CHAPTER 1
INTRODUCTION
1.0 Introduction

Progress in lactation biology of the bovine mammary gland advanced substantially during the 20th century (Bauman et al., 2006). Milk yield has been a major selection criterion for genetic improvement in livestock. In India, almost 53% of total milk produced is sourced from buffaloes and indigenous and crossbred cattle producing the rest of the milk. A great part of the milk production goes unrecorded as the buffalo milk is largely handled by the unorganized sector. Myriad breeds of buffaloes contribute to the rich biodiversity of the farm animals in the country. The milk producing ability of farm animals differ significantly among various breeds. The underlying mechanism behind this variability has a direct correlation with the expression of different genes in the form of signalling proteins, transcription factors, cell survival and death factors in the mammary gland during pre-lactation, lactation and involution. Some breeds of buffaloes are high producers of milk and many are low producers. The numbers and the secretory activity of the mammary epithelial cells reflect milk-producing ability of the farm animals. In India the per capita milk producing ability of buffaloes is low. Although genetic, environmental factors and nutrition play a major role, expression of various mammary derived molecules play an important role in their production potential. It is expected that there will be differential level of expression of some genes in pre-lactation and lactation stages. In this context, identification of the differentially expressed genes will help in the selection of high yielding animals.

Mammary gland development involves a series of complex physiological processes. There are six defined stages involved in development of the mammary gland including embryonic, pre pubertal, pubertal, pregnancy, lactation, and involution. Each of these stages involves a complex interaction of hormones, growth factors, and signal transduction pathways, ultimately leading to expression of developmentally regulated genes. Although some factors involved in mammary development, such as the hormones prolactin, estrogen, and progesterone, have been known for some time, it has only been in the last 10 years that individual genes involved in mammogenesis have been identified. A large number of proteins are synthesized in the mammary gland and exported via the secretory pathway or the milk fat globule. Those proteins that are exclusively synthesized in the mammary gland, the caseins, whey acidic protein (WAP), lactalbumin, are among the most highly expressed genes during lactation. Milk protein gene expression has long been known to increase in pregnancy, and differing profiles for the various milk proteins have been documented. Stat5 has been
accorded a central role in the regulation of milk protein gene expression, and other regulators have also been implicated. Clearly there is much to learn about milk protein gene regulation. Identification of various genetic factors involved in regulating the lactation in farm animals would thus enable researchers to unravel the molecular basis of milk production serving as a platform to direct future research to enhance the productivity of indigenous breeds. Hence it is required to understand the expression pattern of these genes and their differential expression patterns at different stages of mammary development.

Although lactation is believed to be a product of Darwinian selective pressure, little is known of its molecular origins or its regulation. Current knowledge of the molecular regulation of mammary development and lactation has largely been derived from dissection of signaling networks in cell culture systems and phenotypic characterization of genetically altered mice. Some proteins modulated during pregnancy and lactation have been identified and characterized in the context of hormonal and metabolic pathways. Beyond these signalling pathways, the regulation of mammary gland development and lactation is incompletely understood. Of particular interest are the major molecular events that govern macroscopic and histological changes in the mammary gland during secretory differentiation, secretory activation (the lactation switch), and the onset of involution (the involution switch). Unbiased genome-wide approaches are likely to identify novel genes and gene products involved in the regulation of lactation, particularly when incorporated into a larger picture of mammary development and function. The lactation phase, the continuous decline in the milk production can be attributed to apoptosis of the mammary epithelial cells. Therefore, identifying these factors and determining their level of expression will help us to assess animal’s lactation potential. The mechanism of action of a number of proteins involved in mammary development and involution process is not yet clear. Hence studying the regulatory elements of differentially expressed mammary proteins will help us to know mammary function better.

In this context, identification of the differentially expressed genes between high and low producers will help in the selection of high yielding animals. It has also been observed that indigenous farm animals, especially buffaloes and indigenous cattle undergo self-induced dry phase earlier than their average lactation period even without being induced by discontinuation of suckling/milking. The decline in milk yield after peak lactation in dairy animals has long been a biological puzzle for the mammary biologists, as well as a cause of considerable loss of income to the dairy farmers. Recent advances in understanding the control of the mammary cell population now offer new insights on mammary biological phenomena and a potential means of alleviating the farmer’s problems. Many evidences now
indicate that a change in mammary cells number, the result of an imbalance between cell proliferation and cell removal, is a principal cause of declining production. Further, it suggests that the persistency of lactation, the rate of decline in milk yield with stage of lactation, is strongly influenced by the rate of cell death by apoptosis in the lactating mammary gland. Apart from the environmental and management factors, mammary expressed regulatory and signaling proteins play most important role during lactation, which modulates lactation persistency in animals. The switching on and off and a complex interplay of a number of genes regulate the milk yield and the duration of milk production. Identification of various genetic factors involved in regulating the lactation and involution in farm animal would enable us to unravel the molecular basis of milk production. This would serve as a platform to direct future research to enhance the productivity of indigenous breeds. Hence it is required to understand the expression pattern of these genes/proteins and their differential expression patterns at different stages of mammary development and involution. The identification and characterization of these factors will pave the way to design suitable strategy to ameliorate the problem of milk cessation.

Functional genomics studies highlighted the complexity and coordinated set of molecular events that encompass murine, bovine (Bionaz et al., 2007), caprine (Ollier et al., 2007) and porcine (Tramontana et al., 2008) mammary adaptations to lactation, revealing new insights about the underlying transcriptomic regulation. Until recently bovine functional genomics studies were not feasible. However, up-to-date bovine genome sequencing and annotation efforts combined with quantitative PCR have become powerful tools for high precision gene expression analysis. Significant proportion of research on mammary development have been conducted on small model animals like mouse which differ appreciably from the farm animals physiologically and also with respect to differences in mammary gland size. Therefore, it may not be possible to translate the rodent data on large milch animals like buffalo. Hence it is most essential to understand the expression pattern in mammary gland development at various stages of lactation cycles.

With the above background information and knowledge gaps the present study is aimed at elucidating the global gene expression profiling and sequence characterization of specific genes involved in various physiological stages of buffalo mammary gland with the following objectives:

1) To delineate gene expression pattern during pre lactation (heifer) and lactation stages of buffalo mammary gland by global gene expression profiling using microarray technique.
2) To identify differentially expressed genes and gene pathways during heifer and lactation stages of buffalo mammary gland.

3) To validate and characterize differentially expressed transcripts during two physiological stages of lactation.