CHAPTER I
INTRODUCTION

1.1 Background and Justifications

In domestic ruminants, ovarian follicular development occurs in waves of growth and regression of antral follicles (Adams, 1999; Webb et al., 2004). Best characterized in cattle, two or three successive waves of follicular growth occur in most estrous cycles (Townson et al., 2002). Each wave involves the recruitment of a cohort of follicles and the selection of a dominant follicle, which continues to grow and mature to the preovulatory stage while others in the wave undergo atresia. Previous ultrasonographic studies also confirm that there is a distinct wave-like pattern of follicular development in sheep and goats (Ginther and Kot, 1994; de Castro et al., 1999). In ruminants, as in other mammals, preovulatory follicular development begins with the recruitment and growth of primary follicles within the ovary (Armstrong and Webb, 1997). On a cellular level, successful development to the preovulartory stage requires both proliferation and differentiation of follicular granulosa and theca cell (Quirk et al., 2004). During the early preovulatory phase, concentrations of follicle-stimulating hormone (FSH) in peripheral plasma have been shown to be directly correlated with growth of small antral follicles (Jablonka-Shriff et al., 1994; Zeleznik, 2004). Studies in several species have demonstrated that follicular growth and development is mediated by FSH (Jablonka-Shriff et al., 1996; Thomas et al., 2002). Furthermore, administration of exogenous gonadotropic preparations containing FSH-like activity during this period of declining endogenous FSH can override follicular selection and induce growth of multiple preovulatory follicles (Ginther et al., 1986). Moreover, Jablonka-Shariff et al. (1996) demonstrated that the FSH model should prove useful for studying the mechanisms regulating follicular growth and atresia in ewes. Conversely, in goats, Lucci et al. (1999) and Silva et al. (2002) showed that atresia also occurs in preantral follicles, but to our knowledge there is no information available about what regulation of follicular growth and atresia. The model for gonadotropin induced follicular growth and atresia in goat is yet to be established. The knowledge of the model for induced follicular growth and atresia is of importance to understand follicular atresia in goat.

A complex regulatory system must exist to determine which follicles are selected. Although it is well established that the ovarian function is regulated primarily by the pituitary gonadotropins FSH, luteinizing hormone (LH), their receptors (FSHR and LHR), it is also evident that locally produced factors, such as steroid hormones, peptides, insulin-like growth factor (IGF) system (Fortune et al., 2001; Mihm and Bleach, 2003), and angiogenic factors (Redmer and Reynolds, 1996; Grazul-Bilska et al., 2007) have essential modulatory roles in follicular development (recruitment, selection and dominance) and ovulation (Berisha and Schams, 2005). Since the development and regression of follicles are associated with major structural and functional changes, it is important to classify follicles accurately as healthy or atretic at all stages of development (Rodgers and Irving-Rodgers, 2010). Maintenance of follicular health depends on the presence of angiogenic factors and a functional vasculature (Jiang et al., 2003). Changes of vascularization and expression of some regulators, including angiogenic factors, are associated with follicular growth and/or atresia. Several angiogenic factors, including endothelial nitric oxide synthase (eNOS) and vascular endothelial growth factor (VEGF), are expressed in ovarian follicles (Grazul-Bilska et al., 2006). However, all these studies have been conducted in sheep and cattle (*Bos taurus* breeds of European origin), there are little information in *Bos indicus* regarding vascularity, cell proliferation, and eNOS expression of growing follicle during the follicular wave.

Reproductive performance of dairy cows is influenced by ability to resume estrous cycles early postpartum (Stevenson et al., 2008) and, after artificial insemination (AI), to maintain pregnancy (Santos et al., 2004). Slow recovery of ovarian activity and resumption of ovarian activity (Darwash et al., 1999) during the postpartum period are major impediments to insemination of cows immediately after the end of the voluntary waiting period (Thatcher et al., 2003). Hormonal applications as a method to control the estrous cycle have been widely used by dairy producers. Most early systems still required at least some period of detected estrus, however, because fixed-time artificial insemination (TAI) failed to yield acceptable pregnancy rates. Sterry et al. (2006) suggested the need for protocols that synchronized not only estrus, but ovulation of follicle protocols. Consequently, the development of hormonal synchronization protocols that allow for TAI have provided a management tool for initiating first postpartum AI and there by precisely controlling the voluntary waiting period in lactating dairy cows (Navanukraw et al., 2004). A hormonal protocol for synchronizing ovulation in lactating dairy cows (Ovsynch) uses injections of gonadotropin-releasing hormone (GnRH) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and is an effective method for hormonally programming cows to receive TAI (Pursley et al., 1995). However, the Ovsynch protocol has a low pregnancy rate per artificial insemination (PR/AI) to TAI (37.3%) in lactating dairy cows (Navanukraw et al., 2004; Stevenson et al., 2008). This is because postpartum lactating cows have low progesterone (P4) concentrations as a result of low conception rate and high early embryo mortality rate (Santos et al., 2001). Therefore, P4 is required for the survival of the embryo/fetus of cows, and several studies have investigated the effect of treatment with GnRH or human chorionic gonadotropin (hCG) after AI to improve fertility by inducing ovulation and forming an accessory corpus luteum (CL) (Hanlon et al., 2005; Howard et al., 2006).

