean values were found to be statistically highly significant (P<0.01) between 0 and 30th day and no significant difference was noticed between 30th and long term follow up days (Table 10).

In buffaloes the mean ± S.E. values on 0, 30 and long term follow up days in group IIA were 10.77 ± 0.20, 9.42 ± 0.11 and 10.25 ± 0.20 and in group IIB were 9.87 ± 0.21, 9.68 ± 0.08 and 10.05 ± 0.22 respectively. In group IIA the mean values were found to be statistically highly significant (P<0.01) between the treatment days and in group IIB between 0 and long term follow up days (Table 11).

XIV. Teat canal length (Medl's test)

Normal teat condition

The mean ± S.E. values of teat canal length (in cm) of normal left fore, left hind, right fore and right hind teats in group I cows on the day of examination were 1.40 ± 0.06, 1.33 ± 0.08, 1.27 ± 0.06 and 1.25 ± 0.07 and in buffaloes were 1.80 ± 0.05, 2.03 ± 0.10, 1.77 ± 0.08 and 2.07 ± 0.10 respectively. The mean values showed statistically high significant difference (P<0.01) between cows and buffaloes (Table 9).

Diseased teat condition

The mean ± S.E. values of teat canal length (in cm) of diseased teat in cows with obstructive milk flow disorder on 0, 30 and long term follow up days in group IIA were 1.60 ± 0.05, 1.02 ± 0.07 and 1.05 ± 0.05 and in group IIB were 1.48 ± 0.08, 1.23 ± 0.05

and 1.15 ± 0.07 respectively. In group IIA and group IIB the mean values were found to be statistically highly significant ($P<0.01$) between 0 and 30th day with no significant difference between 30th and long term follow up days (Table 10).

In buffaloes the mean ± S.E. values on 0, 30 and long term follow up days in group IIA were 2.10 ± 0.1, 1.87 ± 0.09 and 1.85 ± 0.06 and in group IIB were 1.95 ± 0.05, 1.80 ± 0.04 and 1.80 ± 0.08 respectively. The mean values revealed no statistically significant difference between the treatment groups. The mean values in both the groups between the treatment days were found to be slightly decreased from 0 day to long term follow up days (Table 11).

4.2.1.2 Milk parameters

I. Total milkability by hand

Normal

The mean ± S.E. values of total milkability by hand in milliliters of normal left fore, left hind, right fore and right hind teats in group I cows on the day of examination were 1650.00 ± 99.16, 2150.00 ± 131.02, 1583.33 ± 94.58 and 2066.67 ± 152.02 and in buffaloes were 1100.00 ± 57.74, 1400.00 ± 96.61, 1166.67 ± 66.67 and 1383.33 ± 94.58 respectively. The mean values showed statistically highly significant difference ($P<0.01$) between cows and buffaloes (Table 12).

Diseased condition

In cows the mean ± S.E. values of total milkability by hand in milliliters of cows with diseased teat condition on 0, 30 and long term follow up days in group IIA were 400.00 ± 51.64, 1500.00 ± 141.42 and 1850.00 ± 108.78 and in group IIB were 333.33 ± 66.67, 1366.67 ± 142.98 and 1500.00 ± 146.06 respectively. The mean values were found to be highly significant ($P<0.01$) between the treatment groups. In group I and group II high significant difference ($P<0.01$) was noticed between 0 and long term follow up days and although with a slight raise in the values of group IIA the values of treatment days between groups remained to be similar (Table 13).

In buffaloes total milkability by hand in milliliters with diseased teat condition on 0, 30 and long term follow up days in group IIA were 350.00 ± 56.27, 1133.33 ± 80.28 and 1300.00 ± 57.74 and in group IIB were 283.33 ± 60.09, 1050.00 ± 99.16 and 1166.67 ± 120.19 respectively. The mean values were found to be statistically highly significant ($P<0.01$) between the treatment groups. In group IIA and IIB significant difference of

Table 9 : Mean ± S.E. of the teat tip to floor distance, teat length and teat canal length values of group I normal cows and buffaloes

Parameter	Quarter	Side	Cow	Buffalo	t - value	Significance
Teat tip to floor distance (cm)	Left	Fore	51.13 ± 0.91	48.43 ± 0.36	2.77	*
		Hind	50.07 ± 0.60	47.50 ± 0.33	3.76	**
	Right	Fore	50.17 ± 0.49	48.32 ± 0.37	3.01	*
		Hind	49.65 ± 0.51	47.15 ± 0.42	3.81	**
Teat length (cm)	Left	Fore	7.77 ± 0.12	8.05 ± 0.13	1.56	NS
		Hind	7.37 ± 0.15	8.38 ± 0.22	3.86	**
	Right	Fore	7.72 ± 0.09	7.85 ± 0.10	0.97	NS
		Hind	7.32 ± 0.21	8.22 ± 0.12	3.66	**
Teat girth	Left	Fore	9.27 ± 0.32	10.18 ± 0.26	2.24	*
		Hind	9.58 ± 0.28	10.62 ± 0.21	2.98	*
	Right	Fore	9.03 ± 0.25	9.97 ± 0.21	2.88	*
		Hind	9.73 ± 0.24	10.52 ± 0.21	2.43	*
Teat canal length (cm)	Left	Fore	1.40 ± 0.06	1.80 ± 0.05	4.90	**
		Hind	1.33 ± 0.08	2.03 ± 0.10	5.39	**
	Right	Fore	1.27 ± 0.06	1.77 ± 0.08	5.30	**
		Hind	1.25 ± 0.07	2.07 ± 0.10	6.83	**

Means bearing similar superscripts between groups do not differ significantly
P values of < 0.01 - ** (Highly Significant)
P values of 0.01 – 0.05 - * (Significant)
P values of > 0.05 – NS (Non Significant)

Table 10 : Mean ± S.E. of the teat tip to floor distance, teat length and teat canal length values of group IIA and IIB cows treated by theloscopic surgery with and without bio-implant on 0, 30 and long term follow up days

Parameter	Day	Treatment		F - Value	Significance
		With implant	Without implant		
Teat tip to floor distance (cm)	0	45.83 ± 1.19	47.67 ± 1.02	1.928	NS
	30	48.67 ± 0.42	48.00 ± 0.63		
Teat length (cm)	0	10.32 ± 0.27^{b}	9.95 ± 0.38^{b}	24.450	**
	30	7.78 ± 0.14^{a}	8.40 ± 0.12^{a}		
	Long term follow up	7.67 ± 0.19^{a}	7.90 ± 0.21^{a}		
Teat Girth (cm)	0	10.28 ± 0.35^{c}	10.93 ± 0.27^{c}	27.260	**
	30	8.12 ± 0.18^{a}	9.03 ± 0.17^{b}		
	Long term follow up	8.07 ± 0.19^{a}	8.62 ± 0.13^{ab}		
Teat canal length (cm)	0	1.60 ± 0.05^{c}	1.48 ± 0.08^{c}	13.694	**
	30	1.02 ± 0.07^{a}	1.23 ± 0.05^{b}		
	Long term follow up	1.05 ± 0.05^{ab}	1.15 ± 0.07^{ab}		

Means bearing similar superscripts between groups do not differ significantly
P values of < 0.01 - ** (Highly Significant)
P values of 0.01 – 0.05 - * (Significant)
P values of > 0.05 – NS (Non Significant)

Table 11 : Mean ± S.E. of the teat tip to floor distance, teat length and teat canal length values of group IIA and IIB buffaloes treated by theloscopic surgery with and without bio-implant on 0, 30 and long term follow up days

Parameter	Day	Treatment		F - Value	Significance
		With implant	Without implant		
Teat tip to floor distance (cm)	0	46.72 ± 0.62	49.28 ± 0.66	4.83	*
	30	48.92 ± 0.99	50.63 ± 0.62		
Teat length (cm)	0	9.48 ± 0.22^{c}	9.20 ± 0.19^{bc}	5.94	**
	30	8.75 ± 0.24^{b}	8.62 ± 0.17^{ab}		
	Long term follow up	8.05 ± 0.25^{a}	8.65 ± 0.15^{ab}		
Teat Girth (cm)	0	10.77 ± 0.20^{d}	10.25 ± 0.20^{cd}	6.93	**
	30	10.05 ± 0.22^{bc}	9.87 ± 0.21^{abc}		
	Long term follow up	9.42 ± 0.11^{a}	9.68 ± 0.08^{ab}		
Teat canal length (cm)	0	2.10 ± 0.11^{b}	1.95 ± 0.05^{ab}	2.32	NS
	30	1.87 ± 0.09^{a}	1.80 ± 0.04^{a}		
	Long term follow up	1.85 ± 0.06^{a}	1.80 ± 0.08^{a}		

Means bearing similar superscripts between groups do not differ significantly
P values of < 0.01 - ** (Highly Significant)
P values of 0.01 – 0.05 - * (Significant)
P values of > 0.05 – NS (Non Significant)

mean values was noticed between the 0 and 30th day and the mean values of parallel treatment days between the groups remained to be similar (Table 14).

II. Electrical conductivity

Normal

The mean ± S.E. values of electrical conductivity of milk (in mS/cm) of normal cows related to left fore, left hind, right fore and right hind teats in group I cows on the day of examination were 4.90 ± 0.09, 4.88 ± 0.09, 4.92 ± 0.09 and 5.08 ± 0.07 and in buffaloes were 5.13 ± 0.10, 4.95 ± 0.12, 5.15 ± 0.06 and 5.15 ± 0.05 respectively. The mean values releaved no statistically significant difference between the cows and buffaloes. The mean electrical conductivity values of buffaloes remained to be slightly higher than values of cows (Table 12).

Diseased condition

In cows the mean ± S.E. values of electrical conductivity of milk (mS/cm) in cows with diseased teat condition on 0, 30 and long term follow up days in group IIA were 6.08 ± 0.09, 5.02 ± 0.09 and 4.92 ± 0.09 and in group IIB were 6.07 ± 0.10, 5.15 ± 0.06 and 4.88 ± 0.09 respectively. A statistically high significant difference ($P<0.01$) was noticed between the treatment groups. In group IIA and IIB the mean values were highly significant between 0 and 30th day and no significant difference was noticed between day 30 and long term follow up days (Table 12).

In buffaloes the mean ± S.E. values of electrical conductivity of milk (mS/cm) with diseased teat condition on 0, 30 and long term follow up days in group IIA were 6.07 ± 0.12, 5.22 ± 0.07 and 5.13 ± 0.06 and in group IIB were 6.18 ± 0.08, 5.08 ± 0.10 and 5.12 ± 0.05 respectively. The mean values showed statistically high significant difference ($P<0.01$) between the treatment groups. In group IIA and IIB the mean values were highly significant between 0 and 30th day and no significant difference was noticed between day 30 and long term follow up days (Table 14).

III. Somatic cell count

Normal

The mean ± S.E. values of somatic cell count of milk (10^5cells/ml) identified by Giemsa stain and modified Newman's stain (Plate 29 and Plate 30) of normal cows related to left fore, left hind, right fore and right hind teats in group I cows on the day of examination were 1.31 ± 0.10, 1.28 ± 0.04, 1.22 ± 0.12 and 1.33 ± 0.06 and in buffaloes were 1.09 ± 0.05, 1.16 ± 0.04, 1.11 ± 0.04 and 1.21 ± 0.03 respectively. The mean values revealed no statistically significant difference between cows and buffaloes but the mean somatic cell values of cows were found to be slightly higher than the buffaloes (Table 12 and Fig.5).

Diseased condition

In cows the mean ± S.E. values of somatic cell count of milk (10^5cells/ml) in cows with diseased teat condition on 0, 30 and long term follow up days in group IIA were 27.95 ± 3.43, 4.80 ± 0.73 and 1.74 ± 0.10 and in group IIB were 26.48 ± 2.64, 5.61 ± 0.73 and 2.03 ± 0.10 respectively (Table 13 and Fig.9).

In buffaloes the mean ± S.E. values of somatic cell count of milk (10^5cells/ml) on 0, 30 and long term follow up days in group IIA were 25.29 ± 2.94, 4.33 ± 0.73 and 1.68 ± 0.08 and in group IIB were 27.15 ± 3.12, 4.83 ± 0.94 and 1.82 ± 0.04 respectively (Table 14 and Fig.10).

In both group IIA and IIB cows and buffaloes the mean values showed statistically high significant difference on 0 and 30th day ($P<0.01$) and between the treatment groups. The parallel mean values on 30 and long term follow up days in group IIA and IIB showed slightly higher mean values in group IIB than group IIA.

IV.California mastitis test score

In group IIA cows California Mastitis Test Score (CMT) on day 0 of investigation was found to be 2 in all six cases 100 per cent and score of 1 in four cases (66.67 %) and score of Trace (T) in two cases (33.33 %) on 30th day and on long term follow up day score of T (100 %) was recorded in all the six cases. In group IIB cows on day 0 of examination CMT score of 2 was noticed in all the six cases (100 %). On 30th day score of 1 was recorded in four cases (66.67 %) and score of T was recorded in two cases (33.33 %) and on long term follow up score of T was recorded in all the six cases (100 %)

In group IIA and group IIB buffaloes score of 2 was recorded in six cases (100 %) on day 0 of investigation, score of 1 and T each 3 cases (50 %) on 30th day and score of T in six cases (100 %) on long term follow up day (Table 15).

