CHAPTER II
LITERATURE REVIEW

2.1 Follicular growth and development

Folliculogenesis may be defined as formation of Graafian (mature, preovulatory) follicles from a pool of primordial (non-growing) follicles. The pool of primordial follicles remains stable from birth until about the fourth yr of life, and then subsequently declines (Spicer and Echternkamp, 1986). Primordial follicles represent the source from which follicles will be recruited for growth throughout life, with paired ovaries of an individual containing around 150,000 of these follicles at birth (Webb et al., 1999).

2.1.1 Follicular waves

A follicle wave is characterized by the synchronous growth of a cohort of follicles, one which continues growing while the others regress at variable intervals. There are a number of terms that are particularly relevant when describing follicle waves based on ultrasonographic observations (Evans, 2003). Studies using ultrasonic imaging to monitor follicle populations in different size categories or to monitor individually identified follicles (Adams, 1999) have convincingly documented that follicular growth in cattle occurs in a wave-like fashion and that the majority of estrous cycles in cattle are comprised of two or three such waves (Fortune et al., 2001; Ginther et al., 2004; Adams et al., 2008). Although lactating Holstein dairy cows tend to have two follicle waves per cycle (Townson et al., 2002), and beef and dairy heifers tend to have either two or three waves per cycle (Savio et al., 1988; Ginther et al., 1989). Follicular wave emergence in cattle is characterized by the sudden (within 2 to 3 days) growth of 8 to 41 small follicles that are initially detected by ultrasonography at a diameter of 3 to 4 mm (Adams et al., 2008). In both two- and three-wave estrous cycles, emergence of the first follicular wave occurs consistently on the day of ovulation (day 0, Sirois and Fortune, 1990). Emergence of the second wave occurs on day 9 or 10 in two-wave cycles, and on day 8 or 9 in three-wave cycles. In three-wave cycles, a third wave emerges on day 15 or 16 (Adams et al., 2008). Under the influence of P4 (e.g., di-estrus), dominant follicle of successive

waves undergo atresia. The dominant follicle present at the onset of luteolysis becomes the ovulatory follicle, and emergence of the next wave is delayed until the day of the ensuing ovulation. The CL begins to regress earlier in two-wave cycles (day 16) than in three-wave cycles (day 19) resulting in a correspondingly shorter estrous cycle (19 to 20 days versus 22 to 23 days; Figure 2.1).

Emergence of a follicular wave and selection of the dominant follicles are temporally associated with a rise and fall in circulating concentrations of FSH (Figure 2.1; Adams et al., 2008). Emergence of a follicular wave is preceded by a surge in plasma FSH concentrations in both spontaneous waves and induced waves (Adams et al., 1993). Follicular products, especially those from the dominant follicle, are responsible for suppressing FSH release and, therefore, the emergence of the next follicular wave (Figure 2.1). At the end of the period of dominance (i.e., at ovulation, or the mid-static phase of an anovulatory dominant follicle), circulating concentrations of FSH begin to rise; they increase 1.5 to 2-fold over the next 2 days, and peak approximately 12 to 24 h before emergence of the wave (when the future dominant follicle is 4 to 5 mm in diameter; Bergfelt et al., 1994). Receptors for FSH are present only on granulosa cells, whereas LH receptors are located on both granulosa and theca cells in the wall of antral follicles. The dominant follicle acquires more LH receptors on its granulosa cells than its subordinates and is therefore able to shift its gonadotropin dependence to LH during the FSH nadir, and continue to grow while the subordinates regress (Evan, 2003; Adams et al., 2008).

Studies using repeated ultrasonography suggest that there are between two and six waves of follicle development during estrous cycles in goats with three or four waves being the most prevalent (Menchaca and Rubianes, 2001). On any given day of the estrous cycle there are 5 to 10 follicles $\geq$ 3 mm in diameter in the ovaries and follicles ovulate at between 6 and 9 mm in diameter (Ginther and Kot 1994). A follicle wave involves the emergence of a group of small antral follicles from which commonly 1 or 2 follicles are selected to grow to more than 5 mm in diameter (Menchaca et al., 2002). According to different authors the number of follicular waves ranges between two and five waves per cycle, but the predominant pattern for goats that developed an interovulatory cycle of normal length (19 to 22 days) is of four waves (de Castro et al., 1999; Menchaca and Rubianes, 2002). The emergence of

waves 1, 2, 3 and 4 (the ovulatory wave) occurs on days 0, 5 to 6, 10 to 11 and around day 15 postovulation, respectively. In goats that developed three follicular waves, wave 2 emerges 1 to 2 days later and the ovulatory wave emerges 1 to 2 days earlier (Rubianes and Menchaca, 2003).