Therefore, the research study was designed to investigate the controlling ovarian follicular and CL functions in ruminants.

1.2 Scientific Hypothesis

1.2.1 Differences between the healthy and atretic follicles and size of antral follicles could be partly explained by differences in vascularization, mitotic activity, and expression of eNOS.

1.2.2 The model for induction of follicular growth and atresia using FSH treatment and withdrawal should prove useful for studying the effects of FSH administration on follicular growth, mitotic activity, oocyte quality, and embryo development.

1.2.3 hCG administration within a TAI protocal or hCG or GnRH administration on day 5 after TAI can be effective protocols to increase accessory CL and P4 concentration.

1.3 Objectives

1.3.1 To characterize vascularization, expression of eNOS proteins, and proliferating cell nuclear antigen (PCNA) labeling index and evaluate the relationships among these variables in antral follicles of the first follicular wave.

1.3.2 To determine the effects of FSH treatment and withdrawal on follicular growth, mitotic activity, oocyte quality, embryo development, and gene expression.

1.3.3 To evaluate the effects of GnRH replacement with hCG in TAI protocol on ovulation and conception rates, accessory CL, and subsequent plasma P4 concentrations.

1.4 Scope and Limitations

1.4.1 To determine the relationships among vasculature, cell proliferation, and expression of eNOS of growing follicles in beef cattle, bovine ovaries were obtained on day 6 of the estrous cycle after a synchronized estrus with $PGF_{2\alpha}$ analogue. Follicles were classified by size (small, medium, and large) and by morphology as healthy and atretic follicles. Ovaries were fixed, paraffin-embedded and used for immunofluorescence detection of Factor VIII. Immunostaining of eNOS and PCNA were performed using specific monoclonal antibodies. Vasculature and positive staining of eNOS and PCNA were quantitatively evaluated using the image analysis.

1.4.2 To determine the effects of FSH treatment and withdrawal on follicular growth, mitotic activity, oocyte quality, and development of embryo in goat model, forty female Thai-native goats were allocated randomly to five treatment groups as follows: control day 17, control day 20, a day FSH-P + two days withdrawal (1 d FSH + 2 d W), two days FSH-P + a day withdrawal (2 d FSH + 1 d W), and three days FSH-P (3 d FSH). The number and surface diameter of all visible follicles were recorded and classified by diameter into large (≥7 mm), medium (4-6 mm) or small (≤ 3 mm). The oocytes from visible follicles were aspirated using a 21G needle and evaluated based on morphology and categorized as healthy and nonhealthy. After aspiration of follicles, the remaining ovarian tissues were fixed used for histochemical detection of PCNA. Healthy oocyte only was used for in vitro maturation (IVM), in vitro fertilization (IVF), and in vitro culture (IVC). Total RNA was extracted from morula and blastocyst embryos. The quantification of β-actin, B cell lymphoma-2

(Bcl-2), and connexins43 (Cx43) transcripts was carried out by real-time quantitative RT-PCR.

1.4.3 To evaluate the effects of GnRH replacement with hCG in TAI protocol on ovulation rate, accessory CL, and subsequent plasma P4 concentrations, thirty-six nonpregnant Holstein-Friesian cows were randomly allocated to three groups as follows: Group 1 (modified Ovsynch, GPH), Group 2 (Ovsynch+hCG), and Group 3 (Ovsynch+GnRH). Blood samples were collected according to the hormonal treatments from the coccygeal vein into heparinized vacutainers. Concentrations of P4 were determined by competitive ELISA. The ovaries of cows were examined by transrectal ultrasonography on day -10, day -8, day -3, day -1, day 0, 20 to 48 h after TAI, day 5, day 8, and day 12 and all ovarian structures were mapped to monitor changes in CL and follicles in response to treatment.

1.5 Anticipated outcomes

Gain knowledge on follicular development and CL function related to the relationships among vascularization, mitotic activity, and expression of eNOS in bovine antral follicles of the first follicular wave. Better understands the roles of FSH administration on follicular growth, oocyte quality and embryo development. Lastly, enhancing the efficiency of TAI protocol on ovulation and conception rates, CL development, and subsequent P4 concentrations may lead to controlling ovarian follicular and CL functions in ruminants.