Table 12 : Mean ± S.E. of milkability by hand from the quarter, electrical conductivity of milk and somatic cell count values of group I normal cows and buffaloes

Parameter	Quarter	Side	Cow	Buffalo	t - value	Significance
Milkability by hand from the quarter (ml)	Fore	Left	1650.00 ± 99.16	1100.00 ± 57.74	4.79	**
		Right	2150.00 ± 131.02	1400.00 ± 96.61	4.61	**
	Hind	Left	1583.33 ± 94.58	1166.67 ± 66.67	3.60	**
		Right	2066.67 ± 152.02	1383.33 ± 94.58	3.82	**
Electrical conductivity of milk (mS/cm)	Fore	Left	4.90 ± 0.09	5.13 ± 0.10	1.75	NS
		Right	4.88 ± 0.09	4.95 ± 0.12	0.43	NS
	Hind	Left	4.92 ± 0.09	5.15 ± 0.06	2.12	NS
		Right	5.08 ± 0.07	5.15 ± 0.05	0.81	NS
Somatic cell count X 10^5 (cells per ml)	Fore	Left	1.31 ± 0.10	1.09 ± 0.05	2.02	NS
		Right	1.28 ± 0.04	1.16 ± 0.04	2.01	NS
	Hind	Left	1.22 ± 0.12	1.11 ± 0.04	0.89	NS
		Right	1.33 ± 0.06	1.21 ± 0.03	1.66	NS

Means bearing similar superscripts between groups do not differ significantly
P values of < 0.01 - ** (Highly Significant)
P values of 0.01 – 0.05 - * (Significant)
P values of > 0.05 – NS (Non Significant)

Table 13 : Mean ± S.E. of milkability by hand from the quarter, electrical conductivity of milk and somatic cell count values of group IIA and IIB cows treated by theloscopic surgery with and without bio-implant on 0, 30 and long term follow up days

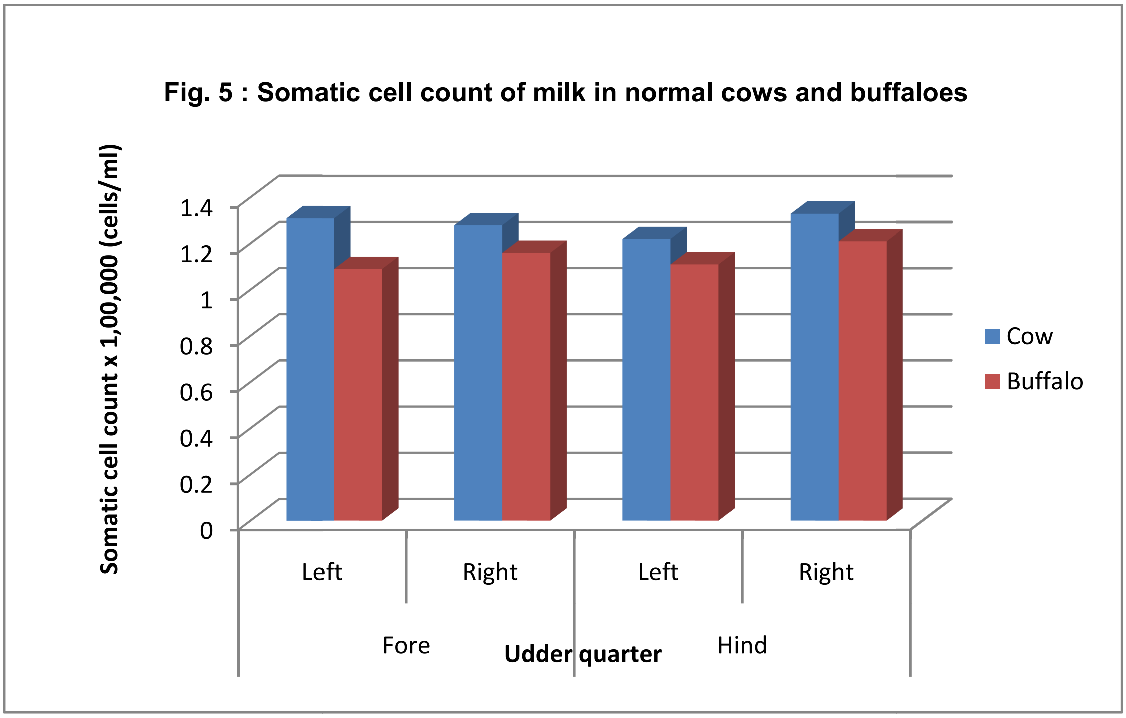
Parameter	Day	Treatment		F - Value	Significance
		With implant	Without implant		
Milkability by hand from the quarter (ml)	0	400.00 ± 51.64^{a}	333.33 ± 66.67^{a}	29.895	**
	30	1500.00 ± 141.42^{bc}	1366.67 ± 142.98^{b}		
	Long term follow up	1850.00 ± 108.78^{c}	1500.00 ± 146.06^{bc}		
Electrical conductivity of milk (mS/cm)	0	6.08 ± 0.09^{b}	6.07 ± 0.10^{b}	41.897	**
	30	5.02 ± 0.09^{a}	5.15 ± 0.06^{a}		
	Long term follow up	4.92 ± 0.09^{a}	4.88 ± 0.09^{a}		
Somatic cell count X 10^{5} (cells per ml)	0	27.95 ± 3.43^{b}	26.48 ± 2.64^{b}	45.934	**
	30	4.80 ± 0.73^{a}	5.61 ± 0.73^{a}		
	Long term follow up	1.74 ± 0.10^{a}	2.03 ± 0.10^{a}		

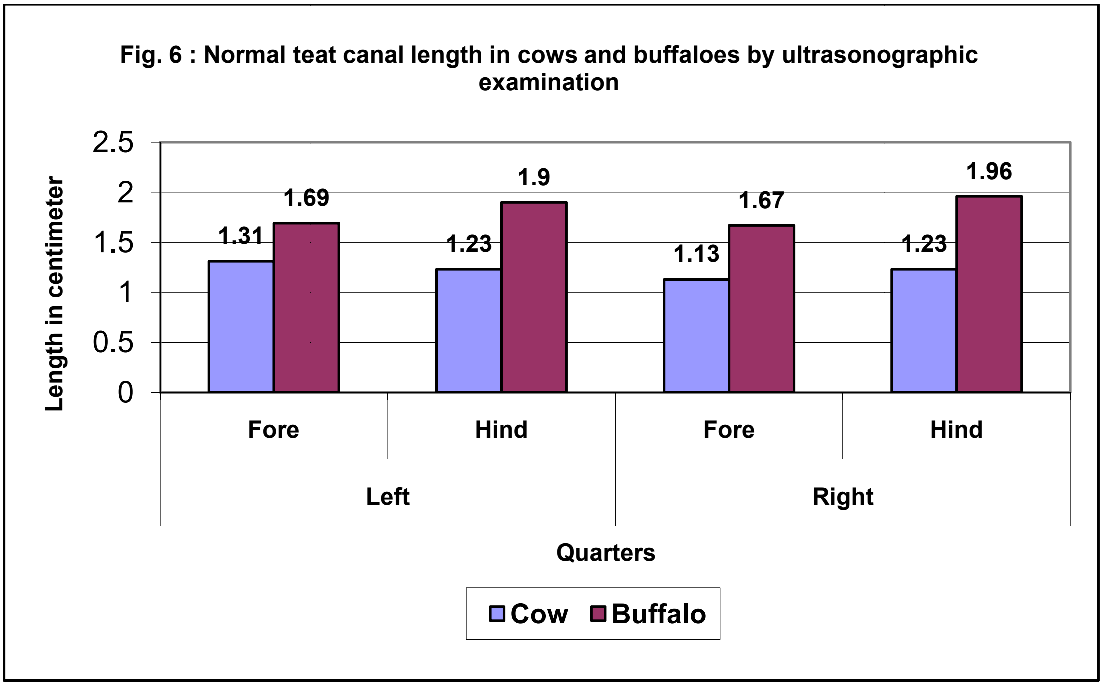
Means bearing similar superscripts between groups do not differ significantly
P values of < 0.01 - ** (Highly Significant)
P values of 0.01 – 0.05 - * (Significant)
P values of > 0.05 – NS (Non Significant)

Table 14 : Mean ± S.E. of milkability by hand from the quarter, electrical conductivity of milk and somatic cell count values of group IIA and IIB buffaloes treated by theloscopic surgery with and without bio-implant on 0, 30 and long term follow up days

Parameter	Day	Treatment		F - Value	Significance
		With implant	Without implant		
Milkability by hand from the quarter (ml)	0	350.00 ± 56.27[a]	283.33 ± 60.09[a]	29.05	**
	30	1133.33 ± 80.28[b]	1050.00 ± 99.16[b]		
	Long term follow up	1300.00 ± 57.74[b]	1166.67 ± 120.19[b]		
Electrical conductivity of milk (mS/cm)	0	6.07 ± 0.12[b]	6.18 ± 0.08[b]	28.23	**
	30	5.22 ± 0.07[a]	5.08 ± 0.10[a]		
	Long term follow up	5.13 ± 0.06[a]	5.12 ± 0.05[a]		
Somatic cell count X 10^5 (cells per ml)	0	25.29 ± 2.94[b]	27.15 ± 3.12[b]	43.58	**
	30	4.33 ± 0.73[a]	4.83 ± 0.94[a]		
	Long term follow up	1.68 ± 0.08[a]	1.82 ± 0.04[a]		

Means bearing similar superscripts between groups do not differ significantly
P values of < 0.01 - ** (Highly Significant)
P values of 0.01 – 0.05 - * (Significant)
P values of > 0.05 – NS (Non Significant)





V. Bacterial isolation and identification

The organisms identified in group IIA cases on day 0 were *Staphylococcus* sp. (33.33 %) (Plate 31 and 32), *Streptococcus* sp. (16.67 %) (Plate 33 and 34) and *E.coli* (50 %) (Plate 35 and 36). On day 30, bacterial organisms were identified in three cases affected with *Staphylococcus* sp., *Streptococcus* sp. and *E.coli* each 16.67 per cent and on long term follow up day presence of *Staphylococcus* sp. and *Streptococcus* sp. each 16.67 per cent respectively. In group IIB on day 0 of examination the organisms identified were *Staphylococcus* sp. (33.33 %), *Streptococcus* sp. (16.67 %), *E.coli* (33.33 %) and *Corynebacterium* (16.67 %). On 30th day the organisms identified were *Staphylococcus* sp. (16.67 %), *Streptococcus* sp. (16.67 %) and *E.coli* (33.33 %) and *Staphylococcus* sp. and *Streptococcus* sp. each 16.67 per cent on long term follow up day (Table 16).

In buffaloes on day 0 of examination the bacterial organisms isolated in group IIA were *Staphylococcus* sp., *Streptococcus* sp. and *E.coli* each 33.33 per cent on day 30 *Staphylococcus* sp. and *Streptococcus* sp. each 16.67 per cent and *Streptococcus* sp. 16.67 per cent on long term follow up day. In group IIB on day 0 of examination the bacterial organisms isolated were *Staphylococcus* sp. and *Streptococcus* sp. each 33.33 per cent and *E.coli* and *Klebsiella* each 16.67 per cent respectively. On day 30 *Staphylococcus* sp. were isolated in two cases (33.33 %) and *Streptococcus* sp. in one case (16.67 %) and on long term follow up day two cases were identified with bacterial infection *Staphylococcus* sp. and *Streptococcus* sp. each with 16.67 per cent (Table 17).

VI. Antibiotic sensitivity test

In Group IIA cows on day 0 of examination the antibiogram showed the following percentage of sensitivity to the antibiotics; enrofloxacin 83.33 per cent (Plate 37), gentamicin 83.33 per cent, ceftrioxone 66.67 per cent, amoxicillin with clavulanic acid 33.33 per cent and amoxicillin 0 per cent and on day 30 in two cases were found to be sensitive to enrofloxacin 100 per cent, gentamicin 100 per cent, ceftrioxone 50 per cent, amoxicillin with clavulanic acid 50 per cent. In group IIB the following sensitivity

Table 15 : California mastitis test score in group IIA and group IIB cows and buffaloes

Animal	Cow						Buffalo					
	IIA			IIB			IIA			IIB		
	0	30	LTF	0	30	LTF	0	30	LTF	0	30	LTF
1.	2	1	T	2	1	T	2	1	T	2	1	T
2.	2	1	T	2	1	T	2	T	T	2	T	T
3.	2	T	T	2	T	T	2	T	T	2	T	T
4.	2	T	T	2	T	T	2	T	T	2	T	T
5.	2	1	T	2	1	T	2	1	T	2	1	T
6.	2	1	T	2	1	T	2	1	T	2	1	T
T %	-	33.33	100.00	-	33.33	100.00	-	50.00	100.00	-	50.00	100.00
1 %	-	66.67	-	-	66.67	-	-	50.00	-	-	50.00	
2 %	100.00	-	-	100.00	-	-	100.00	-	-	100.00	-	

LTF – Long term follow up days

Table 16 : Percentage of organisms isolated from group IIA and IIB cow's milk on 0, 30 and long term follow up days

Organisms isolated	Cow Milk					
	Group IIA (n=6)			Group IIB (n=6)		
	Day 0	Day 30	Long term follow up day	Day 0	Day 30	Long term follow up day
Staphylococcus sp. (%)	33.33 (n=2)	16.67 (n=1)	16.67 (n=1)	33.33 (n=2)	16.67 (n=1)	16.67 (n=1)
Streptococcus sp. (%)	16.67 (n=1)	16.67 (n=1)	16.67 (n=1)	16.67 (n=1)	16.67 (n=1)	16.67 (n=1)
E.coli (%)	50.00 (n=3)	16.67 (n=1)	-	33.33 (n=2)	33.33 (n=2)	-
Corynebacterium (%)	-	-	-	16.67 (n=1)	-	-

Table 17 : Percentage of organisms isolated from group IIA and IIB buffalo's milk on 0, 30 and long term follow up days

Organisms isolated	Buffalo Milk					
	Group IIA (n=6)			Group IIB (n=6)		
	Day 0	Day 30	Long term follow up day	Day 0	Day 30	Long term follow up day
Staphylococcus sp. (%)	33.33 (n=2)	16.67 (n=1)	-	33.33 (n=2)	33.33 (n=2)	16.67 (n=1)
Streptococcus sp. (%)	33.33 (n=2)	16.67 (n=1)	16.67 (n=1)	33.33 (n=2)	16.67 (n=1)	16.67 (n=1)
E.coli (%)	33.33 (n=2)	-	-	16.67 (n=1)	-	-
Klebsiella (%)	-	-	-	16.67 (n=1)	-	-

percentage to antibiotics was noticed; enrofloxacin 83.33 per cent, gentamicin 66.67 per cent, ceftrioxone 50 per cent, amoxicillin with clavulanic acid 16.67 per cent and amoxicillin 0 per cent and on day 30 four cases antibiotic sensitivity in four cases were enrofloxacin 100 per cent, gentamicin 100 per cent, ceftrioxone 50 per cent, amoxicillin with clavulanic acid 75 per cent and amoxicillin 0 per cent.

In buffaloes the percentage of sensitivity to antibiotics in group IIA cases on the day 0 of examination were enrofloxacin 100 per cent, gentamicin 100 per cent, ceftrioxone 66.67 per cent, amoxicillin with clavulanic acid 33.33 per cent and amoxicillin 0 per cent and on day 30 in two cases enrofloxacin 100 per cent, gentamicin 100 per cent, ceftrioxone 50 per cent, amoxicillin with clavulanic acid 100 per cent and amoxicillin 0 per cent of sensitivity was noticed. In group IIB buffaloes on day 0 of examination enrofloxacin was found to be sensitive in 100 per cent followed by gentamicin 83.33 per cent, ceftrioxone 50 per cent, amoxicillin with clavulanic acid 33.33 per cent and amoxicillin 0 per cent and on day 30 in three cases the sensitivity to enrofloxacin was 100 per cent followed by gentamicin 66.67 per cent, ceftrioxone 66.67 per cent, amoxicillin with clavulanic acid 66.67 per cent and amoxicillin 0 per cent respectively. In group IIA and IIB cows and buffaloes on 0 and 30th day of investigation resistance to amoxicillin was noticed (Table 18).

4.2.2 Radiographic Examination of Normal and Diseased Teat

4.2.2.1 Plain radiography

Plain radiographic examination procedure of udder and teat were found to be easy but they fail to demonstrate the obstructive lesions and pathological alterations in the teat and udder tissues (Plate 38).

Normal and diseased udder and teat

In both normal and diseased teat study the approximate alterations in the size of the teat in comparison with the contralateral teats alone could be studied. The radiographs of udder and teat tissues were imaged as uniform shades of grey with lack of soft tissue contrast details.

4.2.2.2 Contrast radiography

Normal and diseased udder and teat

The contrast study of the udder and teat were found to be better diagnostic techniques than the plain radiographic technique.

In positive contrast study of the normal udder and teat the contrast agent injected into the teat cistern masked the internal details of the teat cistern. However this technique demonstrated the size, shape of the teat cistern, pattern of lactiferous ducts of the mammary glands and the length of the teat canal.

