Heat stress is a concern for all livestock production systems and its effect has been studied because of the profound and readily apparent negative impact on production and reproduction. Heat stress causes significant economic loss to animal production system in most tropical countries (Hansen, 2009). High ambient temperature directly or indirectly affects production performance and health of farm animals (Gaughan et al., 2009; Horowitz, 2002). Thermal balance is affected by environmental factors (ambient temperature, relative humidity, solar radiation, air movement and precipitation), animal factors (rate of metabolism, moisture loss) and thermoregulatory mechanisms of animals (Bohmanova et al., 2007; Collier et al., 2008). In homeotherms, the body temperature remains almost constant within relatively narrow limits. Despite repeated challenges due to variation in external environment, this ability to maintain homeothermy has been very useful in developing the higher forms of life. To maintain the ‘internal milieu’ in stressful conditions, number of physiological and behavioural changes occur and they vary in intensity and duration of the environmental stress.

In tropical countries like India, where more than 85% places experience moderate to high temperature humidity index (THI) during summer season, heat stress is a concern for farm animals particularly buffalo which forms the mainstay of dairy agriculture (Sethi et al., 1994). In terms of THI, the value of THI more than 72 is considered as stressful and THI value above 78 is considered as very severe heat stress for buffalo. It is used to evaluate the impact of climatic conditions that contribute to heat stress on the production of livestock all over the world. The THI for dairy cattle has been calculated to be 72 (Thom, 1959), however for high producing dairy cows, this has been shifted down to 68 because of the concurrent rise of metabolic heat production which is associated with vast milk production.

Buffalo are found only in certain regions of the world i.e., Asia, some Mediterranean, Eastern Europe and many Latin America countries. Since these regions are widely different in the geographical conditions, buffalo can thrive very
well and be simultaneously economical in these regions. India has about 105 million buffalo which is the highest figure in the world contributing about 55 million tons of milk to the country’s total milk production. Amongst the different breeds of buffalo, Murrah is the most important and well known breed not only in India but in the entire world. Like all other mammals, buffalo are homeotherms i.e., they maintain a constant body temperature regulating peripheral and internal body temperature with assistance of cutaneous sensors and internal temperature sensors (located in the hypothalamus) along with integration of endocrine system. Tissue, cellular metabolism and the underlying biochemical reactions that sustain life and productive functions need body temperature to be maintained within very narrow limits. An increase in body temperature by 1-2 °C may result in detectable, deleterious effects in metabolism, tissue integrity and a significant depression in production. When the heat load of an animal is greater than its capacity to lose heat, a portion of the metabolizable energy typically used for production must be diverted to assure thermal balance. Therefore, selection for tolerance to environmental stress has traditionally resulted in reduced productivity (Smith et al., 2000).

The physiological state of animals is conducive in a certain comfort zone, immediately crossing this zone, animals experience either hypothermy or hyperthermy. The animals adjust at lower temperature with a variation of about 15–25 °C, however, a rise of temperature by only 3–6 °C over the comfort zone is experienced as heat stress, which explains the higher concern for heat stress compared to cold stress (Collier et al., 1982). Buffalo are more prone to heat stress due to scarcely distributed sweat glands, dark body color and sparse hair on body surface and therefore, get easily distressed reducing their reproductive capacity (Das et al., 1999). The core body temperature of buffalo ranges between 38.5° and 39 °C and may reach up to 41.5 °C on exposure to solar radiation during summer (Das et al., 1999; Sethi et al., 1994; Aggarwal and Upadhyay, 1998).

This is particularly the case for fertility (Hansen, 2009). The disruption of reproduction during heat stress is caused by the failure of the animal to cope with heat stress, leading to a rise in body temperature above its regulated set point, which can compromise the functioning of the germ cells and the viability of an early developing
embryo. The changes which are required to regulate the body temperature can compromise the reproductive function of the animal. Although this adaptive response increases the dissipation of body heat to the environment, it also leads to reduced perfusion of the placental vascular bed and retarded fetal growth (Collier et al., 1982). Furthermore, heat stress reduces the length and intensity of estrus behaviour, modifies endocrine function, alters the oviductal and uterine environments, interrupts early embryonic development and ultimately lowers the conception rates in cattle (Rensis and Scaramuzzi, 2003; Wolfenson et al., 2000).

Studies in cattle suggest that oocytes are directly susceptible to heat stress in addition to the alterations in follicular gonadotropic environment. Exposure to elevated ambient temperature negatively impacts several stages of development to reduce pregnancy rates. These stages include the germinal vesicle (GV)-stage oocyte contained within antral ovarian follicles (Al-Katanani et al., 2002), the ovulated oocyte near the time of fertilization, the zygote just after fertilization (Ju et al., 1999; Ju and Tseng, 2004), and early cleavage-stage embryos (Ealy et al., 1993). The majority of early embryonic losses in cattle in the presence of environmental heat stress occur before day 20 of gestation (Hansen and Arechiga, 1999). Effects of heat stress are particularly prominent during estrus (day-1) when the oocyte is undergoing meiotic maturation in preparation for fertilization (Cavestany et al., 1985). While negative effects of heat stress exposure during meiotic maturation on fertility could result from the effects on the maternal environment such as the follicle or hormonal profiles (Rensis and Scaramuzzi, 2003), direct (in vitro) exposure of bovine oocytes to elevated temperature during maturation has been reported to reduce embryo development (Edwards and Hansen, 1997; Lawrence et al., 2004) to a similar degree as that seen in vivo (Cavestany et al., 1985). Reduced blastocyst development of bovine oocytes after direct exposure to elevated temperature has been associated with accelerated nuclear (i.e., progression to metaphase II (MII) of meiosis) and cytoplasmic (i.e., migration of cortical granules to the oolemma) maturation (Edwards et al., 2005).

