Introduction

1. INTRODUCTION

The increasing food demands of the world has forced man to renew efforts for enhancing the contribution of the domesticate animals. Goats were among the first farm animals to be domesticated. As indicated by the archaeological evidence, this small ruminant has been associated with man in a symbiotic relationship for up to 10,000 years (Ensminger and Parker, 1986). Goats disseminated all over the world because their great adaptability to varying environmental conditions and the different nutritional regimes under which they were evolved and subsequently maintained. They proved useful to man throughout the ages due to their productivity, small size, and non-competitiveness with him for food (Rout, 1999).

Goats are known to be potential genetic resource for meat, milk, skin and fiber throughout the tropical, subtropical and developing countries. These small ruminants contribute substantially in the economic status of peasants and landless poor. In fact, goats play a significant role in human nutrition and food security of pastoral people. The goat milk has pharmaceutical value and is used in the treatment of liver dysfunctions, dengue and pre born immature babies. The goat meat has better qualities of red and white meat that possess minimum quantities of free radicals and low density lipids. The goats have unique ability to adapt to a variety of environments, and are widely distributed in the tropics and subtropics (Devendra, 2003).

The largest number of goats is present in Asia (59.7%), followed by Africa (33.8%), representing 93.5% out of the total number of the world. The Jamnapari goat in India and Pakistan, serves the dual function of meat and milk production. The Jamnapari is the best dairy goat in India (Rout, 1999). It is characterized by varied color, Roman nose, and extremely long ears. Similar long-legged types are also in Africa and South America. Reproduction is a major factor contributing to the efficiency of milk and meat production. Although the goat farming is primarily for milk or fiber production, reproduction makes an important contribution by adding to the number of animals which can be utilized for meat thus contributing to production through influencing culling (Gill and Dev, 1972).
Reproduction, the essence of survival is finely tuned and regulated by coordinated signaling network between gonads, pituitary and hypothalamus. Ovary, the primary female reproductive organ which performs two major functions (i) production of ovum and (ii) secretion of plethora of growth factors, hormones and cytokines for proper manifestation of ovarian functions, secondary sexual characteristics and to help and support pregnancy. There are various cell types within the ovary that supports the growth and development of oocytes until ovulation (Hilier et al., 1994). The mammalian ovary is comprised of defined the 5 components of ovary namely the developing follicles, atretic follicles, corpora lutea, interstitial gland tissue and stroma (Sharma and Batra, 2008). For fertilization and successful propagation of the species, the orchestra of endocrine elaborations of each component is a must. The stroma forms one of the important compartments of the mammalian ovary about which our knowledge is still meager due to the presence of variable amounts and distribution of different types of interstitial cells. The stroma shows great morphological variations in different phases of reproduction and extensive diversity in different regions of the ovary within the same species (Mossman and Duke, 1973; Guraya 1978, 1991, 1997; Guraya and Motta, 1980).

Morphologically stroma is composed of various cellular entities such as fibroblast like stromal cells, interstitial cells of thecal origin and interstitial cells of epithelial origin, medullary interstitial cells, blood vessels, lymphatics and cholinergic and adrenergic nerves. The interstitial cells present in stroma have been classified as the primary and secondary depending on the source and sequence of their development and differentiation (Guraya, 2000). Primary interstitial cells differentiates first from the fibroblast like stromal cells of developing ovary and thus do not have any anatomical relationship with the growth of follicles (Guraya, 1977, 1978; Guraya and Uppal 1978). The formation of secondary interstitial cells is related to follicular growth and atresia in the maturing and adult ovary as these clearly originate from the theca interna of the atretic follicles, therefore designated as thecal type interstitial cells (Guraya, 1977, 1978, 1985; Guraya and Motta, 1980).
Woll et al. (1948) put forward a hypothesis that the cells present in ovarian stroma produce steroids, which affects endometrium. After which various investigations including histochemistry were performed for specific identification the presence of enzymes necessary for the production of steroids in ovarian stroma. The presence of steroid specific enzymes in ovarian stromal tissue was demonstrated by enzyme histochemical techniques. In the case of postmenopausal ovary, aromatase activity was found in stromal tissue, which proved that the post-menopausal ovary can produce substantial amounts of estrogen \textit{in vitro} (Dennefors et al., 1980)