In obstructive milk flow disorder condition, usage of contrast agents into the teat cistern was found to be debatable because of the possible irritant nature of the iodine content of the contrast agent. In conditions up to partial obstructions of the gland and teat cistern this technique was found to be more useful to study the diameter, shape of the cistern, the degree of change in the teat wall thickness, demonstration of pattern of fistulous tract and to measure the approximate length of the teat canal. This technique was more effective to demonstrate the lesions in the teat cistern involving less than 30 per cent of the mucosal surface and also helped to quantify the approximate size and site of the lesion.

The negative contrast study of normal and diseased udder and teat showed similar diagnostic utility as that of positive contrast study and demonstrated better radiographic images than plain radiography. However the usage of the unsterile atmospheric air into the teat and gland cistern leads to debate on possibility of introduction of infection into the mammary gland.

In negative study of normal udder and teat the concentric folds of the teat cistern could be visualised better than the positive contrast study and the area of the gland and teat cistern could be appreciated with the demonstration of pattern of lactiferous ducts. Injection of air into the teat cistern inflated the teat cisternal cavity and the menstruation of the teat cisternal cavity was found to be difficult.

Double contrast radiographic study of the normal udder and teat provided better imaging details than plain, positive and negative contrast radiography. The size, shape and the mucosal fold pattern of the teat cistern and the gland cistern, and tracts of lactiferous ducts was well demonstrated than the other radiographic techniques (Plate 39).

Double contrast radiography enabled better diagnosis than plain, positive and negative contrast techniques. The proliferative lesions in the teat cistern, changes in the internal architecture of mucosal surfaces of teat cistern, teat wall thickness, shape, size, length of the stenotic lesions in the cisternal cavities were well demonstrated and overall had the advantageous features similar to that of double contrast cystographic study.

4.2.2.3 Limitations

Plain contrast radiographic technique was easy to perform but they fail to image the soft tissue contrast details. Usage of positive contrast agent into the teat cistern for diagnostic study can be considered to be a non physiological method and its usage during inflamed conditions into teat cistern and mammary gland is questionable. Positive contrast agent masked the pathological lesions in the cisternal cavities and hence limited diagnostic utility was noticed. Spillage of the positive contrast agents in the outer surface of the teat wall produced artifacts and false positive results. Positive contrast agent could not be injected into the teat cistern in conditions of fibrosis and complete obstruction of the teat canal and distal parts of the teat cistern.

In negative contrast study injection of atmospheric air into the teat cistern is questionable because of the possibility of carrying the infection into the mammary gland. As in positive contrast study, the details of internal lesions of the teat cistern were found to be masked and the inability situation to inject the air during obstructive conditions was also noticed.

Double contrast radiography had the similar limitations encountered with that of positive and negative contrast radiography and the diagnostic sensitivity was found to be more superior to the other radiographic techniques.

4.2.3 Ultrasonographic Examination

Normal and diseased udder and teat

The udder and base of the teat are amenable to sonographic imaging because of its superficial location and had the potential to diagnose different physiological status of the organ. The glandular parenchyma of udder appeared as granular, homogenous hyperechoic with anechoic alveoli, lactiferous ducts as anechoic areas. The gland sinus appeared as homogenous anechoic area in continuous with the teat sinus. The lining of wall of the gland sinus appeared as mixed hyper-echoic folds. During ultrasonography the secretion and let down of milk in the cisternal area was identified as typical whirling effects of the milk constituents as echoic particle movement in the cisternal cavities. In this study these structures were well appreciable by contact with coupling gel technique using 5 MHz convex and 7.5 MHz linear transducers.

The ultrasonographic diagnosis of the udder lesions recorded in this study were udder fibrosis, oedema, abscess and atrophy of the gland. Udder oedema, abscess and haematoma appeared as hypoechoic space occupying images within the homogenous hyperechoic udder parenchyma. Atresia and fibrosis of the udder appeared as hyperechoic appearance and loss of typical echopattern of the udder.

In water bath technique all the teats appeared as hypoechoic structures with anechoic lumens. The teat walls appeared as hyperechoic outer layer, hypoechoic thicker middle layer and more hyperechoic inner layer. The teat canal appeared as thin, bright white line delineated on each side by parallel thick, dark, grey black bands. The teat canal was variably visible at the teat orifice whereas in the proximal part a protruding hypoechoic mass was noticed indicative of the mucosa of the Frustenberg rosette at the junction of the teat sinus and the papillary duct. During ultrasonography the secretion and let down of milk in the cisternal area was identified as typical whirling effects of the milk constituents as echoic particle movement in the cisternal cavities (Plate 40, 41, 42, 43, 52, 53 and 54).

The ultrasonographic diagnosis of the teat lesions included in this study were intracisternal obstructions, lacerations, fistula, fibrosis and thelitis. Intraluminal teat obstructions appeared as hyperechoic shadow located adjacent to the mucosa. Teat canal fibrosis appeared as hyperechoic shadow in the teat canal and the tunica intima was absent in the proximal part of the teat canal area. The extent of trauma on the teat wall was assessed by its proportion of hypoechoic appearance and discontinuity of the hyperechoic outer teat wall and the fistulous tract on the teat wall was assessed by its hypoechoic tubular appearance. Fibrosis of the teat was easily detected by hyperechoic appearance and loss of typical echogenic pattern of teat. Teat stenosis and obstruction appeared as hyperechoic or sometimes hypoechoic. Pus and haematoma had similar echogenicity and movement of these particles during handling of teat was noticed (Plate 44, 45, 46, 47, 48, 49, 50 and 51).

4.2.3.1 Teat quantification

Normal Teat

I. Teat canal length

The mean ± S.E. values of teat canal length (in cm) of normal left fore, left hind, right fore and right hind teats in group I cows on the day of examination were 1.31 ± 0.02, 1.23 ± 0.05, 1.13 ± 0.05 and 1.23 ± 0.04 and in buffaloes were 1.69 ± 0.07, 1.90 ± 0.09, 1.67 ± 0.07 and 1.96 ± 0.10 respectively (Table 19 and Fig.5).

In cows and buffaloes the mean teat canal length of left and right fore and hind teats showed highly significant difference ($P < 0.01$) and the mean values of cow's teat canal length were slightly lower than the buffalo (Plate 56).

II. Teat diameter 1

The mean ± S.E. values of teat diameter 1 (in cm) at the level of Furstenberg rosette of normal left fore, left hind, right fore and right hind teats in group I cows on the day of examination were 2.33 ± 0.03, 2.24 ± 0.06, 2.40 ± 0.02 and 2.34 ± 0.05 and in buffaloes were 2.47 ± 0.05, 2.52 ± 0.04, 2.53 ± 0.04 and 2.52 ± 0.03 respectively (Table 19).

The mean teat diameter values in cows and buffaloes showed statistically significant difference ($P < 0.05$) for the left fore, right and left hind teat and highly significant difference ($P < 0.01$) for the right fore teat. The mean teat diameter values of buffaloes teats were slightly higher than the cow teat.

III. Teat diameter 2

The mean ± S.E. values of teat diameter 2 (in cm) at 1.5 cm proximal to Furstenberg rosette of normal left fore, left hind, right fore and right hind teats in group I cows on the day of examination were 3.33 ± 0.10, 3.46 ± 0.03, 3.31 ± 0.08 and 3.38 ± 0.08 and in buffaloes were 3.64 ± 0.05, 3.70 ± 0.01, 3.67 ± 0.06 and 3.68 ±0.03 respectively. The mean teat diameter values in cows and buffaloes showed statistically significant difference ($P < 0.05$) for left fore, and highly significant difference ($P < 0.01$) for the right fore, right and left hind teat. The mean teat diameter values of buffaloes teats were slightly higher than the cow teat (Table 19).

IV. Teat wall thickness

The mean ± S.E. values of teat wall thickness (in cm) at 1.5 cm proximal to Furstenberg rosette of normal left fore, left hind, right fore and right hind teats in group I cows on the day of examination were 0.36 ± 0.03, 0.37 ± 0.03, 0.38 ± 0.03 and 0.37 ± 0.03 and in buffaloes were 0.49 ± 0.03, 0.47 ± 0.03, 0.49 ± 0.03 and 0.49 ± 0.02 respectively. The mean teat wall thickness in cows and buffaloes showed statistically significant difference ($P < 0.05$) for left and right fore and left fore and highly significant difference ($P < 0.01$) for the right hind teat. The mean teat wall thickness of buffalo teats was slightly higher than the cow teat (Table 19).

V. Teat cistern width

The mean ± S.E. values of teat cistern width (in cm) at 1.5 cm proximal to Furstenberg rosette of normal left fore, left hind, right fore and right hind teats in group I cows on the day of examination were 2.41 ± 0.11, 2.62 ± 0.09, 2.40 ± 0.10 and 2.53 ± 0.10 and in buffaloes were 2.80 ± 0.06, 3.14 ± 0.08, 2.72 ± 0.05 and 2.97 ± 0.14 respectively. The mean teat cistern width in cows and buffaloes showed statistically significant difference ($P < 0.05$) for left fore and left and right hind and highly significant difference ($P < 0.01$) for the right fore teat. The mean teat cistern width of buffalo teats was slightly higher than the cow teat (Table 19).

Table 18 : Percentage of sensitivity to antibiotics in group IIA and IIB cows and buffaloes on 0 and 30th day

Antibiotic Sensitivity	Cow Milk				Buffalo Milk			
	Group IIA		Group IIB		Group IIA		Group IIB	
	Day 0 (n=6)	Day 30 (n=2)	Day 0 (n=6)	Day 30 (n=4)	Day 0 (n=6)	Day 30 (n=2)	Day 0 (n=6)	Day 30 (n=3)
Enrofloxacin (%)	83.33 (n=5)	100.00 (n=2)	83.33 (n=5)	100.00 (n=4)	100.00 (n=6)	100.00 (n=2)	100.00 (n=6)	100.00 (n=3)
Gentamicin (%)	83.33 (n=5)	100.00 (n=2)	66.67 (n=4)	100.00 (n=4)	100.00 (n=6)	100.00 (n=2)	83.33 (n=5)	66.67 (n=2)
Ceftrioxone (%)	66.67 (n=4)	50.00 (n=1)	50.00 (n=3)	50.00 (n=2)	66.67 (n=4)	50.00 (n=1)	50.00 (n=3)	66.67 (n=2)
Amoxycillin - clavulanic acid (%)	33.33 (n=2)	50.00 (n=1)	16.67 (n=1)	75.00 (n=3)	33.33 (n=2)	100.00 (n=2)	33.33 (n=2)	66.67 (n=2)
Amoxycillin (%)	0.00 (n=0)	0.00 (n=0)	0.00 (n=0)	0.00 (n=0)	0.00 (n=0)	0.00 (n=0)	0.00 (n=0)	0.00 (n=0)

Table 19 : Mean ± S.E. of teat canal length, teat diameter 1, teat diameter 2, teat wall thickness and teat cistern width values of group I normal cows and buffaloes

Parameter	Quarter	Side	Cow	Buffalo	t - value	Significance
Teat canal length (cm)	Fore	Left	1.31 ± 0.02	1.69 ± 0.07	5.05	**
		Right	1.23 ± 0.05	1.90 ± 0.09	6.40	**
	Hind	Left	1.13 ± 0.05	1.67 ± 0.07	6.03	**
		Right	1.23 ± 0.04	1.96 ± 0.10	7.03	**
Teat diameter 1 (cm)	Fore	Left	2.33 ± 0.03	2.47 ± 0.05	2.33	*
		Right	2.24 ± 0.06	2.52 ± 0.04	4.15	**
	Hind	Left	2.40 ± 0.02	2.53 ± 0.04	3.03	*
		Right	2.34 ± 0.05	2.52 ± 0.03	2.81	*
Teat diameter 2 (cm)	Fore	Left	3.33 ± 0.10	3.64 ± 0.05	2.77	*
		Right	3.46 ± 0.03	3.70 ±0.01	6.85	**
	Hind	Left	3.31 ± 0.08	3.67 ±0.06	3.39	**
		Right	3.38 ± 0.08	3.68 ±0.03	3.45	**
Teat wall thickness (cm)	Fore	Left	0.36 ± 0.03	0.49 ± 0.03	3.05	*
		Right	0.37 ± 0.03	0.47 ± 0.03	2.75	*
	Hind	Left	0.38 ± 0.03	0.49 ± 0.03	2.90	*
		Right	0.37 ± 0.03	0.49 ± 0.02	3.36	**
Teat cistern width (cm)	Fore	Left	2.41 ± 0.11	2.80 ± 0.06	3.15	*
		Right	2.62 ± 0.09	3.14 ± 0.08	4.17	**
	Hind	Left	2.40 ± 0.10	2.72 ± 0.05	2.74	*
		Right	2.53 ± 0.10	2.97 ± 0.14	2.58	*

Means bearing similar superscripts between groups do not differ significantly
P values of < 0.01 - ** (Highly Significant) P values of 0.01 – 0.05 - * (Significant)
P values of > 0.05 – NS (Non Significant)

Diseased Teat

I. Teat canal length (cm)

The mean ± S.E. values of teat canal length (in cm) of cows with diseased teat condition on 0, 30 and long term follow up days in group IIA were 1.65 ± 0.07, 1.38 ± 0.06 and 1.36 ± 0.08 and in group IIB were 1.59 ± 0.07, 1.47 ± 0.04 and 1.48 ± 0.05 respectively. A statistically significant difference ($P < 0.05$) was noticed between the mean values of treatment groups. In group IIA the mean values on 30 and long term follow up days were lower than the group IIB cows (Table 20 and Fig.7).

In buffaloes the mean ± S.E. values of teat canal length (in cm) with diseased teat condition on 0, 30 and long term follow up days in group IIA were 1.89 ± 0.06, 1.76 ± 0.02 and 1.75 ± 0.01 and in group IIB were 2.01 ± 0.03, 1.89 ± 0.04 and 1.88 ± 0.04 respectively. The mean values showed statistically high significant difference ($P<0.01$) between the treatment groups. In group IIA the mean values were lower than the group IIB buffaloes on day 30 and long term follow up days (Table 21 and Fig.8).

II. Teat diameter 1

The mean ± S.E. values of teat diameter 1 (in cm) at the level of Furstenberg rosette of cows with diseased teat condition on 0, 30 and long term follow up days in group IIA were 2.80 ± 0.04, 2.46 ± 0.11 and 2.41 ± 0.09 and in group IIB were 2.69 ± 0.07, 2.57 ± 0.03 and 2.52 ± 0.04 respectively. A statistically high significant difference ($P < 0.01$) was noticed between the mean values of treatment groups. In group IIA the mean values on 30 and long term follow up days were lower than the group IIB cows (Table 20).

In buffaloes the mean ± S.E. values of teat diameter 1 (in cm) at the level of Furstenberg rosette with diseased teat condition on 0, 30 and long term follow up days in group IIA were 2.79 ± 0.09, 2.54 ± 0.06 and 2.46 ± 0.07 and in group IIB were 2.67 ± 0.16, 2.59 ± 0.09 and 2.54 ± 0.05 respectively. The mean values showed no statistically significant difference between the treatment groups but the mean value of the group IIA treatment group were lower than group IIB buffaloes on 30 and long term follow up days (Table 21).