Heat stress of maturing oocytes also induces alterations on molecular level. Specifically, de novo protein synthesis has been reported to reduce by approximately
30% after exposure to 41 °C for the first 12 h of meiotic maturation, coincident with a 65% reduction in blastocyst development (Edwards and Hansen, 1996). Heat stress has also been demonstrated to induce differential abundance of specific unknown proteins after exposure of bovine oocytes to 41 °C for the first half of meiotic maturation (West-Rispoli et al., 2006). Protein synthesis during the first 12 h of bovine oocyte maturation is critical for proper meiotic maturation and subsequent embryo development after fertilization (Saeki et al., 1997). Thus, heat-induced alterations in protein synthesis after culture of bovine oocytes at 41 °C for the first 12 h of meiotic maturation may be a key factor in explaining the observed reduction in development of heat stressed oocytes. Alterations in protein synthesis may be mediated by several mechanisms at the level of translation. Any perturbation in oocyte transcripts resulting from heat stress at the onset of meiotic maturation could be problematic as oocytes are transcriptionally inactive soon after resumption of meiosis and rely upon stored messages in the ooplasm for protein synthesis (Rodman and Bachvarova, 1976). It can be concluded that heat stress affects the cell which can be either the oocyte, the cumulus cell or the embryo in a direct way through elevated temperature either on the animal level or at the cellular level, or it can act in an indirect manner through the disrupted energy balance and associated metabolic changes which occur in the heat-stressed animal.

Heat stress apart from in vitro culture conditions is considered to cause oxidative stress in oocytes by generating the superoxide anion (O$_2^-$) or hydrogen peroxide (H$_2$O$_2$) (Fisher et al., 1991). Reactive oxygen species (ROS), such as superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (OH$^-$) are oxygen-derived molecules generated within the tissues and cells which have the ability to react with any molecule and modify it oxidatively. Oxidative stress ensues when there is an imbalance between the scavenging capacity of antioxidant defense systems and production of ROS. Free radicals and reactive oxygen species (ROS) are generated as a part of routine cellular metabolism and by various exogenous sources (Blondin et al., 1997). During the past few years, it has been observed that physiological concentrations of ROS participate in normal cell processes as major factors in regulation of growth and development (H Hancock et al., 2001). However, the
overabundance can have deleterious effects on cellular function by inducing oxidative
damage of intracellular components and resulting in structural and functional alteration and inducing apoptosis (Guerin et al., 2001).

Heat stress in oocyte can activate the already existing antioxidative defense system and enzyme synthesis. Enzymatic antioxidants such as SOD, Catalase, GRH and GSH-Px work in concert with non-enzymatic antioxidants such as glutathione occurring in the oocyte, presumably to counter the potentially harmful effects of ROS. Glutathione (GSH) is the most important intracellular antioxidant. The principal role of glutathione is neutralisation of free radicals which are produced in cells at elevated temperature. GSH acts as a substrate for enzymes like glutathione peroxidase, catalase and enables the transformation of free radicals into H₂O (Bray and Taylor, 1993). Without these enzymes, cellular injuries may occur in different forms like chromatin damage, lipid peroxidation of membranes, altered cytoskeletal structures and other detrimental changes.

Although buffalo is the mainstay of dairy agriculture in India, the information on the direct effects of heat stress on buffalo oocytes is meager. At present, little is known regarding the molecular mechanisms operating in bubaline oocyte during periods of heat stress affecting its developmental competence; therefore, research based improvement and addition in knowledge in this regard is need of the hour. The present research will help in identifying the causes at the molecular level that might in some way be responsible for the observed changes in fertility during periods of heat stress. Based on the published data, the working hypothesis of this research work was that physiologically-relevant elevated temperatures may affect the buffalo cumulus oocyte complex (COC) during in vitro maturation which may in turn alter the maternal pools of RNA, subsequently, reducing embryo development. Therefore, keeping this under consideration, this research has been designed with the following specific objectives:

1. To study the effect of heat stress on the developmental potential of buffalo
   Cumulus Oocyte complexes (COCs) during meiotic maturation in vitro.
2. To study the effect of heat stress on the expression of candidate genes related to developmental competence, apoptosis, heat and oxidative stress in COCs.

3. To determine the normal to apoptotic cell ratio in COCs at different temperatures during maturation using TUNEL assay.

4. To determine the effect of heat stress on the biochemical parameters of COCs, denuded oocytes and cumulus cell mass.