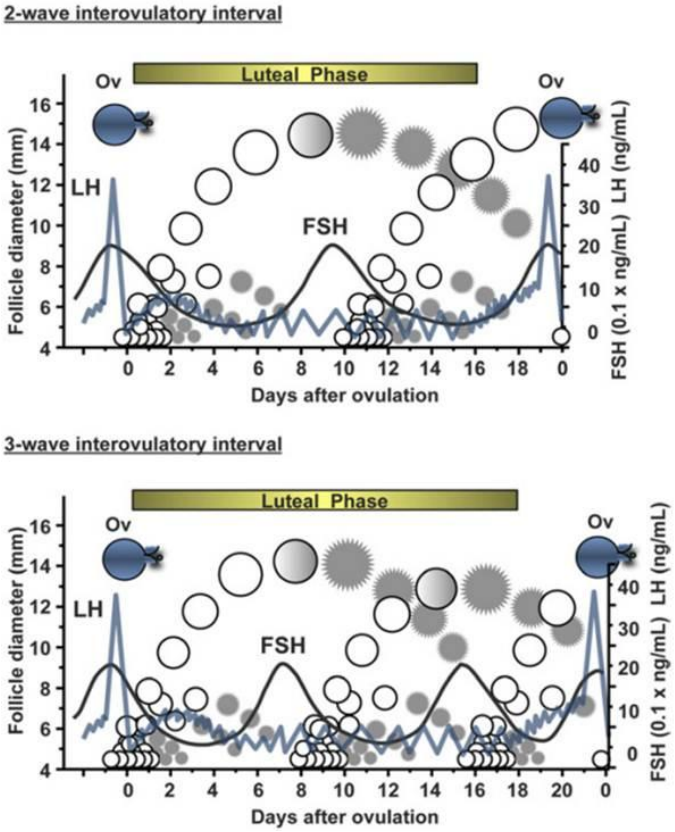

Figure 2.1 Dynamics of ovarian follicular development and gonadotropin secretion during two- and three-wave estrous cycles in cattle. Dominant follicle (DF) and subordinate follicles (SF) are indicated as open (viable) or shaded (atretic) circles. A surge in circulating FSH concentrations (thick line) precedes emergence of each wave. A surge in circulating LH concentrations (thin line) precedes ovulation. The LH surge is preceded and succeeded by a period of high-LH pulse frequency as a result of low-circulating P4 concentrations (i.e., period of luteolysis and luteogenesis, respectively).

Source: Adams et al. (2008)

2.1.2 Stages of follicular development

Based on studies in primates and rodents, the following terms were proposed by Goodman and Hodgen (1983) to describe folliculogenesis.

1) Recruitment

Recruitment is a process whereby a cohort of follicles begins to mature in a milieu of sufficient pituitary gonadotropic stimulation to permit progress toward ovulation (Sirois and Fortune, 1990; Lucy et al., 1992; Fortune, 1994). It is clear that follicular waves are initiated by a rise in circulating FSH. In cattle and other species, follicular waves are preceded or accompanied by a small rise in FSH, and if this rise is inhibited or delayed, the follicular wave is inhibited or delayed (Driancourt, 2001). Exogenous FSH recruits greater than normal numbers of follicles to grow larger than 5 mm in diameter and, in superovulation protocols, increases the number of follicles available for ovulation (Adams et al., 1993). These effects of FSH are, at least to some extent, dose dependent because treatment with small amounts of exogenous FSH can produce codominant follicles (Rivera and Fortune, 2001).

The term recruitment has been given to the growth of follicles beyond the stage at which most follicles undergo atresia (Fortune, 1994). Recruitment is not a random or isolated phenomenon; on the contrary, follicles seem to be recruited as groups or cohorts, suggesting that they have received a signal that allows them to continue growth and development rather than regress (Webb et al., 1999). The signal that stimulates recruitment appears to be a slight elevation in plasma FSH. There are various types of evidence for that hypothesis (Driancourt, 2001). Follicular recruitment is temporally correlated with slight increases in circulating FSH. Rats exhibit a secondary surge of FSH on the day of estrus, just before the next cohort of ovulatory follicles is recruited (Sirois and Fortune, 1988).

In cattle, not only does a secondary surge of FSH on the day of ovulation precede the first follicular wave of the cycle (Adams et al., 1993), but also slight elevations in FSH have been shown to precede the second and third follicular waves of the cycle (Webb et al., 1999). Not only are there temporal correlations between elevations in plasma FSH and the recruitment of follicles, but also perturbations of those increases in FSH lead to concomitant changes in the patterns and/or number of recruited follicles (Fortune, 1994). Since the emergence of waves

or cohorts of follicles seems closely tied to slight elevations in plasma FSH, one could hypothesize that species in which ovulatory-size follicles do not develop during the luteal phase (Zeleznik and Kubik, 1986) have higher luteal-phase levels of feedback regulators like estradiol (E2) and inhibin. Alternatively, hypothalamic/pituitary sensitivity to such feedback may be greater in species in which the development of dominant follicle is suppressed during the luteal phase (Fortune, 1994; Webb et al., 1999).

2) Selection and deviation

Selection is the process by which a single follicle is chosen and avoids atresia with the potential competence to achieve ovulation (Sirois and Fortune, 1990; Fortune, 1994). The use of serial ultrasound imaging has elucidated the patterns of development of large antral follicles in several domestic species (Ginther et al., 2001). These studies have revealed that large ovulatory-sized follicles develop as part of a wave or cohort of antral follicles recruited synchronously to grow beyond the size when follicles normally become atretic and then through their selection as a dominant follicle, capable of ovulation. As follicular development proceeds during the wave, the increasing levels of E2 (and perhaps inhibin) in the circulation reduce FSH to basal levels through negative feedback (Beg et al., 2002). Previous study indicated that combined effects of elevated inhibin (a product of the dominant follicle) and E2 account for the decline in FSH during the estrous cycle (Bleach et al., 2001).