The endocrine functioning of theca, granulosa cells and luteal cells of the ovary is controlled by gonadotropins during different reproductive stages of mammals. Although stroma has largely been ignored as a necessary and vital component of the developmental process, the receptors for gonadotropins however, has been reported in the ovarian stromal cells by Pleuso \textit{et al.} (1976), which were further confirmed by Nakano \textit{et al.} (1989). The presence of the binding sites of FSH in the ovarian stromal cells are suggestive that, there must be some effect of hypothalamo-pitutary hormones on the stromal cell steroidogenesis, which is yet to be established in caprine ovary. The positive or negative feedback mechanism of regulation of steroidogenesis in ovarian stroma is yet to be revealed.

Mc Natty \textit{et al.} (1979) have reported the synthesis of estrogens from the stromal cells in human ovary. The estradiol is naturally occurring estrogen, which is synthesized from cholesterol involving a series of sequential steps that result in the cleavage of side-chains, reorganization of olefinic bonds, and the addition of hydroxyl groups. For estradiol synthesis, this pathway is from cholesterol to pregnanes, then on to androstanes and finally synthesizing the estranes (Strauss \textit{et al.}, 2004). Estradiol synthesis in the premenopausal ovary requires the synergistic efforts of at least two cell types that synthesize their product when stimulated by independent upstream mechanisms. Following menopause, peripheral estradiol levels in the blood are thought to be mainly due to contribution from the adrenals and peripheral aromatization of androgens to estrogen in adipose tissue and skin, where aromatase activity correlates with estradiol production. However, evidences are present, that the
postmenopausal ovary retains the ability to produce both androgens (Havelock et al., 2006) and estrogen.

Androgens, mainly synthesized by the theca cells and ovarian stroma, are converted into testosterone and dihydrotestosterone by mural granulosa cells. Androgen action can affect many components of ovarian development and cyclic function, since their receptors are expressed in fetal ovary and oocytes and in oocytes, granulosa cells, theca cells and ovarian stroma of mature adult ovary. Although, a relatively weak androgen, androstenedione is converted to testosterone by stromal cells (Blaustein et al., 1979). Even during late follicular phase of the menstrual cycle when the estrogens are at the peak, the plasma concentrations of androgens are still greater than estrogens. Circulating estrogens and androgens are mostly bound to plasma albumin and sex hormone binding globulin leaving 2%-3% free. The bound hormones have some ability to enter target tissue, whereas the free hormones are presumed to be completely active (Carr et al., 1993, Pardridge et al., 1986).

Various animal models do indicate that testosterone stimulates the growth in vivo of ovarian surface papillomas and cystadenomas (Silva et al., 1997) and the dysgenetic ovaries of neonatally thymectomized mice (which develop tubular adenomas) produce abnormally large amounts of androstenedione. It is well known that permanent alterations in adult fetal phenotype are induced due to steroid hormone excess including glucocorticoid and sex hormone during fetal life. While lifelong androgen deficiencies do not concomitantly result in fetal estrogen deficiencies, such as in androgen receptor knockout (ARKO), female mice, there are only suitable impairments in adult ovarian functions. Although ovarian follicle counts are similar to those wild type mice, ARKO mice have fewer estrous cycles, smaller litter sizes, yields fewer oocytes upon gonadotropic hyper stimulation, and exhibit a diminished granulosa layer within follicles and smaller sized corpora lutea. Thus, androgen may play a great role in supporting ovarian follicle and oocyte maturation, in adult ovary, when treated the adult monkey with testosterone. Not surprisingly, the ovaries fail to show major follicle development or the presence of corpora lutea, but they do become enlarged due to hypertrophy of stroma under endogenous hypergonadotropic over stimulation.
Introduction

