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Expression of leptin and its receptor in corpus luteum during estrous cycle in buffalo (*Bubalus bubalis*)

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ABSTRACT

Leptin is supposed to play a crucial role in ovarian luteal dynamics. The present study was aimed to investigate the importance of leptin and its receptors in buffalo corpus luteum (CL) obtained from different stages of the estrous cycle. Real-time RT-PCR (qPCR), western blot and immunohistochemistry techniques were applied to investigate mRNA expression, protein expression and localization of examined factors. Additionally to assess the contribution of leptin in progesterone production the expression profiles of StAR, P450scc and HSD were also investigated. In general, we demonstrated presence of leptin and its receptors in buffalo CL during the estrous cycle. The mRNA levels of leptin and its receptors were significantly up regulated in ($P < 0.05$) in all the stages and highest levels were observed in mid and late luteal stages consistent with in vivo luteinization of buffalo CL and declined coincidental to luteal regression. The expression of StAR, P450scc and HSD factors maintained low in early luteal phase, after that level of expression increased steadily to show a significant rise ($P < 0.05$) in mid luteal phase followed by gradual decline in late luteal phase and regressed CL and this correlates well with the Ob and ObR receptor activity, verifying their key role in progesterone and other steroids production in functional CL. As revealed by immunohistochemistry, leptin protein was localized predominantly in large luteal cells however leptin receptor (Ob-R) was localized in large luteal cells as well as in endothelial cells. It can be concluded from our study that leptin via its autocrine/paracrine effects play a significant role in promoting angiogenesis, steroidogenesis and also acts as key survival factor in bubaline CL.

1. Introduction

Leptin, a 16.4 kDa peptide hormone, product of the obese gene, is secreted primarily in adipocytes and is known to play a critical role in the regulation of body weight by inhibiting food intake and stimulating energy expenditure (Zhang et al., 1994). Apart from their role in the regulation of body weight and energy expenditure, evidence suggests that leptin also plays an important role in reproduction. Its role in reproduction includes important actions on the hypothalamus to bring about release of LH-releasing hormone, thereby triggering gonadotropin release and leading to development of the reproductive tract and induction of puberty (Caro et al.,

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1996). Administration of leptin to obese leptin-deficient mutant mice caused decreased food intake, body weight loss, increased ovarian weight, increase in ovarian follicles and restoration of fertility (Barash et al., 1996; Chehab et al., 1996; Kikuchi et al., 1999).

Leptin signaling is accomplished via receptors which have six isoforms with sequence homology placing it in the Class I cytokine family (Tartaglia et al., 1995). Signaling of leptin receptors is brought about through JAK-STAT pathway. Leptin dose dependently modulate SREBP1 and StAR transcription and in turn steroidogenesis in cells. Leptin receptors have been found in the hypothalamic center responsible for satiety (Tartaglia et al., 1995). In addition, leptin receptors exhibit widespread distribution in mammalian tissue, including liver, heart, kidney, lung, small intestine, testes, ovaries, spleen, pancreas and adipose tissues (Lee et al., 1996).

Although the general view has been that the principal effects of leptin are on the neuroendocrine component of reproduction (Chehab et al., 1996), evidence has emerged to indicate direct involvement of leptin in ovarian function. The expression of the leptin and its receptor in ovarian cells of many species suggest that leptin plays a role in an autocrine/paracrine fashion and take part in important processes concerning reproduction. The expression of leptin receptors has been demonstrated in human, mouse, rat, pig and bovine ovaries (Karlsson et al., 1997; Kikuchi et al., 1999; Duggal et al., 2000; Ruiz-Corte's et al., 2000; Nicklin et al., 2007; Sarkar et al., 2010). Additionally, there are reports which indicate that reproductive dysfunction in aging obese mice is related to modified intra-ovarian leptin gene expression that is related to acquired obesity and declining fertility in this species and is related to progressive hyperleptinemia and leptin resistance (Brannian et al., 2005, 2009). Given its positive effects on gonadotropin secretion and fertility, leptin is expected to have either a positive local effect or no effect at all. The majority of researchers have suggested that the direct effects of leptin on ovarian cells are inhibitory and can be attributed to attenuation of gonadotropin, insulin, insulin-like growth factor 1 (IGF-I) and/or glucocorticoid-mediated steroidogenesis (Spicer and Francisco, 1997, 1998; Zachow and Magoffin, 1997; Zachow et al., 1999; Duggal et al., 2000; Spicer et al., 2000; Ghizzoni et al., 2001; Guo et al., 2001). Contrasting studies have revealed direct stimulatory effects of leptin in rat and human ovaries in the form of induction of angiogenesis and proliferation of ovarian cells (Spicer and Francisco, 1997; Bouloumie et al., 1998) and more specifically progesterone production from the bovine corpus luteum (CL) (Nicklin et al., 2007). Moreover, the presence and turnover of mRNA and protein for leptin have been described in human granulosa and cumulus cells and the presence of leptin in mature human oocytes (Cioffi et al., 1997) and bovine ovaries (Sarkar et al., 2010).