Time of deviation is defined as the beginning of the greatest difference in growth rates (diameter changes between adjacent examinations) between the two largest follicles at or before the examination when the second-largest follicle reached its maximum diameter (Ginther et al., 2000). The definition was developed to allow objective assignment of the time of deviation in individual waves (Rhodes et al., 1995a). Either deviation of follicles is a major event in the selection process, or the terms deviation and selection are synonymous (Bergfeld et al., 1996). Follicle diameter deviation during follicular waves in cattle begins with a reduction in growth rates of developing subordinate follicle, in contrast to the maintenance of a constant growth rate by a developing dominant follicle (Beg et al., 2002). In retrospect, the following two faulty assumptions have hampered progress in studies of follicle selection: (1) the follicles of the cohort are equivalent in diameter at the time of

deviation, and (2) between days 0 and 4 the dominant follicle and largest subordinate follicle diverge gradually in diameter (Ginther, 2000). Deviation in individual waves is defined as beginning at the examination before the first examination with an apparent change in the differences in diameter between the two largest follicles (Ginther et al., 2001). Several reports, the mean diameters of the two largest follicles at the beginning of deviation were 8.5 and 7.7 mm in heifers (Ginther et al., 2003).

It has been proposed (Ginther et al., 2000) that a close, two-way functional coupling between FSH and the follicles is an integral component of the deviation mechanism. During the common growth phase that precedes deviation, FSH concentrations decline, and the FSH/follicle two-way coupling involves multiple follicles (Gibbons et al., 1999). However, the coupling involves only the future dominant follicle by the beginning of deviation (Ginther et al., 2000). The change from multiple- to single-follicle coupling is attributed to the development of greater responsiveness by the future dominant follicle than by the future subordinate follicle to the low FSH concentrations at the beginning of deviation (Beg et al., 2002). The wave-stimulating FSH surge reaches peak concentrations, on average, when the largest follicle is about 5 mm in heifers (Ginther et al., 2003). The declining concentration of FSH fails to support the continued growth of the subordinate follicle, but is required and sufficient for the dominant follicle to continue to grow. However, LH also begins to play a role in the continued growth of the dominant follicle at the end of cycle (Ginther et al., 2001; Beg et al., 2002).

3) Dominance

Dominance is the means by which the selected follicle dominates through inhibition of recruitment of a new cohort of follicles ovulation (Ginther et al., 1989; Fortune, 1994). In 75% of the waves, the first follicle to emerge during the wave becomes the dominant follicle. Thus, the dominant follicle often has a slight size advantage over the other follicles in the wave, and this size advantage may allow it to reach the point of deviation earlier than the other follicles of the wave (Ginther and Kot, 1994). The decline in FSH plays a role in stimulating the follicle to synthesize intrafollicular factors (e.g., E2, growth factor, and inhibin). These intrafollicular factors and perhaps others may account for the responsiveness of the largest follicle to the low concentrations of FSH (Mihm and Bleach, 2003).

The selected dominant follicle becomes increasingly responsive to LH (Ginther et al., 2000) and continues growth in the face of decreasing FSH concentrations. Irrespective of the stage of the estrous cycle during which follicles develop, the switch from FSH (Adams et al., 1993) to LH dependency (Kulick et al., 2001) is propagated through the presence of LHR on the granulosa cells (Xu et al., 1995). LHR are localised to the theca and granulosa cells of healthy follicles, at different stages of follicle development (Camp et al., 1991). As the follicle grows, the theca cell LHR increases and LHR is acquired by the granulosa cells of the follicle undergoing selection to become the dominant follicle (Bao et al., 1997; Braw-Tal and Roth, 2005). Moreover, evidence suggests transient increases in circulating LH concentrations that occur at or around the time of follicle selection (Ginther et al., 2003), allows the dominant follicle to continue E2 production and grow in a lesser FSH environment (Ireland and Roche, 1983). During the early luteal phase lesser amplitude and greater frequency LH pulses occur, in the mid-luteal period LH pulses are of greater amplitude and lesser frequency both of which are of insufficient amplitude and frequency for final maturation and subsequent ovulation of the dominant follicle (Rahe et al., 1980). Thus, the dominant follicle produced during the luteal phase of the estrous cycle undergoes atresia, E2 and inhibin production decreases, and removes this negative feedback block to the hypothalamus/pituitary, FSH secretion can increase and a new follicle wave emerges (Forde et al., 2011).

2.2 Angiogenesis and angiogenic factors in the ovary

Angiogenesis is a key aspect of normal cyclical ovarian function (Ferrara, 2004). In adult tissues, capillary growth (angiogenesis) occurs normally during tissue repair, such as in the healing of wounds and fractures. The female reproductive organs exhibit marked, periodic growth and regression, accompanied by equally striking changes in their rates of blood flow (Redmer and Reynolds, 1996). Ovarian follicles and corpora lutea have been shown to contain and produce angiogenic factors (Jiang et al., 2003).

2.2.1 Angiogenesis

Angiogenesis refers to the formation of new blood vessels, or neovascularization, and is essential for normal tissue growth and development

(Folkman and Klagsbrun, 1987; Klagsbrun and D'Amore, 1991). The angiogenic process begins with capillary proliferation and culminates in formation of a new micro-circulatory bed composed of arterioles, capillaries and venules (Redmer and Reynolds, 1996). The initial component of angiogenesis, capillary proliferation, consists of at least three processes: (1) fragmentation of the basement membrane of the existing vessel, (2) migration of endothelial cells (the primary cell type constituting capillaries) from the existing vessel toward the angiogenic stimulus and (3) proliferation of endothelial cells (Folkman and Klagsbrun, 1987; Klagsbrun and D'Amore, 1991). Neovascularization is completed by formation of capillary lumina and differentiation of the newly formed capillaries into arterioles and venules (Redmer and Reynolds, 1996). Pervious study suggested that angiogenesis and capillary degeneration are both evident during ovarian follicle growth (Robinson et al., 2009). The process of selection of a dominant follicle in monovular species has been also associated with angiogenesis, as there is evidence that selected follicles possess a more elaborate microvascular network than other follicles (Zeleznik et al., 1981). In bovine, Jiang et al. (2003) reported that active angiogenesis increases during ovarian follicular growth; it is first evident in the follicular apical region of the inner theca layer; and capillary degeneration, a consequence of apoptosis of outer theca interna capillaries, occurs unevenly and first appears in the outer vascular layers of the follicles and is likely to be associated with the process of atresia.

