Chapter- I

Introduction
CHAPTER 1

INTRODUCTION

Buffalo plays a pivotal role in livestock and agriculture economy of many countries across the globe. The current world buffalo population is 194 million. About 97.13% world buffalo is in Asia, with 76.92% in South Asia. India and Pakistan have 57% and 43% of the population, respectively. Buffalo world population is steadily increasing at the rate of 2% per annum during the last two decades (Moaeen-ud-Din, 2014). Out of 90 million heads, approximately 70% buffaloes have been grouped as nondescript since these animals do not resemble any of the nine morphologically well characterized breeds, namely Nili-Ravi, Murrah, Bhadawari, Mehsana, Jaffarabadi, Surati, Nagpuri, Pandharpuri and Toda (Kumar et al., 2006). Amongst all the buffalo breeds, Murrah is a distinguished milch breed with Jaffarabadi and Nili-Ravi close-by (Sadana, 2010).

A larger part of the human population depends on domestic water buffalo than on any other livestock species in the world. This species was distributed from southern Asia to Europe during the Pleistocene. Later on with increasing dry climatic conditions, its distribution was restricted to the Indian subcontinent and Southeast Asia (Kumar et al., 2006). The domestic or water buffalo belongs to kingdom Animalia, phylum Chordata, class mammalian, order Artiodactyla, family Bovidae and subfamily Bovinae.

A characteristic of water buffalo is the high percentage of both protein and fat in the milk, although average milk yield per lactation is quite low if compared to dairy cattle (Rosati and Van Vleck, 2002). The water buffalo (Bubalus bubalis) contributes immensely to the agricultural economy through milk, meat, hides and draught power. According to phenotypes, karyotypes and recent mitochondrial DNA work the water buffalo has been divided into two subspecies; the river buffalo (2N=50) and the swamp buffalo (2N=48) differing in their genetic constitution. The river buffalo possess five pairs of submetacentric and 20 of acrocentric chromosomes, whereas the Swamp buffalo possess 19 pairs of metacentric chromosomes. The riverine buffalo, generally large in size, with curled horns found in the Indian subcontinent, middle East and eastern Europe. They
prefer to enter clear water, and are primarily used for milk production, but are also used for meat production and for draught purposes. The swamp buffalo, found in China and other Southeast Asian countries, are stocky animals with marshy land habitats primarily used for draught power in paddy fields and haulage but are also used for meat and milk production. The two subspecies are inter-fertile and produce progeny with 49 chromosomes (Pasha and Hayat, 2012). River buffalo have high lactation yields and are more suited to ploughing and drafting on dry plane land, along with for milk and meat purposes (Amaral et al., 2007). Murrah in India, Nili Ravi and Kundi in Pakistan, Beheri and Saidi in Egypt and Italian and Shumen in Europe are well known types of river buffalo (Rosati and Van Vleck, 2002). India possesses the best River buffalo breeds in Asia, which originated from the north-western states of India and have a high potential for milk and fat production apart from their use as a work animal and as a supplementary stock for use as meat production (Sethi, 2003). In India, all descriptions of buffalo’s breed structure and diversity in the country is around only River buffaloes as compared to Swamp buffaloes. By virtue of their numbers and relevance the ‘Buffalo' in India is synonym to ‘River Buffalo'.

Buffalo contribute more than fifty percent milk of the total milk produced in India but constitute less than half of the cattle population (Mitra et al., 2012). Despite having more than fifty percent of milk production, efficiency of milk production remains low due to absence of appropriate selection of genetically superior animals. Genetic improvement for milk production is limited to selection of bulls based upon their dams’ yield and such bulls are primarily used through natural mating. However, grading up and substitution of local breeds by the Murrah, a well-known dairy breed originally from North India, is a common practice (George et al., 1988). Among all the buffalo breeds, Murrah (popularly known as black gold) occupies prominent position being the highest milk producer (about 2200 kg per lactation) and has been widely used for improving native buffalo populations in India and also imported by several countries for improvement programmes of their native breeds, therefore a special attention has to be focused on Murrah breed. The home tract for Murrah is mainly considered as southern part of Haryana comprising districts of Rohtak, Jind, Hisar and Gurgaon. However, this breed has now spread to almost all parts of the country (Sodhi et al., 2006).
Steroid hormones play important roles in the reproductive biology of vertebrates, including mammals. The majority of the actions of steroid hormones, including estrogens, androgens, and progestrogen, are mediated by specific receptors that are localized in or near the nucleus of target cells. Steroid hormone receptors form a super family of nuclear transcription factors that include estrogen, progestrogen, androgen, glucocorticoid, mineralocorticoid, the vitamin D, and the retinoic acid receptors (Katsu et al., 2010).

Estrogen and progesterone hormone actions are mediated through intracellular receptors that are members of the nuclear receptor (NR) superfamily, namely estrogen receptor (ESR) and progesterone receptor (PGR) (King and Greene, 1984 and Perrot-Applanat et al., 1985). ESR and PGR are expressed in the ruminant oviduct and the complexity of physiological effects on gametes and early embryo scan be explained by segmental differences in the regulation of steroid hormone receptor expression (Bage et al., 2002; Ulbrich et al., 2003; Garcia-Palencia et al., 2007 and Valle et al., 2007). This superfamily consists of 18 receptor members, which are divided into class I and class II NR. Class I NR includes ESR and PGR, which are considered as candidate genes involved in biology of reproductive traits.