Several major roles are played by progesterone produced by the mammalian corpus luteum, during the reproductive cycle in females, including regulation of secretion of gonadotropins, preparation of the uterus for implantation and maintenance of pregnancy. Albeit at different levels, granulosa cells, thecal/stromal cells also secrete progesterone (Csapo et al., 1971; Gore Langton et al., 1988). Progesterone acts different levels, when secreted from the ovary for eg. a) hypothalamus-pituitary axis, to regulate gonadotropin secretion (Muldoon and Mahesh, 1987) and mating behavior, b) the mammary gland to stimulate development and c) the uterus. As it regulates all these essential physiological processes, considerable research has been focused on the mechanism through which progesterone mediates its action in these tissues. Unlike the well characterized progesterone responsive tissue, it has been difficult to determine whether progesterone influence ovarian functions because the ovary synthesizes progesterone in high amounts. However in his reviews Rothchildid (1981, 1996) put forth the concept that progesterone has an intraovarian site of action.

Low levels of androstenedione and progesterone have been reported in ovarian stromal cells of human, mouse and rat (Craig, 1967; Serment et al., 1969; Laffargue and Serment, 1973; Guraya and Uppal, 1978; McNatty, 1979; Barbieri et al., 1984; Brook and Clarke 1989; Nagamani et al., 1992; Thompson and Adelson, 1993). In young cycling animals this low level of stromal cell steroid production are probably physiologically significant. The older animals whose ovaries contain negligible follicles however are proposed to be more affected by ovarian stromal cells steroid production (Serment et al., 1969; Laffargue and Serment, 1973; Dennefors et al., 1980; Snowden et al., 1989; Nagamani et al., 1992; Thompson and Adelson, 1993). In humans, ovarian stromal cells are the primary source of androgens during perimenopausal and postmenopausal life. The stromal cells androgen production has been proposed to influence cancers of the ovary and endometrium (Nagamani, 1992; Thompson and Adelson, 1993). It has been established during the various studies that for the normal and pathological conditions of humans, the hormonal regulation of ovarian stromal steroid production may be important. Thus, for studying
perimenopausal and postmenopausal ovarian stromal cell function these cells may provide a useful model system.

Correlative morphological (ultrastructural) *in vitro* biochemical studies on interstitial cells of the ovarian stroma in different species of mammals remains to be carried to identify their secretory products as well as to define more precisely the regulatory mechanisms involved in the function of steroid synthesis. Ovarian interstitial gland can hardly be excluded as a contributing site for the production of progestins during pregnancy, but the exact function remains obscure. However, it was difficult to isolate the interstitial tissue that do not contain other type of cells, as they are embedded in the connective tissue (stroma), the hormonal regulation of interstitial cells could not be performed during the present study.

All the available information on ovarian stroma is restricted to primates and some rodents. However to the best of the knowledge of author information of stromal endocrinology, morphology, histochemistry and molecular regulatory mechanisms of small and large ruminants has yet not been initiated. It is therefore the present study on goat ovary has been augmented.

The present study was thus, proposed with the following objectives:

1. Histochemical changes in the stromal cells of normal cycling goat ovaries.
2. Ultrastructural variations of the stromal cells in goat ovary.
3. Influence of endocrine manipulations (FSH, LH, Estrogen, Progesterone etc.) on transdifferentiation of stromal cells.
4. Changes in endocrine output and their possible role in reproduction.
5. Change in western blotting after endocrine manipulation

The results of the present investigation will reveal the physiological obligations of stroma and provide better insight in endocrine elaborations of stroma and interstitial cells and their regulations by intra or extra ovarian hormones and growth factors. The outcome of the research will find applications in *in vitro* folliculogenesis, oocyte maturation, and fertilization.