2.2.2 Ovarian angiogenic factors

In adults, it is largely limited to pathological situations such as tumour growth and wound healing (Robinson et al., 2009). However, the ovary undergoes continual cyclical changes and so requires continual angiogenesis (Reynolds and Redmer 1999; Fraser and Lunn, 2001). An established vasculature consists of an inner lining of endothelial cells, associated mural cells such as pericytes and vascular smooth muscle cells (vSMC; Redmer et al., 2001). These vessels remain quiescent until there is an angiogenic stimulus such as hypoxia or wounding, which then upregulates proangiogenic factors, such as VEGF (Gerhardt and Betsholtz, 2003). After this stimulus, the existing vessels start to destabilise through the disruption of endothelial and mural cellular contacts. At the same time, numerous proteases are activated and the extra-cellular matrix (ECM) is degraded (Robinson et al., 2009).

Endothelial cells, then, migrate towards the angiogenic stimuli and proliferate under the influence of pro-angiogenic factors (Bruno et al., 2009). Once connected and aligned, the endothelial cells form a lumen and the newly formed vessel is then stabilised by the recruitment of pericytes (Gerhardt and Betsholtz, 2003). Thus, angiogenesis is a highly regulated process involving a balance between pro- and anti-angiogenic factors (Robinson et al., 2009).

The principal pro-angiogenic factors include fibroblast growth factor 2 (FGF2), VEGF, plaletet-derived growth factor (PDGF) family, and angiopoietin (ANGPT) system (Redmer and Reynolds, 1996; Maisonpierre et al., 1997; Ferrara et al., 2003; Presta et al., 2005). Blockade of VEGF/PDGF signalling has highlighted the critical roles that these factors play in controlling not only angiogenesis but also ovarian function (Robinson et al., 2009). For example, inhibition of VEGF signalling by various methods disrupted ovulation, completely blocked the vascularisation of the subsequent CL and prevented the post-ovulatory rise in P4 (Fraser and Lunn, 2001). Conversely, much less is generally known about the anti-angiogenic factors. They generally associate with the ECM and suppress angiogenesis by inhibiting endothelial migration or stimulating apoptosis in endothelial cells (Armstrong and Bornstein, 2003; Wahl et al., 2005).

2.2.3 Development of vasculature in follicle

In the ovary, the vascular supply is formed on a cyclic basis. The vasculature is not distributed equally among the population of follicles in the adult ovary (Hanahan, 1997).

1) Preantral follicle

Non-growing primordial follicles and slow-growing preantral follicles do not have a vascular supply of their own, but instead rely on vessels in the surrounding stroma (Stouffer et al., 2001). The smaller, primordial follicles do not possess an independent capillary network and are dependent on their proximity to the stromal vessels (Geva and Jaffe, 2003). While primordial and primary follicles receive nutrients and oxygen by passive diffusion from stromal blood vessels, follicular growth is associated with the development of an individual capillary network and continued angiogenesis to nourish the rapidly expanding follicle (Fraser, 2006). However, the formation of an individual capillary network around each

follicle is required for follicles to grow beyond these stages (Robinson et al., 2009). This network is initially thin, roughly structured and has a single layer (Jiang et al., 2003).

2) Antral follicle

As an antrum develops in the follicle, the theca layer acquires a vascular sheath consisting of two capillary networks located in the theca interna and externa, respectively (Martelli et al., 2006). Arterioles and venules from the theca externa branch into the single-layer capillary plexus of the theca interna, but neither the basement membrane nor the granulosa layer of the follicle are traversed by capillaries (Jiang et al., 2003). Hence, the granulosa layer receives nutrients and hormones by diffusion from the capillary network located in the peripheral theca layer (Bruno et al., 2009). The use of vascular corrosion casts have suggested that the nature of angiogenesis in the theca layer changes during follicular development (Grazul-Bilskaet al., 2006; 2007). Initially, there is budding which is followed by predominantly sprouting during early antral follicle stages and then capillary elongation in the later stages (Jiang et al., 2003).

3) Preovulatory follicle

The mature vascular sheath, found in the theca layer of preovulatory follicles, consists of two concentric networks of vessels in the theca interna and externa layers (Wulff et al., 2002). Neovascularisation is crucial for antral follicle growth, dominance and preovulatory development since numerous studies have shown that anti-angiogenic compounds (e.g. VEGF trap) reduced the thecal vascularity and consequently severely comprised follicular development (Wulff et al., 2002). This hypothesis is supported by the observation that, during dominant follicle selection, those follicles that were estrogen-active (EA) had vastly greater vasculature and VEGF concentrations than their EA counterparts (Fraser and Duncan, 2009). This was despite the EA follicle being larger in diameter (Grazul-Bilska et al., 2007). There is also strong evidence that, shortly after selection, there is a rapid degeneration of the thecal vasculature, once atresia has been initiated in the subordinate follicle (Jiang et al., 2003).