Fig. 7: Changes in mean teat canal length of group IIA and IIB cows treated by theloscopic surgery with and without bio-implant in on 0, 30 and longterm follow up days

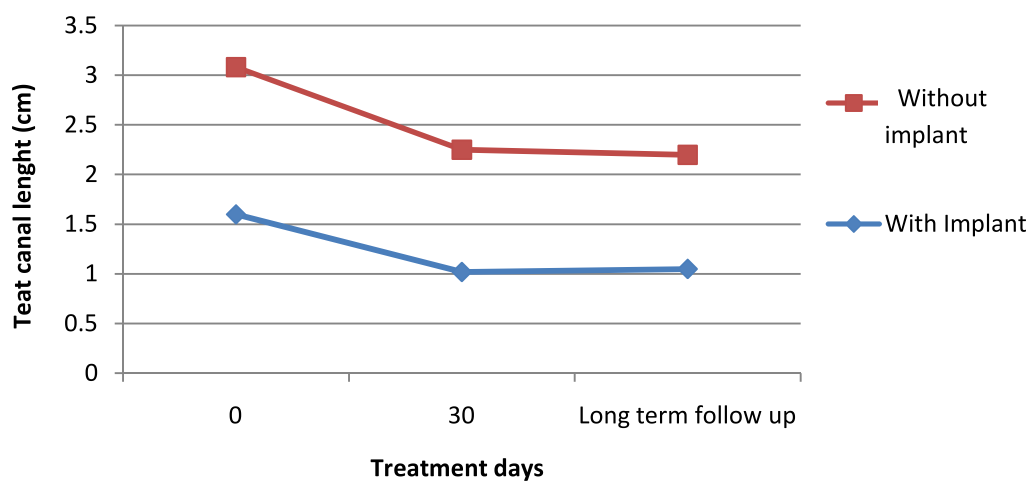


Fig. 8 : Changes in the mean teat canal length of group IIA and IIB buffaloes treated by theloscopic surgery with and without bio-implant on 0, 30 and long term follow up days

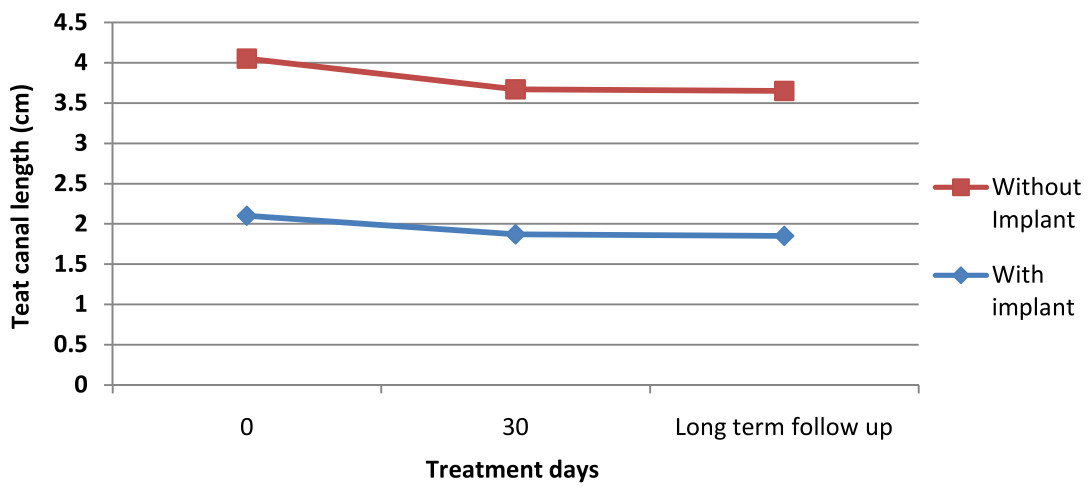


Fig. 9 : Changes in the mean somatic cell count values of milk in group IIA and IIB cows on 0, 30 and long term follow up days

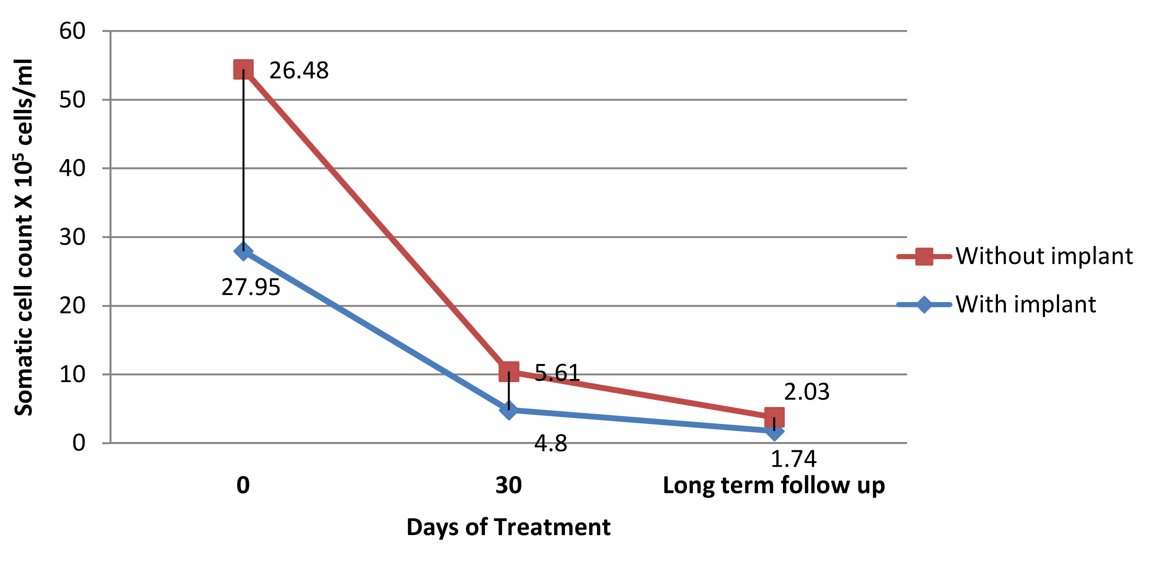
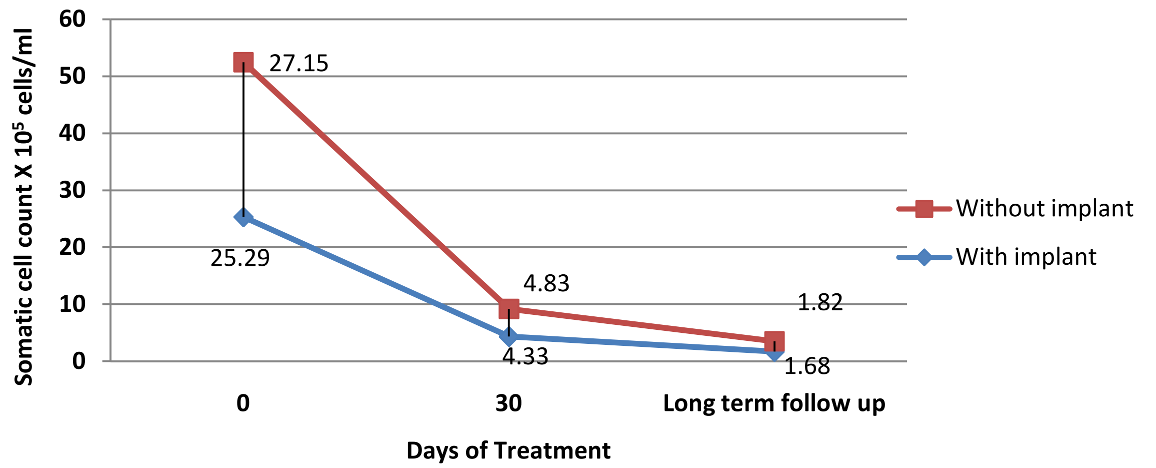


Fig. 10 : Changes in the mean somatic cell count values of milk in group IIA and IIB buffaloes on 0, 30 and long term follow up days



III. Teat diameter 2

The mean ± S.E. values of teat diameter 2 (in cm) at 1.5 cm proximal to Furstenberg rosette of cows with diseased teat condition on 0, 30 and long term follow up days in group IIA were 3.87 ± 0.13, 3.30 ± 0.08 and 3.30 ± 0.03 and in group IIB were 3.58 ± 0.12, 3.43 ± 0.05 and 3.35 ± 0.08 respectively. A statistically high significant difference ($P < 0.01$) was noticed between the mean values of treatment groups. In group IIA the mean values on 30 and long term follow up days were lower than the group IIB cows (Table 20).

In buffaloes the mean ± S.E. values of teat diameter 2 (in cm) at 1.5 cm proximal to Furstenberg rosette with diseased teat condition on 0, 30 and long term follow up days in group IIA were 3.98 ± 0.04, 3.72 ± 0.07 and 3.64 ± 0.08 and in group IIB were 3.87 ± 0.04, 3.65 ± 0.02 and 3.61 ± 0.02 respectively. The mean values showed statistically high ($P < 0.01$) significant difference between the treatment groups and the mean value of the group IIA treatment group were lower than group IIB buffaloes on 30 and long term follow up days (Table 21).

IV. Teat wall thickness

The mean ± S.E. values of teat wall thickness (in cm) at 1.5 cm proximal to Furstenberg rosette of cows with diseased teat condition on 0, 30 and long term follow up days in group IIA were 0.29 ± 0.02, 0.40 ± 0.02 and 0.39 ± 0.02 and in group IIB were 0.35 ± 0.02, 0.40 ± 0.01 and 0.38 ± 0.02 respectively. A statistically high significant difference ($P < 0.01$) was noticed between the mean values of treatment groups. In group IIA and IIB the mean values on day 30 and long term follow up were higher than day 0 of investigation (Table 20).

In buffaloes the mean ± S.E. values of teat wall thickness (in cm) at 1.5 cm proximal to Furstenberg rosette with diseased teat condition on 0, 30 and long term follow up days in group IIA were 0.60 ± 0.04, 0.55 ± 0.02 and 0.52 ± 0.02 and in group IIB were 0.70 ± 0.04, 0.62 ± 0.04 and 0.60 ± 0.01 respectively. The mean values showed statistically high ($P < 0.01$) significant difference between the treatment groups and the mean value of the group IIA treatment group were lower than group IIB buffaloes on 30 and long term follow up days (Table 21).

V. Teat cistern width

The mean ± S.E. values of teat cistern width (in cm) at 1.5 cm proximal to Furstenberg rosette of cows with diseased teat condition on 0, 30 and long term follow up days in group IIA were 3.37 ± 0.13, 2.55 ± 0.06 and 2.49 ± 0.03 and in group IIB were 3.49 ± 0.14, 3.03 ± 0.04 and 2.96 ± 0.07 respectively. A statistically high significant difference ($P < 0.01$) was noticed between the mean values of treatment groups. In group IIA and IIB the mean values on day 30 and long term follow up were lower than day 0 of investigation (Table 20).

In buffaloes the mean ± S.E. values of teat cistern width (in cm) at 1.5 cm proximal to Furstenberg rosette with diseased teat condition on 0, 30 and long term follow up days in group IIA were 3.18 ± 0.07, 3.03 ± 0.04 and 3.02 ± 0.02 and in group IIB were 3.11 ± 0.04, 2.88 ± 0.09 and 2.59 ± 0.05 respectively. The mean values showed statistically high ($P < 0.01$) significant difference between the treatment groups and the mean value of the group IIA treatment group were higher than group IIB buffaloes on 30 and long term follow up days (Table 21).

4.2.3.2 Limitations

In standing restrained position of the animal complete ultrasonographic scanning of hind quarters by contact with gel application technique was difficult. As the hind quarters lie in between the hind limbs the scanning procedure was hindered.

Ultrasonographic imaging of the proximal teat cistern and annular fold area by water bath method was found to be slightly difficult. Quantification of teat canal diameter of normal teat was found to be limited. The lack of contrast details and thinnest diameter between the walls of teat canal impedes the quantification procedure of the teat canal. Maintenance of closer distance between the transducer and the teat position in the water bath is needed to produce desired ultrasound image details and a firm contact on the polyethylene cup with acoustic coupling gel is needed. Dipping of teat in the water bath

Table 20 : Mean ± S.E. of teat canal length, teat diameter 1, teat diameter 2, teat wall thickness and teat cistern width ultrasonographic values of group IIA and IIB cows treated by theloscopic surgery with and without bio-implant on 0, 30 and long term follow up days

Parameter	Day	Treatment		F - Value	Significance
		With implant	Without implant		
Teat canal length (cm)	0	1.65 ± 0.07^{b}	1.59 ± 0.07^{b}	3.094	*
	30	1.38 ± 0.06^{a}	1.47 ± 0.04^{ab}		
	Long term follow up	1.36 ± 0.08^{a}	1.48 ± 0.05^{ab}		
Teat diameter 1 (cm)	0	2.80 ± 0.04^{c}	2.69 ± 0.07^{bc}	4.467	**
	30	2.46 ± 0.11^{a}	2.57 ± 0.03^{ab}		
	Long term follow up	2.41 ± 0.09^{a}	2.52 ± 0.04^{ab}		
Teat diameter 2 (cm)	0	3.87 ± 0.13^{b}	3.58 ± 0.12^{a}	6.306	**
	30	3.30 ± 0.08^{a}	3.43 ± 0.05^{a}		
	Long term follow up	3.30 ± 0.03^{a}	3.35 ± 0.08^{a}		
Teat wall thickness (cm)	0	0.29 ± 0.02^{a}	0.35 ± 0.02^{ab}	4.451	**
	30	0.40 ± 0.02^{b}	0.40 ± 0.01^{b}		
	Long term follow up	0.39 ± 0.02^{b}	0.38 ± 0.02^{b}		
Teat cistern width (cm)	0	3.37 ± 0.13^{c}	3.49 ± 0.14^{c}	21.316	**
	30	2.55 ± 0.06^{a}	3.03 ± 0.04^{b}		
	Long term follow up	2.49 ± 0.03^{a}	2.96 ± 0.07^{b}		

Means bearing similar superscripts between groups do not differ significantly
P values of < 0.01 - ** (Highly Significant)
P values of 0.01 – 0.05 - * (Significant)
P values of > 0.05 – NS (Non Significant)

Table 21 : Mean ± S.E. of teat canal length, teat diameter 1, teat diameter 2, teat wall thickness and teat cistern width ultrasonographic values of group IIA and IIB buffaloes treated by theloscopic surgery with and without bio-implant on 0, 30 and long term follow up days

Parameter	Day	Treatment		F - Value	Significance
		With implant	Without implant		
Teat canal length (cm)	0	1.89 ± 0.06^{b}	2.01 ± 0.03^{c}	7.25	**
	30	1.76 ± 0.02^{a}	1.89 ± 0.04^{b}		
	Long term follow up	1.75 ± 0.01^{a}	1.88 ± 0.04^{b}		
Teat diameter 1 (cm)	0	2.79 ± 0.09^{b}	2.67 ± 0.16^{ab}	1.61	NS
	30	2.54 ± 0.06^{ab}	2.59 ± 0.09^{ab}		
	Long term follow up	2.46 ± 0.07^{a}	2.54 ± 0.05^{ab}		
Teat diameter 2 (cm)	0	3.98 ± 0.04^{b}	3.87 ± 0.04^{b}	8.68	**
	30	3.72 ± 0.07^{a}	3.65 ± 0.02^{a}		
	Long term follow up	3.64 ± 0.08^{a}	3.61 ± 0.02^{a}		
Teat wall thickness (cm)	0	0.60 ± 0.04^{ab}	0.70 ± 0.04^{b}	3.44	*
	30	0.55 ± 0.02^{a}	0.62 ± 0.04^{ab}		
	Long term follow up	0.52 ± 0.02^{a}	0.60 ± 0.01^{ab}		
Teat cistern width (cm)	0	3.18 ± 0.07^{c}	3.11 ± 0.04^{c}	13.73	**
	30	3.03 ± 0.04^{bc}	2.88 ± 0.09^{b}		
	Long term follow up	3.02 ± 0.02^{bc}	2.59 ± 0.05^{a}		

Means bearing similar superscripts between groups do not differ significantly
P values of < 0.01 - ** (Highly Significant)
P values of 0.01 – 0.05 - * (Significant)
P values of > 0.05 – NS (Non Significant)

and positional change of the water bath by the handler or due to animal movement caused the outflow of water from the water bath. This outflow dissolved the externally applied gel and reduced the image visualisation during the scanning process. Use of tap water or sterile physiological solutions, mineral water etc., hindered the image visualisation because of the echogenic particles present in them and use of triple glass distilled water is needed to favour better visualisation of the sonographic images.