Infertility in animals may be caused by a defective corpus luteum (CL), which can be attributed, in part, to incomplete vascularization (angiogenesis) of the corpus luteum, causing a decrease in progesterone production. The angiogenic process is regulated by proangiogenic factors including vascular endothelial growth factor (VEGF), angiopoietin-1 (Ang-1) and fibroblast growth factor 2 (FGF-2) that are expressed at different times during the luteal phase. Nevertheless, it is known that leptin induces angiogenesis (Bouloumie et al., 1998) and this may be reflected in the correlation between the luteinization and leptin and leptin receptor in the bovine ovary. Leptin, a potent satiety hormone that influences the gene expression of some of these angiogenic hormones in non-ovarian tissues, has also been identified in the porcine and caprine corpus luteum. Therefore, leptin regulates the production of VEGF, Ang-1, and FGF-2 in developing luteal tissue and ultimately corpus luteum formation and progesterone production (Robin et al., 2009; Jessica et al., 2008).

Several studies have shown that leptin has crucial role in steroidogenesis. The key factors of progesterone synthesis in corpus luteum of various species have been found to be StAR, P450scc, and 3β-HSD. In the process of progesterone synthesis, the formation of pregnenolone from cholesterol catalyzed by the cholesterol side-chain cleavage enzyme complex (P450scc) that resides in the inner mitochondrial membrane is the rate-limiting enzymatic step in steroidogenesis (Miller, 1988). This step in the biosynthesis of steroid hormones is stimulated by trophic hormones but is not effective in augmenting steroidogenesis unless there is concurrently increased translocation of cholesterol from the outer to the inner mitochondrial membranes (Simpson et al., 1978; Toaff et al., 1979). Effective transport of cholesterol to the mitochondrial P450scc is believed to be an important step of steroidogenic regulation. Findings now suggest that a specific mitochondrial protein steroidogenic acute regulatory protein (StAR), mediates this critical function of cholesterol transport (Clark et al., 1994; Stucco and Clark, 1996). The in vivo data in cattle has shown that increased plasma leptin concentrations are associated with elevated proegesterone secretion in dairy cows (Mann and Blache, 2002).

Given their role in controlling ovarian function, leptin and their receptors are hypothesized to be involved in corpus luteum formation, function and development during estrous cycle in an autocrine/paracrine manner in buffalo. To test this hypothesis we evaluated (a) the mRNA and protein expression of leptin and its receptor (b) the mRNA expression of key steroidogenic factors viz. p450scc, StAR and 3β-HSD in bubaline CL during different stages of estrous cycle applying qPCR, Western blot, ELISA and immunohistochemistry.

2. Materials and methods

2.1. Collection of CL during the estrous cycle

Entire reproductive tracts from buffalo cows were collected at a local slaughterhouse within 10–20 min after slaughter and were transported on ice to the laboratory. The stage of the estrous cycle was defined by macroscopic observation of the ovaries (color, consistency, CL stage, number and size of follicles) and the uterus (color, consistency and mucus) as described previously (Sarkar et al., 2010). CL were assigned to the following stages; days 1–4, 5–10, 11–16 and >17 of estrous cycle. Luteal tissue was