Gonadotropin dependent follicular growth is characterized by the expansion of the antrum through accumulation of fluid in the follicular cavity, cell

division in the theca and granulosa layers, and expansion of the inner capillary plexus (Macchiarelli et al., 2006). The follicle selected for maturation and ovulation possesses a denser microvascular network than others in the same cohort (Zeleznik et al., 1981). Differential neovascularization is likely important in selection of the dominant follicle. Increased blood flow results in an increased supply of gonadotropins as compared to less vascular secondary follicles in the cohort (Bruno et al., 2009). High levels of E2 produced by granulosa cells in the dominant follicle stimulate the LH surge which triggers ovulation. The collapse of the basement membrane that accompanies ovulation allows penetration of the luteinized granulosa layer by invading microvessels originating from the theca layer (Miyamoto et al., 2009). The vascular sheath that develops around each follicle is confined to the thecal layer by the presence of the membrane until the breakdown of the basement membrane at ovulation (Redmer and Reynolds, 1996). It is confined to the theca layer with the granulosa layer remaining avascular throughout folliculogenesis (Tamanini and De Ambrogi, 2004). Because all capillaries remain outside the basement membrane of the follicle, the granulosa layer with its fluid-filled antrum and the cumulus cell-oocyte complex remain avascular until after ovulation (Stouffer et al., 2001).

2.3 Vascular function in the ovary

2.3.1 Vascular endothelial growth factor

VEGF is a basic, heparin-binding, homodimeric glycoprotein of 45 kd, which binds specifically to receptors on endothelial cells (Geva and Jaffe, 2003). Analysis of the VEGF transcripts by RT-PCR shows that follicle and CL tissue express predominantly the smallest isoforms (Berisha and Schams, 2005). VEGF mRNA expression in both follicle compartments, theca interna and granulosa cell, VEGF protein concentration in total follicle tissue and VEGF protein concentration in follicular fluid (FF) increased significantly with developmental stage of follicle growth (Berisha et al., 2006). The expression of VEGF receptor (VEGFR1 and VEGFR2) mRNA was without any regulatory change during final follicle growth. As shown by immunohistochemistry, VEGF protein was clearly localized in theca interna and granulosa cell of preovulatory follicles (Doyle et al., 2010).

Development of the preovulartory follicle relies on the support of a vascular network (Kaczmarek et al., 2005) regulated by the VEGF system. Follicle cell mitosis, angiogenesis and permeability are mediated by the VEGFR (Ferrara, 2004). During the follicular growth and development, the presence of VEGF could determine the fate of destabilized blood vessel (Fraser and Duncan, 2009). When VEGF is high, a destabilization of blood vessels results in the formation of a new vascular network, whereas a lack of VEGF support results in a regression of blood vessels (Hanahan, 1997). Numerous studies have shown that VEGF and its receptors, VEGFR1 and VEGFR2 are expressed in granulosa cells, and dynamic changes in their expression have been reported throughout follicle development in primates (Fraser and Wulff, 2001) and cattle (Greenaway et al., 2004), with a consistent increase in both VEGF production and expression of VEGFR in dominant follicle. Further, previous study demonstrated that intra-ovarian VEGF injection can stimulate development of pre-antral and antral follicles up to the preovulatory stage (Shimizu et al., 2007). The actions of VEGF are undoubtedly associated with the induction of an extensive vascular system in follicular walls, studies using ruminant cells suggest that VEGF may also modulate granulosa cell function directly by promoting cell survival, proliferation and/or migration (Greenaway et al., 2004). Recent study suggested that, in general, cell responses to VEGF are mediated primarily through binding of VEGF to VEGFR2; whereas VEGFR1 is believed to act as a decoy receptor that regulates VEGF binding to VEGFR2 (Doyle et al., 2010).

2.3.2 Nitric oxide and endothelial nitric oxide synthase

Nitric oxide (NO) is a diatomic free-radical gas involved in a number of physiologic processes (Moncada et al., 1991) and is synthesized from L-arginine by a family of NO synthases (NOS; Wu and Morris, 1998). There are three isoforms including the constitutively expressed eNOS, neuronal NOS (nNOS) and inducible isoform NOS (iNOS; Daff, 2010). eNOS is mostly targeted to caveolae in the plasma membrane, where its activity is highest compared to golgi, cytoskeletal and actin associated forms (Haque et al., 2007). Regulation of endothelial NO synthesis by multi-site eNOS phosphorylation occurs in response to a wide variety of physiological action (Blair et al., 1999). This regulation involves numerous kinases and phosphatases (Chatterjee and Catravas, 2008).