4.2.4 Theloscopic Examination

4.2.4.1 Preparation, anaesthesia and positioning of the animal

For theloscopic examination and surgery aseptic handling of teat tissues and instruments was found be most essential concept. In this study, repetitive cleaning of the outer surface of the teat tissues and flushing of the teat cisternal cavities with normal saline was highly helpful for the avoidance of mastitis in the immediate post examination and treatment period. Ring block, intravenous regional analgesia and local analgesia of the teat cistern was found to produce satisfactory analgesia during the theloscopic examination and surgical procedure. In this study procedures involving delicate handling and short term period of examination or treatment only intracisternal analgesia alone was attempted and for procedures such as lateral theloscopy involving teat tissue invasiveness and excision of larger tissues, intracisternal and regional intravenous analgesia was attempted. For theloscopic examination the lateral recumbency rather than standing posture was preferred because of the following reasons. During visualisation of teat by axial theloscopy on standing posture, the small amount of fluids stagnated at the distal part of the teat cistern due to gravity hindered the visualisation of the teat due to splashing of the fluid contents on the lens. The problem was avoided by removing the scope, cleaning the tip of the scope and the blow pipe and milking the contents intermittently. Hence, animal in lateral recumbency is more preferable than standing posture for axial theloscopic examination procedures. In lateral theloscopic procedure creating an artificial opening in the lateral wall of the teat and introduction of the scope into the cistern was easy and the visualisation problems as encountered in the axial theloscopy was not noticed and the clear endoscopic visualisation of the teat and udder structures were maintained. The contents if stagnated were milked out without any break in visualisation procedure. For this procedure examination of teat structures in standing and mammary gland in lateral recumbency postures were found to be more favourable for effective visualisation.

4.2.4.2 Procedure

I. Axial theloscopy

In this procedure insertion of the theloscope through the teat canal was more easy in inflated condition of the teat rather than in the empty teat cistern condition. However, in this study during insertion of the blow pipe into the teat cistern through the teat canal, the tip of the blow pipe caused consistent damage to the teat canal and the blow pipe with diameter lesser than 3 mm OD may be useful to avoid this complication.

II. Lateral theloscopy

In lateral theloscopy the artificial opening made in the teat wall favoured full visualization of the Furstenberg rosette, proximal to distal parts of the teat canal area and the teat cistern was visualized to the limited extent. The artificial opening made on the lateral wall opposite to the side of teat lesion favoured full and easy visualization of the obstructive lesions located at the mid to distal cisternal area and enabled easy excision of the lesions through the teat canal. Through this approach the gland cistern and lactiferous ducts were effectively visualized and the obstructive tissues involving the proximal part of the teat cistern and at gland cistern could be excised.

Normal and abnormal teats

Through axial thelosopy the lactiferous ducts, annular folds were visualised, and the whole length of the teat cistern was visualized (Plate 57 and Plate 58). Numerous prominent circular/concentric folds were noticed in case of cows than in buffaloes. In some cases of buffaloes during theloscopic examination, the mucosa of the teat cistern was found to be coloured due to pigmentation with melanin. At the end stage of withdrawing the scope from the teat cistern the inner opening and the mid part of the teat canal was visualized.

In teat with obstructive milk flow disorders cisternitis was noticed in most of the cases and the cisternal wall was found to be inflammed with numerous raised areas rather than smooth contour of a normal teat.

In lateral theloscopy the structures that were visualized was similar to that of the axial theloscopy. Through lateral theloscopic view of the normal teat the area of Furstenberg rosette was visualized as 4 to 6 longitudinal folds converging over the proximal part of the teat canal area and the opening of the proximal part of the teat canal was visualized. The teat canal was visualized as whitish passage with irregular surfaces. Access to visualisation of the gland sinus and lactiferous ducts through lateral theloscopy was more easier than axial theloscopy (Plate 59, 60, 61, 62, 63, 64 and 65).

4.2.4.3 Post operative care

In the post operative period depending on the clinical status of the milk secretion and teat tissue condition, repetitive flushing of the teat and gland cistern with normal saline was carried out. The waxy teat implants were found to be dissolved between 24 and 48 hours and in this study no teat obstructive condition due to blockage by the implant was recorded. In this study, the group IIA and IIB cows and buffaloes were subjected to diagnosis and lateral theloscopic surgical treatment on the same day of report to the hospital and were kept as inpatient for observation for three days. Depending upon the milk parameters from the affected quarters the milk was either passively drained out continuously for the first three days after treatment or the treated teat was provided rest for the first three days. During postoperative care use of wider diameter metallic implants were avoided and milking tube of less than 2 mm diameter or a sterile blunt 18G hypodermic needle were used for draining the milk.

In all the cases of group IIA and IIB cows and buffaloes treated by lateral theloscopic procedure, milkability by full hand milking and the outflow of milk was found to be normal and satisfactory on 3, 30 and long term follow up day of post treatment period and no obstructive condition was noticed. The wound made on the lateral wall of the teat as an artificial opening healed satisfactorily within a week and no complications were noticed.

4.2.4.4 Limitations

In axial theloscopic examination/treatment procedure introduction of the scope with blow pipe through the teat canal was found to damage the teat canal area. In conditions with complete obstruction due to teat canal fibrosis, fibrosis of the distal, middle part of the teat cistern, introduction of the theloscope for examination was found to be impossible. Through axial theloscopy visualisation of the entire part of teat canal and the mammary gland was limited. Through this approach mammary gland visualisation was limited due to the insufficient length of the scope. Also clearing of the lens on obstruction during examination process needed withdrawal from the teat cistern, cleaning and reintroduction of the scope. Such repeated withdrawal damaged the teat canal and caused transient enlarged teat canal diameter and ultimately to a transient leaky teat condition. From the observations of this study axial theloscopy procedure was mainly helpful for rapid diagnosis of the etiology for teat obstructive condition involving the teat cistern and to a limited extent to diagnose the lesions involving the teat canal area.

In lateral theloscopy creation of artificial opening, in the teat wall with cisternitis condition need to be limited. For performing this procedure, an additional assistant was needed to hold the scope in fixed position and the dexterity of the surgeon were limited. This procedure was found to be more time consuming than the axial theloscopy and needed aseptic handling of the teat tissues. The access and visualisation of the lactiferous ducts through lateral theloscopy was easier than axial theloscopy

4.2.5 Evaluation of Theloscopic Surgical Procedure

4.2.5.1 Lateral theloscopic surgery – utility, limitations and complications

In this study lateral theloscopy was found to be more useful both in the diagnosis and treatment of the obstructive lesions involving the teat canal and teat cistern area (Plate 63, 64 and 65). With this technique the lesions involving more than 30 per cent of the cisternal area, complete excision of these tissues was found to be difficult and time consuming and severe postoperative complications such as cisternitis and teat canal damage were noticed. The area of visualization and tissue handling access was more in lateral theloscopy than in axial theloscopy and enabled an easy and useful approach for the diagnostic and treatment procedures.

4.2.6. Evaluation of Wound Healing with Ultrasound and Theloscopy

4.2.6.1 Group IIA cows and buffaloes

Postoperatively, in group IIA cows and buffaloes the efficacy of minimally invasive lateral theloscopic surgery with the usage of bio-implants was assessed based on the ultrasonographic appearance pre and post treatment and the results of teat quantification that was already discussed above in detail. The parameters considered were teat canal length (cm), teat diameter 1 (cm), teat diameter 2 (cm), teat wall thickness (cm) and teat cistern width (cm).

Ultrasonographic images revealed mild hyperechoic nature of the healed status of the wound made on the lateral wall of the teat and normal teat echogenicity on 30 and long term follow up days. Based on the results of the teat quantification parameters and from the ultrasonographic appearance of teat during pre and post treatment it is inferred that group IIA cows and buffaloes treated by lateral theloscopy with implants showed favourable healing on 30 and long term follow up days.

In this group, on day 30 three cows and two buffaloes were subjected to axial theloscopic procedure and the postoperative healing was assessed. All the cows and buffaloes had normal appearance of the teat cistern and at the operated sites the wounds were found to be healed with fibrous scar tissue formation (Plate 66 and 67).

4.2.6.2 Group IIB cows and buffaloes

In group IIB cows and buffaloes, based on the ultrasonographic appearance and quantification of the following parameters teat canal length (cm), teat diameter 1 (cm), teat diameter 2 (cm), teat wall thickness (cm) and teat cistern width (cm) wound healing after theloscopic surgery was assessed. The ultrasonographic appearance of the healed tissues were similar to that of the group IIA cows and buffaloes and in two cows the teat canal echogenicity as noticed on day 0 of examination could not be visualized. The mean values of teat quantification parameters on day 30 and long term follow up days revealed lesser significant reduction in the mean values than group IIA cows and buffaloes. From this it could be inferred that the recovery of teat tissues after lateral theloscopic surgery in group IIB cows and buffaloes were less superior to the group IIA cows and buffaloes.

4.2.7 Evaluation of Utility of Natural Teat Insert Bio-Implants

4.2.7.1 Group IIA cows and buffaloes

The utility of teat bio-implants in group IIA cows and buffaloes on comparison with group IIB cows and buffaloes were assessed based on the above mentioned results of the following parameters on 0, 30 and long term follow up days that were already discussed in detail. The parameters considered were clinical observations of teat tip to floor distance (cm), teat length (cm), teat girth, teat canal length by Medl's test (cm), milk parameters such as milkability by hand (ml), electrical conductivity (mS/cm), somatic cell count (10^5 cell per ml) and parameters of ultrasound quantification of teat such as teat canal length (cm), teat diameter 1 (cm), teat diameter 2 (cm), teat wall thickness (cm) and teat cistern width (cm). The results of these parameters reveal that usage of teat bio-implant in cows and buffaloes favoured better clinical outcome than the cows and buffaloes treated by theloscopic surgery without implant.

CHAPTER 5

DISCUSSION

5.1 INCIDENCE OF UDDER AND TEAT AFFECTIONS WITH OBSTRUCTIVE MILK FLOW DISORDERS

Internal lesions caused by contusions of the tip of the teat or distal teat wall (Kubicek 1975 and Seeh and Hospes, 1998) were recorded as the major cause for obstructive milk flow disorders in 68.37 per cent of cows and 66.67 per cent of buffaloes and external in 31.63 and 33.33 per cent respectively. Rambabu (2007) reported an incidence of 28.80 per cent of internal lesions in buffaloes.

In both cows and buffloes hind teats were more frequently affected with 59.18 per cent and 54.17 per cent respectively than the fore teats 40.82 per cent and 45.83 per cent and left sided affections were more common 56.12 per cent and 83.33 per cent than the right sided affections 43.88 per cent and 16.67 per cent respectively. In cows the highest incidence was recorded in left hind 36.73 per cent followed by right hind 22.45 per cent, right fore 21.43 per cent and right hind 19.39 per cent and in buffaloes left hind 50.00 per cent followed by left fore 33.33 per cent, right fore 12.50 per cent and right hind 4.17 per cent. Bleul *et al.* (2005) recorded 76.9 per cent of teat affection in the hind teat with the highest incidence in the left hind teat 46.2 per cent, followed by right hind teat 30.8 per cent, 13.5 in the left front teat and 9.6 per cent in the right front teat respectively. Ducharme *et al.* (1987) reported an incidence of teat affections with obstructive disorders in left and right fore teat each 31 per cent followed by left hind teat 23 per cent and right hind teat 15 per cent. The major incidence in the hind teats could be due to the close location of teats with the hind limbs causing crush injury during sternal recumbency posture . maintained mostly in the right side leaving the left sided udder and teat structures exposed freely and susceptible for traumatic injuries (Waller *et al.,* 2003 and Rovai *et al.*, 2007).

In cows, fore quarters (68.00 per cent) were found to be more affected than the hind quarters (32.00 per cent) and left sided udder was found to be more affected (52.00

per cent) than the right sided udder (48.00 per cent). In buffaloes an equal incidence of each 50.00 per cent was noticed with regard to fore/hind and left/right udder affections. High incidence of fore quarter affection could be due to free exposure of the quarters to the ground and environment and left sided affections could be due to animal maintaining right sided sternal recumbency posture enabling them to susceptible for traumatic and other injuries. The hind quarters are less affected than the fore quarters could be due to safe envelope of the quarters by the hind limbs.

Among the teat lesions in cows, obstructive disorder at the teat canal area was recorded to be the highest incidence 15.30 per cent. The other conditions recorded with decreasing order of incidence were fibrosis condition of the teat canal, hard milker condition, fibrosis condition of the whole teat, obstructive disorder at the distal part of the teat cistern, teat wall wound, teat laceration, teat cistern haematoma, teat fistula, teat tip injury, leaky teat, obstructive disorder at the teat base, obstructive disorder at the mid teat cistern, obstructive disorder at the Furstenberg rosette, teat canal agenesis, thelitis and obstructive disorder at the proximal part of teat cistern. Seeh and Hospes (1998) stated that in 80 to 90 per cent of covered teat injuries teat stenosis was caused by mucosal detachment in the streak canal or Furstenberg's rosette and the free ends of the tissue were found to be projected into the papillary sinus and obstructed the streak canal in a valve-like fashion. Agger and Willeberg. (1986) documented maximum incidence of teat lesions in cows in the distal extremity portion of teat, followed by distal half, proximal half and an involvement of whole teat. Of the distal extremity affections major incidence was noticed in the papillary canal area. The observations on the incidence of teat lesions are in accordance with Fremont *et al.* (2002), Querengasser *et al.* (2002) Steiner (2004) and Geihauser *et al.* (2005).

In buffaloes obstructive disorder at the teat canal area was recorded to be the highest incidence followed by the obstructive disorder at the mid teat cistern, teat laceration, teat tip injury, hard milker, fibrosis of the whole teat, teat cistern haematoma, teat wall wound, leaky teat and thelitis were noticed. Rambabu (2007) recorded the incidence of teat affections in buffaloes with the observations of high incidence of intraluminal obstructions of teat followed by polythelia, oligothelia, teat lacerations/ fistulas and intraluminal foreign bodies each, allergic mastitis and papilloma/neoplasms, fused teats and pox lesions respectively.

5.2 PARAMETERS STUDIED

5.2.1 Clinical Evaluation

5.2.1.1 Udder and teat parameters

I. Udder shape

In group IIA and IIB cows and group IIB buffaloes, majority of the animals had long abdominal type of udder and this type of udder conformation could have predisposed the animal for traumatic teat injury leading to obstructive milk flow disorders. The present observations were in aggrement with the reports of Klasss *et al.* (2004) who stated that small udder conformation in cows might be less susceptible to mastitis and traumatic injuries than with the normal or deep udder shapes.