Estrogens play a central role in normal female reproduction physiology, as well as in the pathology of female reproductive organs. Estrogens exert their actions on target cells through protein receptor (ESR) having two isoforms- ER α and ER β, each of them coded by a separate gene, localised on different chromosomes (in cattle – BTA 9 and 10, respectively). Most tissues of female reproductive organs express both ER α and ER β and their relative expression levels may play a major role in mediating estrogen actions in particular tissue. Estrogen receptors, similarly as other nuclear receptors, are transcription factors which, after binding of a proper ligand (17β-estradiol, estron, estriol) regulate transcription of target genes (Rollerova and Urbancikova 2000). ERα is expressed in a wide variety of reproductive tissues including endometrium, breast tissue, ovarian stroma cells, and the hypothalamus in females (Couse et al., 1997 and Yaghmaie et al., 2005), as well as the prostate, testes, and epididymis in males (Takeyama et al., 2001). ER β has high homology to ERα in the DNA- and ligand binding domains, but encodes a distinct transcriptional activating function-1 domain (Mosselmen et al., 1996 and Sand et al.,
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It is expressed in uterus, breast, ovary, testis, prostate, kidney, bone, blood vessels, brain, lungs, and intestinal mucosa (Hodges et al., 2001; Enmark et al., 1997 & 1999 and Critchley et al., 2001). Due to the functions that estrogens play in the regulation of reproduction, development of the mammary gland, growth and differentiation of cells, estrogen receptors and their genes are considered candidates for markers of production and functional traits in farm animals, in particular female reproduction (Szreder et al., 2011). The ESR gene has been studied for polymorphism in pig (Agnieszka et al., 1999; Depuydt et al., 1999; Cassady et al., 2001; Drogemullar et al., 2001; Chen et al., 2000; Gibson et al., 2002 and Zhang et al., 2002), mouse (Georgiou et al., 1997) and human (Kazuhiro et al., 1998; Keiji and Masanobu, 1999; Maria et al., 1999; Ho et al., 2000; Holmberg et al., 2000; Jason et al., 2000; Kunnas et al., 2000 and Timo et al., 2000).

Progesterone plays a central role in the reproductive events associated with pregnancy establishment and maintenance (Conneely et al., 2002) and the complex regulation of the reproductive events. The progesterone participates in release of mature oocytes, facilitation of implantation, and maintenance of pregnancy, by promotion of uterine growth and suppression of myometrial contractility (Graham and Clarke, 1997). Progesterone receptor (PGR) was tested as candidate gene due to its role in the reproductive functions (Gutierrez-Sagal et al., 1993). The protein encoded by this gene is a nuclear receptor that binds specifically to progesterone and mediates its biological actions. Receptors for progesterone are expressed as two distinct isoforms, PR-A and PR-B that arise from a single gene (Conneely et al., 1989 and Kastner et al., 1990). The expression of both isoforms is conserved in rodent and humans and overlaps spatiotemporally in female reproductive tissues. However, the ratios of the individual isoforms vary in reproductive tissues as a consequence of developmental (Shyamala et al., 1990) and hormonal status (Duffy et al., 1997) and during carcinogenesis (Brandon et al., 1993 and Graham et al., 1996). The PR gene in human, located on chromosome 11q22–23, comprises eight exons and seven introns (Romano et al., 2007) and are mediated by the two progesterone receptor (PR) isoforms (PRA and PRB), which are ligand-dependent transcription factors (Beato and Klug, 2000 and Li and O’Malley, 2003). The PGR gene has been studied for polymorphism in chicken (Conneely et al., 1989; Huckaby et al.,
Reproductive efficiency is the primary factor influencing productivity and is hampered in female buffaloes by delayed maturation, silent estrus, low conception rates and prolonged inter calving intervals (Perera, 1999). Improvement of reproductive efficiency in female buffaloes requires a better understanding of their reproductive physiology under steroid hormonal control, especially in the oviduct, during estrous cycle. Several critical events have to take place under optimal conditions in the oviduct before the final establishment of pregnancy in the uterus (Ellington, 1991). In order to enhance genetic merit of animals with increased milk production, enhanced reproductive efficiency, disease resistance etc, it is important to identify and locate responsible gene quantitative trait loci (QTL) in the genome. To design rational breeding strategies for optimum utilization and conservation of available genetic variability in Indian buffaloes, it is essential to understand their genetic architecture and relationships among various breeds (Navani et al., 2002). Genetic polymorphisms are playing an increasingly important role as genetic markers in many fields of animal breeding. With the development of molecular genetic techniques it has become possible to establish a new class of gene markers based upon the variability at DNA sequence level. The discovery of RFLP generated renewed interest in the use of gene marker loci as an aid to selection programmes. A number of techniques were adopted to detect polymorphism at structural loci, of which polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) is the most preferred one because of its simplicity, quickness, economical, very high repeatability and non-use of hazardous radioactive material. PCR-restriction fragment length polymorphism (RFLP) assay is a cost-effective method for SNP genotyping and mutation detection.

With the understanding of reproductive physiology, the challenge is now to build on this knowledge a cost effective way to deliver molecular tools to enhance genetic improvement programs for reproductive efficiency. Therefore, the present investigation entitled “Molecular characterization of major candidate genes associated with reproductive
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traits in buffalo (*Bubalus bubalis*)” was aimed to test the hypothesis that polymorphism at progesterone receptor (PGR) and estrogen receptor (ESR) loci has some association with reproductive traits of buffaloes that can be used as marker for reproductive efficiency in marker assisted selection and proposed the following objectives:

1. To find out allelic variations in PGR and ESR genes in buffaloes using PCR-RFLP.
2. To sequence the amplified region of PGR and ESR gene and find variations at nucleotide sequence level.
3. To associate these allelic variations with reproductive traits.
4. To compare the sequence of these genes in buffalo with published sequences of cattle and other animals.