It is well known that both folliculogenesis and ovulation are regulated by a variety of factors, such as cytokines, growth factors, and locally produced substances, among which NO seems to play an important role (Tamanini et al., 2003). The above findings provide evidence that intra-ovarian eNOS regulate the ovulatory process simultaneously. Because theca cells, granulosa–luteal cells and cells of the CL are involved in steroidogenesis, it is feasible that NO also regulates steroid synthesis (Rosselli et al., 1998). In addition, a growth-promoting effect of NO is supported by the observation (Hattori et al., 1996) that NO increases endothelial growth factor (EGF) receptors in granulosa cells and interleukin-1β stimulated NO production is effective in promoting muscle cell growth in presence of basic fibroblast growth factor (bFGF; Tamanini et al., 2003). A further mechanism through which NO may be involved in the control of follicular development is its effects on apoptosis, the programmed cell death by which the majority of ovarian follicles are lost during postnatal life (Kiess and Gallaher, 1998). High NO levels have been shown to reduce apoptosis in bovine (Basini et al., 1998) granulosa cells. NO may also influence follicle development by mediating the effects of gonadotropins on the blood-follicle barrier, thus influencing its permeability to different substances (Powers et al., 1995). The overall results on the effects of NO on folliculogenesis suggest that locally produced NO contributes to modulate follicle development and possibly prevents apoptosis (Tamanini et al., 2003). Moreover, results from recent study suggested that in bovine ovaries with atretic follicle number, a defective eNOS/NO system is related to a reduced follicle vasculature and may affect oocyte quality, thus inducing a premature decline of fertility (Tessaro et al., 2011).

Reactive oxygen and nitrogen species (ROS and RNS, respectively) include superoxide anion radicals (O_2^-), hydrogen peroxide (H_2O_2), peroxynitrite (NO_3^-), nitrous oxide (N_2O), and others (Devine et al., 2012). ROS are formed through leakage of electrons from the inner mitochondrial membrane during oxidative phosphorylation and ATP generation. In steroidogenic tissues such as the ovary, steroidogenic cytochrome P450 enzymes are also sources of ROS (Hanukoglu, 2006). Oxidative and nitrosative damage occur when ROS and RNS react with cellular lipids, proteins, and nucleic acids (Roberts et al., 2009). Oxidative stress occurs when increased ROS levels disrupt cellular redox circuits, resulting in disturbances of

redox-regulated cellular processes and/or oxidatively damage cellular macromolecules (Jones, 2008). Antral follicles appear to be highly sensitive to oxidative stress-induced apoptosis of granulosa cells (Devine et al., 2012). Direct measurements of rising ROS levels prior to any increases in markers of apoptosis further support the contention that ROS are involved in the initiation of apoptosis by various toxicants and ionizing radiation in antral follicles and granulosa cells (Hoang et al., 2009). Lipid peroxidation (LPO) is a process that involves interaction of ROS with the cell membrane lipids generating the lipid peroxy radical, which can propagate further oxidation (Bedaiwy et al., 2002).

2.4 Measurements of follicular vasculature

Measurements of vascularity quantitative have been available using physiological image of color Doppler ultrasonography (Acosta, 2007) or immunohistochemistry (Grazul-Bilska et al., 2006; 2007).

2.4.1 Physiological image of color Doppler ultrasonography

Measurement of ovarian blood flow can be achieved non-invasively by color and pulsed Doppler ultrasonography in species with sufficiently large and accessible ovaries such as horses and cattle (Acosta et al., 1983). This demonstrates increased flow to the ovary containing the dominant follicle. In addition, there is increased peak flow velocity with increasing follicular size and high vascularity and flow velocity of the dominant follicle before ovulation (Acosta et al., 2003).

2.4.2 Immunohistochemistry

The most widely employed approach to study changes in angiogenesis during follicular development is to use ovarian sections in which endothelial cells are stained with a specific marker (Fraser, 2006). The most commonly used marker is platelet endothelial cell adhesion molecule, a membrane protein that mediates cell-cell adhesion and is reliably detected in endothelial cells in the follicles of, e.g., the macaque (Christenson and Stouffer, 1996), rat (Zimmermann et al., 2003), marmoset (Taylor et al., 2004), and mouse (Nakhuda et al., 2005). Unfortunately, platelet endothelial cell adhesion molecule antibodies seem to cross-react less in other species (Fraser, 2006). Alternatively, blood vessels may be localised in all species by attachment of carbohydrate lectins that have been biotinylated or fluorescein-labelled

as demonstrated e.g., for bovine (Augustin et al., 1995), sheep (Redmer et al., 2001), and buffalo (Feranil et al., 2004) ovaries. Where neither of these markers are detectable with available antibodies, antibodies to Factor VIII/von Willebrand factor may be used successfully to localise the follicular endothelium, e.g. in the cow (Augustin et al., 1995), pig (Wandji et al., 2000), mare (Watson and Al-Zi'abi, 2002), and sheep (Seekallu et al., 2010). A recent study on the microvascular network of bovine ovarian follicles indicated that Factor VIII (a specific marker for endothelial cell) positive areas varied from region to region in the same follicles and between follicles according to health or atresia (Grazul-Bilska et al., 2007). New opportunities will arise from advances in technology of high resolution imaging systems for research on ruminants, together with the use of contrast agents to enable the imaging of the ovarian vasculature more effectively on a wider scale (Fraser, 2006). Changes in endothelial cell area can then be quantified using image analysis (Grazul-Bilska et al., 2006; 2007). In cattle, angiogenesis was observed mainly in the apical part of the inner capillary layer of medium follicles and the middle or basal part of the capillary layer of healthy dominant follicles. In atretic follicles large avascular areas were observed in the inner thecal layer associated with apoptosis (Grazul-Bilska et al., 2007). Follicular atresia is associated with inadequate development and/or regression of the thecal vasculature in most species studied, it has been suggested that this is related to the longer time taken for the atretic process (Wulff et al., 2001).

2.5 Follicle–luteal transition: a period of intense angiogenesis

The transition from preovulatory follicle to CL is a dynamic process involving a series of biochemical and morphological changes following the LH surge that includes angiogenesis (Reynolds and Redmer, 1999). These processes are under the influence of numerous growth factors and the temporal regulation of the key factors during this period is shown in Figure 2.2 (Robinson et al., 2009).