II. Udder tissue condition

In group IIA and IIB cows and buffaloes firm udder tissue condition was more commonly noticed. The percentage of connective and secretory tissues differed among individual dairy cows and genotypes and was influenced by parity and stage of lactation (Michel, 1994). Udders appearing fleshier after milking had a heavier stroma or connective tissue than udders with a small amount of secretory tissue (Smith, 1959). Furthermore, the hardness of the udder was measured from behind with both hands palpating the udder close to the ventral abdomen where ligaments and connective tissue were strongest and percentage of secretory tissue was lowest. Cows with a strong ligament and udder capsule might also have had a stronger connective tissue in the parenchyma, leading to a higher degree in hardness than cows and buffaloes with a weaker ligament structure (Akers, 2000).

III. Assymetry between fore/between hind quarters

In group IIA and IIB cows pronounced asymmetry of the udder affected with milk flow disorder was noticed. The reason for asymmetry could be due to increased volume of milk loaded in the cistern causing distension of the quarter when compared with the normal quarters and such similar observations were made by Klasss *et al.* (2004). In group IIA and IIB buffaloes no marked asymmetry after teat obstructive disorders was documented on the day 0 of examination and the reason could be due to low cisternal storage capacity of milk as reported by Thomas *et al.* (2004).

IV. Udder oedema

In group IIA and IIB cows, oedema of the most parts and large area of the udder, noticed on the day 0 of examination could be due to inflammatory condition secondary to the obstructive disorders similar to acute mastitis condition as reported by Klaas *et al.* (2004). In buffaloes no such marked oedema was noticed and these observations were similar to that of Dhakal (2006).

V. Teat cleanliness score

In group IIA and IIB cows and buffaloes teat cleanliness score was found to be higher and the reason could be due to improper hygiene practice and maintaining the animals in contaminated barn conditions (Cook, 2002).

VI. Teat shape

In group IIA and IIB cows and buffaloes, normal, conical and fleshy types of teats were documented. Bristol (1989a and 1989b) hypothesized that cone-shaped teat ends were more predisposed to mastitis than round shaped teat ends, because the funnel could hold the milk and might act as substrate for bacterial contamination and growth. Slettbakk *et al.* (1995) stated that some udder and teat-end shapes were associated with milk leakage, resulted in significant risk for clinical mastitis condition Boge (1965) reported that the highest incidence of mastitis in cows with funnel-shaped teat ends followed by plate-shaped teat-ends and round-shaped teat-ends. Chrystal *et al.* (2001) stated that the teat ends with plate-or inverted funnel-shaped were associated with higher incidence of clinical and subclinical mastitis.

VII. Teat wound

In group IIA and IIB cows and buffaloes, presence of wound on the external surface of the teat was noticed. Agger and Willeberg (1986), Burmeister *et al.* (1998) and Larsen *et al*. (2000) opined that rough teat skin and wounds on skin were the reservoir for *S. aureus* and even superficial lesions of the teat skin increased the risk of subclinical and clinical mastitis. Chrystal *et al.* (2001) stated that most mastitis causing pathogens were thought to gain entry to the mammary gland through the streak canal, the presence and shape of teat-end lesions played an important role in the pathogenesis of the disease.

VIII. Scar tissue in the teat canal

In group IIA and IIB cows and buffaloes, presence of scar tissues in the teat canal was noticed. Kubieck (1975) and Seeh and Hospes (1998) stated that 70 to 90 per cent of the teat injuries were due to contusions of the tip of the teat or the distal teat wall and resulted in teat stenosis caused by mucosal detachment in the region of the streak canal.

IX. Skin quality of teats

In group IIA and IIB cows and buffaloes on the day 0 of examination in majority of the animals smooth typed teat skin was recorded. Agger and Willeberg (1986), Burmeister *et al.* (1998) and Larsen *et al*. (2000) opined that rough teat skin were the reservoir for *S. aureus* and increased the risk of subclinical and clinical mastitis.

X. Teat palpation score

In group IIA and IIB cows and buffaloes moderate thickening was noticed in most of the cases on the day 0 of examination. The reason could be due to decreased blood flow to the teat walls following teat injury as reported by Zecconi *et al.* (1992) and Hamann *et al.* (1994). In cases of severe teat wall thickening greater than 5 per cent could be considered for the presence of microbial colonization in the teat canal.

XI. Teat tip to floor distance

Normal teat condition

The mean values of teat tip to floor distance in group I cows were between 49.65 cm and 51.13 cm and in buffaloes were between 47.15 cm and 48.43 cm respectively and a statistically significant difference was noticed between fore and hind quarters in cows and buffaloes. In cows the fore teat lengths were shorter than the hind teats and vice versa in case of buffaloes. In buffaloes the teat tip to floor distance was found to be lower than the cows and the reason could be attributed to the short and stumpy nature of the appendicular skeleton of the Indian cross bred buffaloes than the cross bred cows. It has been widely recognized that the udder suspended below 45 cm from the floor hinders the application of teat cup during milking process and also lead to crush injury to the teats by the claws of the animal. These observations were in accordance with the findings of Kuczaj (2003) and Thomas *et al.* (2004). A reduction in teat-end-to-floor distance was associated with and increased somatic cell count (Slettbakk *et al.,* 1995).

Diseased teat condition

In between group IIA and IIB cows the mean values of teat tip to floor distance in centimeter of diseased teat with obstructive milk flow disorder showed no statistically significant difference and between group IIA and IIB buffaloes a statistically significant difference was noticed on 0 and 30th day of observation. The reason could be attributed due to increased volume of milk stored in the cistern causing downward distension of the quarter and teat when compared with the normal quarters and minor differences in change of the length due to low cisternal storage of milk. These observations were in accordance with the findings of Klaas *et al.* (2004) and Thomas *et al.* (2004).

XII. Teat length

Normal teat condition

The mean values of teat length in centimeter in group I cows and buffaloes showed a non significant difference for the left and right fore teats and highly significant difference for the left and right hind teats respectively. The differences in teat length of fore teats in cows and hind teats in buffaloes are in accordance with McDonald, (1973), Schumer *et al.* (1981), Hamann (1987), Geishauser and Querengasser (2000) and Paulrud and Rasmussen (2004) and Thomas *et al.* (2004).

Diseased teat condition

The mean values of teat length in centimeter of diseased teat in cows with obstructive milk flow disorder in group IIA and IIB cows and buffaloes a statistically highly significant difference on 0, 30 and long term follow up days was noticed indicating that the teat length was restored to normal after theloscopic surgery. These findings are in accordance with Querengasser *et al.* (2002).

XIII. Teat girth

Normal teat condition

The mean values of teat girth in centimeter in group I cows and buffaloes on the day of examination showed a statistically significant difference on day 0 of examination. The reason could be due to an increased internal diameter of the teat cistern in buffaloes than in cows and concurs with the observations made by Schumer *et al.* (1981) and Thomas *et al.* (2004).

Diseased teat condition

The mean values of teat girth of group IIA and IIB cows and buffaloes showed statistically highly significant difference on 0 and 30th day with no significant difference between 30th and long term follow up days. The reason could be due to drainage of the stored milk from the teat cistern and changes in teat wall thickness after theloscopic surgery as suggested by Zecconi *et al.* (1992), Hamann *et al.* (1994), Klasss *et al.* (2004) and Thomas *et al.* (2004).

XIV. Teat canal length (Medl's test)

Normal teat condition

The mean values of teat canal length in centimeter of normal left fore, left hind, right fore and right hind teats in group I cows and buffaloes showed statistically high significant difference between cows and buffaloes on the day 0 of examination. The mean values ranged from 1.25 to 1.40 cm in cows and in buffaloes between 1.77 and 2.07 cm respectively. As evidenced earlier with the difference in teat length of cows and buffaloes the fore teat and hind teat canal length of cows and buffaloes were recorded to be longer in this study and confirmed with the findings of Schumer *et al.* (1981) and Thomas *et al.* (2004).

Diseased teat condition

In group IIA and group IIB cows the mean teat canal length in centimeter showed statistically highly significant difference between 0 and 30th day of examination and with no significant difference between 30th and long term follow up days.

In buffaloes no statistically significant difference between treatment groups of IIA and IIB were noticed and the mean values in both the groups between the treatment days were found to be slightly decreased from 0 day to long term follow up days.

The reason for the significant difference in cows could be due to the restoration of the normal teat canal length after theloscopic surgery on 30th day and sustenance of recovered teat canal length on long term follow up day and these findings were in accordance with Querengasser *et al.* (2002). However, the mean teat canal length in group IIA and IIB cows and buffaloes were found to slightly higher (greater than 2 mm) and the reason could be due to rupture in the teat canal area with inversion of tissue into the teat cistern and this finding was in conformity with that of Querengasser and Geishauser (2000) who stated that this teat canal lengthening was an indicative for obstructive disorder at the teat canal area.

5.2.1.2 Milk parameters

I. Total milkability by hand

Normal

The mean values of total milkability by hand in milliliters of normal left fore, left hind, right fore and right hind teats were higher in cows than in buffaloes. In both cows and buffaloes the mean milkability from hind quarters was higher than the fore quarters and this could be due to differences in the milk secretion capacity between the quarters. Schumer *et al.* (1981) and Thomas *et al.* (2004) stated the the hind quarters account for 55 to 60 per cent of the milk production and this statement was in accordance with the findings of this study.

Diseased condition

In group IIA and IIB cows and buffaloes, the mean total milkability by hand in milliliters on 0, 30 and long term follow up days showed statistically highly significant difference between 0 and 30th day of examination and with no significant difference between 30th and long term follow up days and the mean values of parallel treatment days between the groups remained to be similar. However, the mean values of group IIA cows and buffaloes showed greater restoration of milkability and revealed the effectiveness of implant usage with lateral theloscopic surgery for the treatment of obstructive milk flow disorders in cows and buffaloes. These findings are in accordance with the report of Miller *et al.* (1993), Wellnitz *et al.*(1999), Querengasser *et al.* (2002), Wagner and Ruegg (2002) and Inderwies *et al.* (2003).

II. Electrical conductivity

Normal

The mean electrical conductivity of milk in mS/cm of group I normal buffaloes on the day of examination showed no statistically significant difference but the mean values were found to be higher in buffaloes than the cows. The mean values in cows ranged from 4.88 to 5.08 mS/cm in cows and in buffaloes 4.95 to 5.15 mS/cm. The reason could be due to differences in milk composition of cows and buffaloes. The mean value findings in cows were in accordance with Kitchen *et al.* (1980).

Diseased condition

In group IIA and IIB cows and buffaloes the mean values of electrical conductivity of milk (mS/cm) on 0, 30 and long term follow up days showed statistically highly significant difference between 0 and 30th day of examination and with no significant difference between 30th and long term follow up days and the mean values of parallel treatment days between the groups remained to be similar. The increased electrical conductivity value on day 0 of examination could be due to increased sodium and chloride concentration due to infection and these findings were in accordance with Kitchen *et al.* (1980) and Pyorala (2003). The mean values of group IIA cows and buffaloes showed lower electrical conductivity values than group IIB. From this it could be inferred that usage of implant restored normal milk yield and teat canal patency and normal milk composition is maintained following lateral theloscopic surgery for the treatment of obstructive milk flow disorders in cows and buffaloes.

III. Somatic cell count

Normal

The mean values of somatic cell count of milk (10^5cells per ml) of normal cows in group I were between the range of 1.22 and 1.33 X 10^5 cells per ml and in buffaloes were 1.09 to 1.21 X 10^5 cells per ml respectively. The mean values revealed no statistically significant difference between cows and buffaloes but the mean somatic cell values of cows were found to be slightly higher than the buffaloes. These observations were in agreement with the findings of Silva and Silva (1994), Greut *et al.* (2001), Ludwiczuk *et al.* (2001), Dhakal, (2004) and Moroni *et al.* (2006), Kavitha *et al.* (2009) and Syed *et al.* (2009).

.**Diseased condition**

In group IIA and IIB cows and buffaloes the mean values of somatic cell count of milk (10^5 cells per ml) on 0, 30 and long term follow up days showed statistically highly significant difference between 0 and 30th day of examination and with no significant difference between 30th and long term follow up days and the mean values of parallel treatment days between the groups remained to be similar showed slightly higher mean values in group IIB than group IIA. The lower somatic cell count in group IIA revealed that the maintenance of teat canal patency was found to be more superior than group IIA and thus indicated that the implant usage favoured to restore normal teat canal patency after theloscopic surgery in cows and buffaloes. These findings were in accordance with the report of Reneau (1986), Dhakal and Kapur (1992), Pednekar *et al.* (1992) and Mahendra and Dang (2001), Ormian *et al.* (2001) and Querengasser *et al.* (2002).

IV.California mastitis test (CMT) score

In group IIA and IIB cows on 30th day of examination the CMT score was found to be less in 66.66 per cent of animals than 50 per cent in group IIB animals. In group IIA and IIB buffaloes CMT score remained equal. The results indicated that in group IIA cows the usage of implant favoured to restore normal milk composition and normal teat canal patency after theloscopic surgery in cows and buffaloes. These findings were in accordance with the report of Reneau (1986), Dhakal and Kapur (1992), Pednekar *et al.* (1992) and Querengasser *et al.* (2002), Ruegg (2003) and Dhakal (2006).

V. Bacterial isolation and identification

Jankus and Baumann (1986), Zecconi *et al.* (1992) and Hamann *et al.* (1994) stated that a decreased blood flow period after milking was associated with an increase in teat thickness and greater microbial colonization in the teat canal and (Hamann, 1987), Grindal and Hillerton (1991), (Nickerson, 1994), Sordillo *et al.* (1997) and Sordillo *et al.* (2003) stated that any teat injury such as teat canal protrusion greatly increased the degree of exposure of the mammary gland to pathogens. Changes in the pliability of teat tissue caused by congestion or edema may change the resistance of the teat canal to bacterial invasion (O'Shea, 1987). A relationship between peak flow rate and intramammary infection has been found in several studies (Van de Geer *et al.,* 1988; Grindal *et al.,* 1991; Grindal and Hillerton, 1991; Duda, 1995; Lacy-Hulbert and Hillerton, 1995; Roth *et al.,*1998; Dodenhof *et al.,* 1999 and Rupp and Boichard, 1999). In group IIA cows and buffaloes on comparison with the group IIB cows, the percentage of organism isolated on day 0 of examination was found to be decreased on day 30 and on long term follow up periods. The results indicated that in group IIA cows the usage of implant favoured to restore normal milk composition and normal teat canal patency after theloscopic surgery in cows and buffaloes. These findings were in accordance with the report of Hibbit *et al.* (1969), Dohoo and Meek (1982), Reneau (1986), Capuco *et al.* (1992), Dhakal and Kapur (1992), Pednekar *et al.* (1992), Querengasser *et al.* (2002), Khan and Muhammad (2005), Flock and Winter (2006) and Piepers (2007).

VI. Antibiotic sensitivity test

In Group IIA cows and buffaloes on 0 and 30th day of examination the antibiogram showed the highest sensitivity to enrofloxacin followed by gentamicin, ceftrioxone, amoxicillin with clavulanic acid. In all the groups amoxicillin was found to be resistant on all days of examination. The antibiogram results in cows were in accordance with the report of Bengtsson *et al.* (2009).