2.5.1 Preovulatory follicle

In the preovulatory follicle, there is likely to be a shift away from vascular expansion to vessel maturation and this notion is supported by increases in the ANGPT1:ANGPT2 ratio at this time in cows (Hayashi et al., 2004). Moreover, the injection of ANGPT2 into preovulatory follicles of rhesus monkeys attenuated

follicular maturation and prevented ovulation presumably by disrupting pericyte–endothelial cell interactions (Xu and Stouffer, 2005). This highlighted the importance of the recruitment of pericytes and/or vSMC during the latter stages of follicular development. These cells, through their contractile properties, are likely to influence the follicular blood flow as well as stabilizing the vasculature (Robinson et al., 2009). The dominant localization of FGF2 in endothelial cells and pericytes at the early stage suggests FGF2 is an important factor for endothelial growth (Gospodarowicz et al., 1985). There are also indications for synergistic effects of angiogenic growth factors (Berisha and Schams, 2005).

2.5.2 Periovulatory period

LH might also have some direct effects on angiogenesis (Robinson et al., 2009). For example, follicular FGF2 mRNA and protein concentrations dramatically increase following the LH surge in cows (Robinson et al., 2007). At the same time, FGF2 also spatially translocates from thecal endothelial cells to the nucleolus of granulosa cells (Berisha et al., 2006). The ANPT system is thought to act in concert with growth or survival factors, such as VEGF (Figure 2.2). ANPT1 is necessary to maintain and stabilize blood vessels (Yancopoulos et al., 2000). On the other hand, ANPT2 which acts as natural antagonist for ANPT1, appears to cause endothelial cells to undergo active remodeling, thus it destabilizes vascular structure (Berisha and Schams, 2005). The ANGPT2:ANGPT1 ratio in follicles also increases after the LH surge in cows and this may induce the destabilisation of existing vessels (Shimizu et al., 2007). Whether LH can upregulate follicular VEGFA remains unresolved. In most in vitro studies, LH or hCG stimulated VEGFA production by granulosa cells in cows (Schams et al., 1994). Conversely, recent study suggested that VEGFA is in abundance in the periovulatory follicle in preparation for the intense angiogenesis that occurs after ovulation (Robinson et al., 2009).

During the periovulatory period, there is also hyperaemia and increased ovarian blood flow (Acosta et al., 2003). This is probably due to increase NO production (Mitsube et al., 2002) following the upregulation of eNOS in the thecal vasculature (Figure 2.2). However, this is more likely to be an E2 mediated upregulation rather than the effect of LH since E2 is a potent, rapid stimulator of eNOS in endothelial cells (Kim et al., 2008). VEGFA also plays a role since it

stimulates vascular permeability. Increases in blood flow would normally result in increased supply of oxygen to the tissue, however, hypoxia-induced factor 1α (HIF1A) is upregulated in the periovulatory follicle which suggests that the tissue is hypoxic (Duncan et al., 2008). Since hCG was a more potent stimulator of HIF1A than hypoxia itself in luteinising granulosa cells (van den Driesche et al., 2008), it is possible that the LH surge induces HIF1A expression directly (Figure 2.2). Thus, it is possible that any increases in VEGFA following the LH surge are mediated through the induction of HIF1A mRNA (Duncan et al., 2008). To date no studies have investigated HIF1A expression in ruminants during the follicular–luteal transition (Robinson et al., 2009).

Previous study suggested that protease activity and/or breakdown of the basement membrane is important for the initiation of luteal angiogenesis and is likely to have numerous effects: firstly, it removes the physical block to the vascularisation of the granulosa layer (Robinson et al., 2007). Secondly, it could fragment and spread ECM components as well as creating a more spacious environment. This would generate conditions that are more conducive to endothelial (and other cells) motility and migration (Berisha et al., 2006). Thirdly, any angiogenic factors sequestered in the basement membrane would be released (Ferrara et al., 2003). Finally, it could stimulate the differentiation of the follicular cells (e.g. granulosa cells exposed to fibronectin undergo luteinisation). The increased proteolytic activity would also stimulate the degradation of the ECM surrounding the existing vasculature, which is a pre-requisite for angiogenesis (Robinson et al., 2009).

2.5.3 Endothelial proliferation and formation of vascular networks

The majority of the proliferating cells in the collapsed follicle are of vascular origin (Reynolds and Redmer 1999). Both FGF2 and VEGFA are potent mitogens of endothelial cells and FGF2 and VEGFA stimulate bovine endothelial network formation in vitro (Robinson et al., 2008). FGF-2 expression is highest during very early luteal phase (days 1 to 2), and for FGFR during days 1 to 4 and decrease thereafter to a lower level (Figure 2.2; Schams et al., 1994). Interestingly, treatment with the FGF receptor signaling inhibitor almost completely blocked endothelial network formation, by decreasing both the number of endothelial clusters and their size (Woad et al., 2009). This occurred even in the presence of exogenous