5.2.2 Radiographic Examination of Normal and Diseased Teat

5.2.2.1 Plain radiography

Plain radiographic examination procedure of udder and teat were found to be easy but they fail to demonstrate the obstructive lesions and pathological alterations in the teat and udder tissues. Due to the lack of basic soft tissue contrast details this technique of examination of udder and teat was found to be less superior technique of all diagnostic techniques.These findings were in agreement with the previous reports of McDonald (1968), McDonald (1973), McDonald (1975) and Franz *et al.* (2009).

Normal and diseased udder and teat

In both normal and diseased teat study the approximate alterations in the size of the teat in comparison with the contralateral teats alone could be studied. The radiographs of udder and teat tissues were imaged as uniform shades of grey with lack of soft tissue contrast details and was in accordance with the reports of Witzig *et al.* (1984), Ducharme *et al.* *(*1987), Couture and Mulon (2005) and Franz *et al.* (2009).

5.2.2.2 Contrast radiography

Normal and diseased udder and teat

Contrast radiography techniques of the udder and teat were found to be better diagnostic techniques than the plain radiographic technique. In positive contrast study of the normal udder and teat the contrast agent injected into the teat cistern masked the internal details of the teat cistern and demonstrated the size, shape of the teat cistern, pattern of lactiferous ducts of the mammary glands and the length and diameter of the teat canal. The contrast study was found to be more useful to study the diameter, shape of the cistern, the degree of change in the teat wall thickness, demonstration of pattern of fistulous tract and to measure the approximate length of the teat canal. This technique was more effective to demonstrate the lesions in the teat cistern involving less than 30 per cent of the mucosal surface and also helped to quantify the approximate size and site of the lesion.

In negative contrast study of normal udder and teat the concentric folds of the teat cistern could be visualised better than the positive contrast study and the area of the gland and teat cistern could be appreciated with the demonstration of pattern of lactiferous ducts. Injection of air into the teat cistern inflated the teat cisternal cavity and the menstruation of the teat cisternal cavity was found to be difficult.

Double contrast radiographic study of the normal udder and teat provided better imaging details than plain, positive and negative contrast radiography. The size, shape and the mucosal fold pattern of the teat cistern and the gland cistern and tracts of lactiferous ducts was well demonstrated than the other radiographic techniques. The proliferative lesions in the teat cistern, changes in the internal architecture of mucosal surfaces of teat cistern, teat wall thickness, shape, size, length of the stenotic lesions in the cisternal cavities were well demonstrated and overall had the advantageous features similar to that of double contrast cystographic study. The above radiographic features observed in this study were also documented by McDonald (1968, 1973 and 1975), Witzig *et al.* (1984), Ducharme *et al.* *(*1987), Couture and Mulon (2005) and Franz *et al.* (2009).

5.2.2.3 Limitations

Plain contrast radiographic technique was easy to perform but they fail to image the soft tissue contrast details. Usage of positive contrast agent into the teat cistern for diagnostic study can be considered to be a non physiological method and its usage during inflammed conditions into teat cistern and mammary gland was questionable. Positive contrast agent masked the pathological lesions in the cisternal cavities and hence limited diagnostic utility was noticed. Spillage of the positive contrast agents in the outer surface of the teat wall produced artifacts and false positive results. Positive contrast agent could not be injected into the teat cistern in conditions of fibrosis and complete obstruction of the teat canal and distal parts of the teat cistern.

In negative contrast study injection of atmospheric air into the teat cistern is questionable because of the possibility of carrying the infection into the mammary gland. As in positive contrast study, the details of internal lesions of the teat cistern were found to be masked and the inability situation to inject the air during obstructive conditions was also noticed.

Double contrast radiography had the similar limitations encountered with that of positive and negative contrast radiography and the diagnostic sensitivity was found to be more superior to the other radiographic techniques. The above limitations were also revealed by Ducharme *et al.* (1987), Coutre and Mulon (2005) and Franz *et al.* (2009).

5.2.3 Ultrasonographic Examination

Normal and diseased udder and teat

In this study ultrasonographic imaging with machines attached with 7.5 to 10 MHz linear array transducer provided good ultrasonographic image details and was in agreement with the results of Bruckmaier and Blum (1992), Dinc *et al.* (2000), Franz *et al.* (2001), Twardon *et al.* (2001), Santos *et al.* (2004) and Flock and Winter (2006). Cartee *et al.* (1986) used 5 MHz transducers and reported the ultrasonographic details of udder and teats. In this study, 5 MHz convex transducers were found to be useful for ultrasound scanning of udder than for the teats and the observations were in accordance with Hoque *et al.* (2004). Ultrasonography of udder and teat cows and buffaloes was conducted by gel application method by Franz et *al.* (2001), Santos *et al.* (2004), Flock and Winter (2006) and Rambabu *et al.* (2008). During sonography the acoustic coupling gel improved the contact of the transducer and the air between the probe and tissue was avoided. The appearance of mammary gland, gland sinus, teat wall, teat cistern, teat canal as observed in this study was in accordance with the reports of Franz *et al.* (2001), Santos *et al.* (2004). In water bath technique as reported by Nak *et al.* (2005), Rambabu (2007) all the three layers of teat wall could be clearly demarcated and the teat canal and the rosette of Furstenberg showed a pattern of clarity. Cartee *et al.* (1986) reported that the use of water bath increased the acoustic impedance difference between the teat wall and the surrounding medium and the presence of milk in the teat cistern also acted as window of acoustic impedence for imaging the deeper structures and far wall of the teat. In the present study the ultrasonographic image pattern of udder lesions, oedema, hameatoma, abscess and fibrosis and teat lesions such as intracisternal obstructions, teat canal fibrosis, lacerations and fistula were similar to the reports of Cartee *et al.* (1986), Takeda (1989), Stocker *et al.*(1989), Trostle and O'Brien (1998), Dinc *et al.* (2000), Nudda *et al.* (2000) and Franz *et al.* (2001) Thomas *et al.* (2004), Hoque *et al.* (2004), Santos *et al.* (2004), Flock *et al.* (2004), Flock and Winter (2006) and Rambabu (2007).

5.2.3.1 Teat quantification

Normal Teat

I. Teat canal length

In group I cows the mean teat canal length of fore teats were found to be longer than the hind teats and in buffaloes, hind teats were longer than the fore teats. The mean values ranged from 1.23 to 1.31 cm in cows and in buffaloes between 1.67 and 1.96 cm respectively. In cows and buffaloes the mean teat canal length of left and right fore and hind teats showed highly significant difference and the mean values of cow's teat canal length were slightly lower than the buffalo. These results were in accordance with Schumer *et al.* (1981), Cartee *et al.* (1986), Stocker *et al.,* (1989), Takeda (1989), Gourreau (1995), Trostle and O'Brien (1998), Nudda *et al.* (2000), Franz *et al.* (2001), Thomas *et al.* (2004), Klein *et al.* (2005) and Flock and Winter (2006). Sastry *et al.* (1988), Singh and Singh (1994) and Uppal *et al.* (1994) stated that the longer teat canals and a stronger teat sphincter were the important reasons for a lower incidence of mastitis in buffaloes than in cows.

II. Teat diameter 1

The mean teat diameter 1 values in centimeter of group I cows and buffaloes on day 0 of examination showed statistically significant difference for the left fore, right and left hind teat and highly significant difference for the right fore teat. The mean teat diameter values of buffalo teats ranged from 2.47 to 2.53 cm and the values were slightly higher than that of the teat diameter of cow from 2.24 to 2.40 cm. Similar observations were recorded by Franz *et al.* (2001), Thomas *et al.* (2004), Klein *et al.* (2005) and Flock and Winter (2006).

III. Teat diameter 2

The mean values of teat diameter 2 in centimeter at 1.5 centimeter proximal to Furstenberg rosette in group I cows and buffaloes on day 0 of examination showed statistically significant difference for left fore, and highly significant difference for the right fore, right and left hind teat. The mean teat diameter values of buffalo teats ranged from 3.64 to 3.68 cm and these values were slightly higher than the diameter of cow from 3.31 to 3.46 cm. These values were slightly lower in accordance than that reported by Alieve (1970), Lind *et al.* (1997), Thomas *et al.* (2004), Klein *et al.* (2005) and Flock and Winter (2006).

IV. Teat wall thickness

The mean values in centimeter of teat wall thickness at 1.5 centimeter proximal to Furstenberg rosette of in group I cows and buffaloes on the day of examination showed statistically significant difference for left and right fore and left fore and highly significant difference for the right hind teat. The mean teat wall thickness of buffalo teats ranged from 0.47 to 0.49 cm and these values were slightly higher than the cow teat with values of 0.36 to 0.38 cm. These values recorded were slightly lower in similarity to that reported by Thomas *et al.* (2004), Klein *et al.* (2005) and Flock and Winter (2006). Zecconi *et al.* (1992) and Hamann *et al.* (1994) stated that a decreased blood flow period after milking was associated with an increase in teat thickness and increase or decrease in thickness greater than 5 per cent resulted in greater microbial colonization in the teat canal.

V. Teat cistern width

The mean values of teat cistern width in centimeter at 1.5 centimeter proximal to Furstenberg rosette in group I cows and buffaloes on the day of examination showed statistically significant difference for left fore and left and right hind and highly significant difference for the right fore teat. The mean teat cistern width of buffalo teats ranged from 2.80 to 2.97 cm and these values were slightly higher than the cow teat with values of 2.40 to 2.62 cm. These documented values were slightly lower than that reported by Thomas *et al.* (2004), Klein *et al.* (2005) and Flock and Winter (2006).

Diseased Teat

I. Teat canal length

The mean values of teat canal length in centimeter of cows with diseased teat condition on 0, 30 and long term follow up days in group IIA and IIB cows showed a statistically significant difference between the mean values of treatment groups. In buffaloes the mean values of teat canal length in centimeter with diseased teat condition on 0, 30 and long term follow up days in group IIA and group IIB showed statistically high significant difference between the treatment groups. In both group IIA cows and buffaloes the low mean values indicated that the teat canal length was restored to normal without any deterioration of the teat canal morphology showing beneficial effect of natural teat implant which favours postoperative wound healing of teat canal. Such similar observations were recorded by Seeh *et al.* (1997) and Querengasser *et al.* (2002). The results of ultrasound teat quantification data as documented in this study were in accordance with the findings of Cartee *et al.* (1986), Stocker *et al.,* (1989), Nudda *et al.* (2000), Franz *et al.* (2001), Thomas *et al.* (2004). Klein *et al.* (2005) and Flock and Winter (2006).

II. Teat diameter 1

The mean values of teat diameter 1 in centimeter at the level of Furstenberg rosette in group IIA and IIB cows with diseased teat condition on 0, 30 and long term follow up days showed statistically high significant difference between the mean values of treatment groups.

In buffaloes the mean values of teat diameter 1 in centimeter at the level of Furstenberg rosette with diseased teat condition on 0, 30 and long term follow up days in group IIA and IIB showed no statistically significant difference between the treatment groups. In both group IIA cows and buffaloes the mean values on 30 and long term follow up days were lower than the group IIB animals. The low mean values in both group IIA cows and buffaloes revealed that the implant did not induce any inflammatory response and helped to restore the normal teat diameter at the level of Furstenberg rosette. These observations were in accordance with the findings of Seeh *et al.* (1997) and Querengasser *et al.* (2002) who reported that the teat implant maintained the normal architecture of the teat tissues after implantation following theloscopic surgery. The results of the teat quantification parameter study on the teat diameter showed similar findings as documented by Stocker *et al.* (1989), Takeda (1989), Nudda *et al.* (2000), Coutre and Mulon (2005), and Flock and Winter (2006).

III. Teat diameter 2

The mean values of teat diameter 2 in centimeter at 1.5 centimeter proximal to Furstenberg rosette of cows with diseased teat condition on 0, 30 and long term follow up days in group IIA and group IIB showed statistically high significant difference between the mean values of treatment groups. In buffaloes the mean values of teat diameter 2 on 0, 30 and long term follow up days in group IIA and group IIB showed statistically high significant difference between the treatment groups. In both group IIA cows and buffaloes the mean values on 30 and long term follow up days were lower than the group IIB animals. The low mean values in both group IIA cows and buffaloes revealed that the teat canal patency was normal and helped to restore normal milkability and milk storage capacity. Thus it could be inferred that the teat implant provided a favourable response to maintain the teat diameter at the mid cistern level and these findings were in agreement with the reports of Stocker *et al.* (1989), Takeda (1989), Seeh *et al.* (1997), Nudda *et al.* (2000), Querengasser *et al.* (2002), Coutre and Mulon (2005), and Flock and Winter (2006).

IV. Teat wall thickness

The mean values of teat wall thickness in centimeter at 1.5 centimeter proximal to Furstenberg rosette of cows with diseased teat condition on 0, 30 and long term follow up days in group IIA and group IIB showed a statistically high significant difference between the mean values of treatment groups. In buffaloes the mean of teat wall thickness on 0, 30 and long term follow up days in group IIA and group IIB showed statistically high significant difference between the treatment groups. In both group IIA cows and buffaloes the mean values on 30 and long term follow up days were lower than the group IIB animals. The low mean values in both group IIA cows and buffaloes revealed that teat canal patency was normal and helped to restore the normal milkability and milk storage capacity with less pathological strain on the teat wall function after theloscopic procedure. Changes of the teat wall such as increased congestion and edema resulted in slower closure of the teat canal and/or hypoxia in teat tissues (Hamann *et al.,* 1994). Zecconi *et al.* (1992 and 1996) showed that an increase of teat thickness of over 5 per cent after milking is associated with an increased incidence of intramammary infection. Thus it could be inferred that after lateral theloscopic procedure with the usage of natural teat insert implant did not induce any pathological alterations and the normal teat wall thickness was maintained. The findings of the study and the teat quantification results were in accordance to the findings of Stocker *et al.* (1989), Takeda (1989), Seeh *et al.* (1997), Nudda *et al.* (2000), Querengasser *et al.* (2002), Coutre and Mulon (2005), and Flock and Winter (2006).

V. Teat cistern width

The mean values of teat cistern width in centimeter at 1.5 centimeter proximal to Furstenberg rosette of cows with diseased teat condition on 0, 30 and long term follow up days in group IIA and group IIB showed a statistically high significant difference between the mean values of treatment groups. In buffaloes the mean values of teat cistern width on 0, 30 and long term follow up days in group IIA and group IIB showed statistically high significant difference between the treatment groups. In both group IIA cows and buffaloes the mean values on 30 and long term follow up days were lower than the group IIB animals. The low mean values in both group IIA cows and buffaloes revealed that teat canal patency and normal milkability was restored and milk storage capacity was normal after theloscopic procedure. From the cistern width and the teat wall thickness maintenance it could be concluded that the utility teat implant was more advantageous to restore the normal teat canal function. These results were in accordance to the findings of Stocker *et al.* (1989), Takeda (1989), Seeh *et al.* (1997), Nudda *et al.* (2000), Querengasser *et al.* (2002), Coutre and Mulon (2005), and Flock and Winter (2006).

5.2.3.2 Limitations

In standing restrained position of the animal complete ultrasonographic scanning of hind quarters by contact with gel application technique was difficult. As the hind quarters lie in between the hind limbs the scanning procedure was hindered. Ultrasonographic imaging of the proximal teat cistern and annular fold area by water bath method was found to be slightly difficult. Use of tap water or sterile physiological solutions, mineral water etc., hindered the image visualisation because of the echogenic particles present in the water and hence triple glass distilled water was found to be ideal. Such similar limitations were also revealed by Cartee *et al.* (1986), Franz et *al.* (2001), Ayadi *et al.* (2003), Hoque *et al.* (2004), Santos *et al.* (2004) and Rambabu (2007).

5.2.4 Theloscopic Examination

5.2.4.1 Preparation, anaesthesia and positioning of the animal

Ring block, intravenous regional analgesia and local analgesia of the teat cistern was found to produce satisfactory analgesia during the theloscopic examination and surgical procedure. In this study procedures involving delicate handling and short term period procedures of examination or treatment only intracisternal analgesia alone was attempted and for procedures such as lateral theloscopy involving teat tissue invasiveness and excision of larger tissues, intracisternal and regional intravenous analgesia was attempted. For axial theloscopic examination the lateral recumbency rather than standing posture was preferred for examination procedures. For lateral theloscopic procedure visualisation of teat structures in standing posture and mammary gland examination in lateral recumbency postures were found to be more favourable for effective visualisation. Similar suggestions were revealed by Medl and Querengasser (1994), Bleul *et al.* (2005) and Geishausser *et al.* (2005).

5.2.4.2 Procedure

I. Axial theloscopy

In this procedure insertion of the theloscope through the teat canal was more easy in inflated condition of the teat rather than in the empty teat cistern condition. However, in this study during insertion of the blow pipe into the teat cistern through the teat canal, the tip of the blow pipe caused consistent damage to the teat canal and the blow pipe with diameter lesser than 3 mm OD may be useful to avoid this complication which was in correlation with the findings of Tulleners and Hamir (1990), Medl and Querengasser (1994), Geishauser and Guerengasser (2001), Bleul *et al.* (2005) and Geishausser *et al.* (2005).

II. Lateral theloscopy

In lateral theloscopy the artificial opening made in the teat wall favoured full visualization of the Furstenberg rosette, proximal to distal parts of the teat canal area and the teat cistern was visualized to the limited extent. The artificial opening made on the lateral wall opposite to the side of teat lesion favoured full and easy visualization of the obstructive lesions located at the mid to distal cisternal area and enabled easy excision of the lesions through the teat canal. Through this approach the gland cistern and lactiferous ducts were effectively visualized and the obstructive tissues involving the proximal part of the teat cistern and at gland cistern could be excised. Similar advantages were observed by Tulleners and Hamir (1990), Medl and Querengasser (1994), Geishauser and Guerengasser (2001), Bleul *et al.* (2005) and Geishausser *et al.* (2005).

Normal and abnormal teats

In buffaloes during theloscopic examination colouration of teat cistern mucosa with melanin pigment was noticed and similar observations were reported by Uppal *et al.* (1994) and Ludewig (1998).

5.2.4.3 Post operative care

In all the cases of group IIA and IIB cows and buffaloes treated by lateral theloscopic procedure, milkability by full hand milking and the outflow of milk was found to be normal and satisfactory on 3, 30 and long term follow up day of post treatment period and no obstructive condition was noticed. The wound made on the lateral wall of the teat as an artificial opening healed satisfactorily within a week and no complications were noticed. The outcome as recorded in this study were found to be better than Hospes and Seeh (1998), Bleul *et al.* (1999), Hirsbrunner *et al.* (2001), Zulaul and Steiner (2001), Steiner (2004), Bleul *et al.* (2005) and Geishauser *et al.* (2005).

5.2.4.4 Limitations

The limitations encountered in axial theloscopic were restricted visualisation of entire part of teat canal and the mammary gland and inability to perform surgical procedures through the single port via the teat canal. This technique was limited only for the diagnostic purpose rather than for the surgical treatment and was in accordance with Tulleners and Hamir (1990), Medl and Querengasser (1994), Geishauser and Guerengasser (2001), Bleul *et al.* (2005) and Geishausser *et al.* (2005).

In lateral theloscopy creation of an artificial opening, on the lateral parts of the teat wall diseased with sinusitis condition limited its application and for performing this procedure, an additional assistant was needed to hold the scope in fixed position and the dexterity of the surgeon was limited. This procedure was found to be more time consuming than the axial theloscopy and needed aseptic handling of the teat tissues to avoid postoperative complications and these observations were similar to that stated by Tulleners and Hamir (1990), Medl and Querengasser (1994), John *et al.* (1998), Hirsbrunner and Steiner (1999), Geishauser and Guerengasser (2001), Bleul *et al.* (2005) and Geishausser *et al.* (2005). In this study, the usage of the theloscope with 3 mm OD for theloscopic examination was found to cause considerable damage to teat canal structures of Indian cross bred cows and buffaloes. Hence, based on the observations of the study a recommendation is placed to design scopes with OD less than 3mm so as to enable the surgeons to perform routine theloscopic examination and surgical procedures in Indian dairy cows and buffaloes.

5.2.5 Evaluation of Theloscopic Surgical Procedure

5.2.5.1 Lateral theloscopic surgery – utility, limitations and complications

In this study lateral theloscopy was found to be more useful both in the diagnosis and treatment of the obstructive lesions involving the teat canal and teat cistern area. With this technique the lesions involving more than 30 per cent of the cisternal area, complete excision of these tissues was found to be difficult and time consuming and severe postoperative complications such as cisternitis and teat canal damage were noticed. The area of visualization and tissue handling access was more in lateral theloscopy than in axial theloscopy and enabled an easy and useful approach for the diagnostic and treatment procedures and this was in agreement with the findings of Hospes and Seeh (1998), Medl and Querengasser (1994), Hirsbrunner *et al.* (2001), Zulaul and Steiner (2001), Steiner (2004), Bleul *et al.* (2005) and Geishausser *et al.* (2005).

5.2.6. Evaluation of Wound Healing with Ultrasound and Theloscopy

5.2.6.1 Group IIA cows and buffaloes

Postoperatively, the wound healing in group IIA and IIB cows and buffaloes studied by ultrasonography on day 30 and long term follow up days revealed normal teat canal and teat echogenicity as described by Twardon *et al.* (2001), Flock *et al.* (2004) and Nak *et al.* (2005) and a negligible mild hyperechoic healing nature of the wound created on the lateral wall of the teat was noticed. Based on the results of the teat quantification parameters and from the ultrasonographic images of teat structures during pre and post treatment it is inferred that group IIA cows and buffaloes treated by lateral theloscopy with implants showed favourable healing on 30 and long term follow up days and these observations were in accordance with Querengasser *et al.* (2002).

In group IIA, on day 30 three cows and two buffaloes that were subjected to axial theloscopic procedure had normal appearance of the teat cistern as described by Geishauser and Guerengasser (2001), Bleul *et al.* (2005) and Geishausser *et al.* (2005) and at the operated sites the wounds were found to be healed with fibrous scar tissue formation and similar observation was recorded by Saifzadeh *et al.* (2005).

5.2.6.2 Group IIB cows and buffaloes

In group IIB cows and buffaloes, the ultrasonographic examination on day 30 and follow up period revealed normal teat echogenicity as stated by Twardon *et al.* (2001), Flock *et al.* (2004) and Nak *et al.* (2005) and in two cows the teat canal echogenicity as recorded on the day 0 of examination could not be visualized. The reason could be due to the damage to the keratin layer by the metallic instruments during surgical procedure and decreased keratin production during the postsurgical period (Woolford, 1997; Geishauser and Guerengasser, 2001).

In group IIB theloscopic examination of two cows and two buffaloes revealed normal appearance of the teat cistern as described by Geishauser and Guerengasser (2001), Bleul *et al.* (2005) and Geishausser *et al.* (2005) and no apparent changes in the mucosal wall layers of the teat was noticed.

5.2.7 Evaluation of Utility of Natural Teat Insert Bio-Implant

5.2.7.1 Group IIA cows and buffaloes

In group IIA cows and buffaloes, usage of natural teat insert implant after theloscopic procedure, showed normal milk quality and recovery of udder health on day 30 and long term follow up days. The implants disintegrated on its own and caused no obstructive disorders after theloscopic procedures and these observations were in accordance with Seeh *et al.* (1997). The utility of these implants were judged based on the milkability from the quarter, changes in milk parameters, milk outflow pattern from the teat and post treatment ultrasonographic evaluation of the diseased teat. Postoperatively, the teats treated with natural teat insert implant had no incidence of mastitis up to the long term follow up period and only less microbial pathogenic organisms were isolated from the milk than the group IIB cows and buffaloes. The somatic cells count on day 30 was found to be maintained at low count but at slightly higher level than the clinical limit and restored to normal within the clinical limit on long term follow up period. No alteration in the flow of milk was noticed and the teat canal patency was maintained on the long term follow up day and an increased total milkability from the quarter was noticed. These observations with high success rate after theloscopic surgery were in accordance with the reports of Hospes and Seeh (1998), Bleul *et al.* (1999), Geishauser *et al.* (1999), Hirsbrunner *et al.* (2001), Zulaul and Steiner (2001), Steiner (2004), Bleul *et al.* (2005) and Geishauser *et al.* (2005) revealing an advantageous utility of the usage of natural teat insert implant. Bleul *et al.* (2000) reported the occurance of teat sinusitis following the usage of teat dilators or plastic catheters after conventional surgery and no such incidence were recorded in this study.

SUMMARY

The study was carried out on the clinical cases of lactating cows and buffaloes with apparently healthy mammary gland reported for minor surgical affections and on the lactating clinical cases reported with milk flow disorders to the Large Animal Surgical Outpatient Unit of Madras Veterinary College Teaching Hospital, Chennai. A total of 98 cows and 24 buffaloes were reported during the period of study. Out of which, based on the parity of third calving with early to mid lactation phase and irrespective of the breed the cases 18 lactating cows and 18 she buffaloes were selected for the detailed clinical study and were divided into groups I and IIA and IIB. In group I six healthy cows and six buffaloes were subjected to normal udder and teat study and in group IIA six cows and six buffaloes were treated for the teat obstructive disorders by theloscopic surgery with implant and in group IIB six cows and six buffaloes were treated by theloscopic surgery without implant.

In cows and buffaloes teat affections were recorded in 78.57 per cent 92.31 per cent quarter affections 18.37 per cent in cows and quarter and teat affections 3.06 and 7.69 per cent repectively. In cows single, double and triple quarter affections were also recorded as 83.33 per cent, 11.11 per cent and 5.56 per cent and in buffaloes no such incidence of quarter affection was recorded. The percentage incidence of single, double, triple and quadrant affections of teat in cows and buffaloes were 84.42 and 91.67, 7.80 and 8.33, 3.89 and 3.89 per cent respectively

The etiology of obstructive milk flow disorders in cows and buffaloes recorded in this study were physical trauma 46.94 and 66.67 per cent, as a squeal to mastitis 31.63 and 25.00 per cent and other or unknown reasons in 18.37 and 8.33 per cent of cases respectively and congenital affections of 3.06 per cent was recorded in cows.

In both cows and buffaloes hind teats were found to be more affected 59.18 and 54.17 per cent than the fore teats 40.82 and 45.83 per cent and left sided affections were more common 56.12 and 83.33 per cent than the right sided affections 43.88 and 16.67 per cent respectively.

In cows fore quarters (68.00 per cent) were found to be more affected than the hind quarters (32.00 per cent) and left sided udder was found to be more affected 52.00 per cent than the right sided udder 48.00 per cent. In buffaloes an equal incidence of each 50.00 per cent was noticed with regard to fore/hind and left/right udder affections.

Among the teat lesions in cows, obstructive disorder at the teat canal area was recorded to be the highest incidence 15.30 per cent followed by fibrosis condition of the teat canal 13.30 per cent and hard milker condition 11.22 per cent. The other conditions noticed were fibrosis condition of the whole teat, obstructive disorder at the distal part of the teat cistern, teat wall wound, teat laceration, teat cistern haematoma, teat fistula, teat tip injury, leaky teat, obstructive disorder at the teat base, obstructive disorder at the mid teat cistern, obstructive disorder at the Furstenberg rosette, teat canal agenesis, thelitis and obstructive disorder at the proximal part of teat cistern were noticed .

In buffaloes obstructive disorder at the teat canal area was recorded to be the highest incidence 33.33 per cent followed by the obstructive disorder at the mid teat cistern 16.66 per cent and teat laceration. Teat tip injury, hard milker and fibrosis of the whole teat, teat cistern haematoma, teat wall wound, leaky teat and thelitis were noticed.

In normal cows and buffaloes the mean teat tip to floor distance was higher in cows than in buffaloes. The mean fore teat length in cows and the mean hind teat length in buffaloes were found to be higher than their respective mean teat lengths. The mean teat girth and mean teat canal length measured by graduated probe was higher in buffaloes than in cows.

The mean milkability by hand was found to be higher in cows than in buffaloes and in both the cows and buffaloes the mean milkability was higher in hind quarters than in fore quarters. The mean electrical conductivity values were found to be higher in buffaloes than in cows and the mean somatic cell count was found to be lower in buffaloes than in cows.

Of the radiographic techniques of examination of diseased udder and teat double contrast radiography enabled better diagnosis than plain, positive and negative contrast techniques. The proliferative lesions in the teat cistern, changes in the internal architecture of mucosal surfaces of teat cistern, teat wall thickness, shape, size, length of the stenotic lesions in the cisternal cavities were well demonstrated and the limitations encountered during radiographic examination were as follows. In plain radiography tissue contrast details were absent, positive contrast radiography was found to be nonphysiologic method of examination, and in negative contrast radiography injection of atmospheric air may potentiate the infection for mastitis.

Ultrasonographic examination of teat was done by water bath technique and gland cistern and udder examination was done by contact with gel application technique was followed. In water bath technique use of triple glass distilled water filled in polyethylene cup were found to be ideal for effective visualisation of teat structures.

Ultrasonographic quantification of teat revealed longer mean teat canal length, increased mean teat wall thickness and cistern width in buffaloes than in cows.

In the diseased cows and buffaloes that were treated with natural teat implant after theloscopic surgery showed higher mean milkability, lowered electrical conductivity and somatic cell count than those that were not treated with implants. Ultrasonographic examination of the diseased teats of cows and buffaloes treated with implants after theloscopic surgery showed normal restoration of mean teat canal length, teat diameters, teat cistern width and teat wall thickness than those animals that were not treated with implants.

To conclude based on the observations of this study both ultrasonography and theloscopy were useful in the diagnosis of udder and teat obstructive disorders. Theloscope was useful as an effective and less-invasive diagnostic and therapeutic tool for the treatment of teat lesions and found to have limited application depending on the severity of the pathological lesions of the udder and teat. Whereas ultrasonography aided in identifying the udder parenchymal diseases early changes in the obstructive disorders of teat and to study the healing pattern following removal of the obstructions. However the size of the scope along with the blow pipe used in this study (3mm OD) could be modified to reduced diameters to avoid the incidence of transient incontinentia lactis condition in Indian cross bred dairy cattle.

Use of natural teat insert implant after theloscopic surgery in cows and buffaloes restored normal milkability, milk somatic cell count, teat canal length and patency and normal teat tissue structure and function were maintained on long term follow up than those animals wherein the implants were not used.

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