VEGFA and indicates that FGF2 is critical for the formation of luteal endothelial networks (Kano et al., 2005). It also suggests that these factors must have complementary rather than redundant actions, since the remaining factors were unable to compensate for the loss of VEGF/FGF signalling (Woad et al., 2009). Combined with the dynamism of FGF2 during the follicular–luteal transition, this emphasises the importance of FGF2 in controlling and possibly initiating luteal angiogenesis in the cow (Robinson et al., 2007). The final step in angiogenesis is vessel stabilisation, which occurs by the secretion of PDGFB by endothelial cells, which acts in a paracrine manner to recruit pericytes (Gerhardt and Betsholtz, 2003). Previous studies reported that VEGFA and FGF2 may further influence PDGF signalling and pericyte function (Sleer and Taylor, 2007). For example, VEGFA promoted PDGFB while FGF2 increased PDGFRB production (Zhang et al., 2009). Recently, Woad et al. (2009) have shown that inhibition of PDGFR signalling reduced the formation of bovine luteal endothelial networks in vitro. During the estrous cycle in the cow, eNOS protein within the CL is maintained at high levels and expression of eNOS mRNA is increased during the early luteal phase (days 1 to 3; Miyamoto et al., 2009)

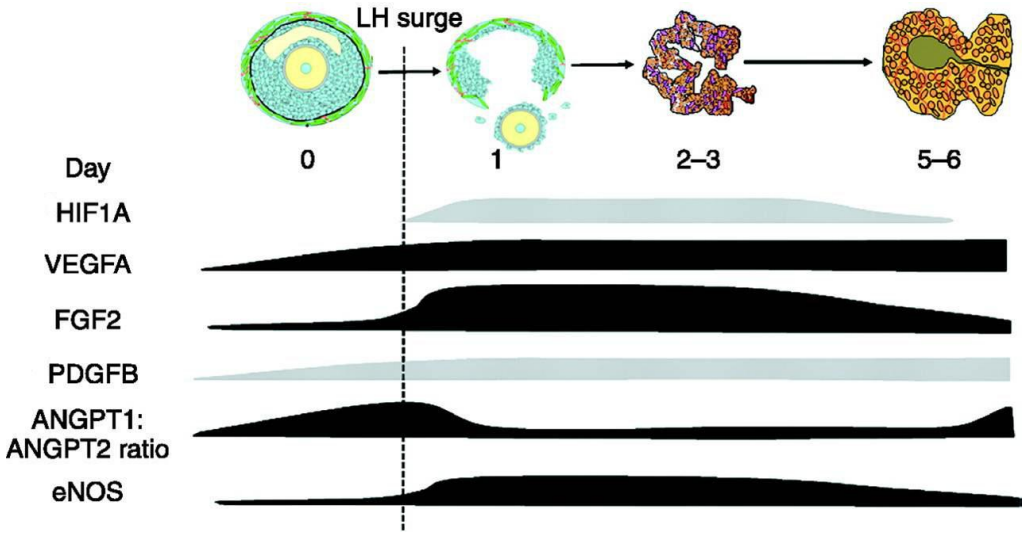

Figure 2.2 Schematic representation of the temporal changes in the levels of angiogenic factors during the bovine follicular–luteal transition.

Source: Robinson et al. (2009)

2.6 Corpus luteum development

The CL is a transient organ in the ovary in mammals (Miyamoto et al., 2009). The CL is the main source of P4; CL morphology and plasma P4 concentration are good indicators of P4 synthesis within the CL (Singh et al., 2003). The bovine CL rapidly develops within 2 to 3 days after ovulation, which is accompanied by angiogenesis and vascularization from the preovulatory follicle (Miyamoto et al., 2009). Intense angiogenesis, proliferation of granulosa and theca cells from the wall of ovulated follicle, and their differentiation (luteinization) during the first 5 to 6 days after ovulation results in a progressive increase in plasma P4 concentration from <1 ng/ml at 3 days after ovulation to approximately 3 ng/ml by 6 days (Adams et al., 2008). If the cow does not become pregnant, the CL is only functional for 17 to 18 days and it must regress within a few days to induce the next chance of ovulation (Adams et al., 2008). In ruminants, it is well known that a pulsatile release of $PGF_{2\alpha}$ from the uterus on days 17 to 18 of the estrous cycle is essential to induce regression of the CL (McCracken et al., 1981). Luteolytic $PGF_{2\alpha}$ induces a drastic decrease in P4 release from the CL as well as CL volume and blood flow to the CL in the non-pregnant cow (Acosta et al., 2002). Thus, it is easily considered that development and regression of the bovine CL have well designed mechanisms and are effectively controlled (Miyamoto et al., 2009).

2.6.1 Corpus luteum function during the estrous cycle

The function of the CL is to produce sufficient concentrations of P4 throughout the luteal phase of the estrous cycle to maintain pregnancy (if a conceptus is present) and during pregnancy, to decrease gonadotrophin secretion and prevent behavioral estrus occurring (Forde et al., 2011). Moreover, sustained increased concentrations of P4 during the luteal phase of the estrous cycle alter the expression pattern of genes in the uterus (Forde et al., 2009). During the mid-luteal phase, these sustained high concentrations of circulating P4 down regulate the nuclear P4 receptor (PR) in the luminal epithelium of the endometrium (Kimmins and MacLaren, 2001). This is a critical switch in allowing the synchronous increase or decrease in genes of the endometrium that are required to initiate uterine receptivity – regardless of the pregnancy status of the animal (Spencer et al., 2008). If, by day 16 of the estrous cycle, the maternal recognition of pregnancy signal (interferon tau; INF-τ) has not

been detected in sufficient quantities, luteolysis of the CL occurs. $PGF_{2\alpha}$ is secreted by the uterus in the bovine (Lamothe et al., 1977) and is the major luteolytic hormone in ruminants (Nett et al., 1976). Oxytocin receptor in the uterus binds oxytocin which propagates the episodic secretion of $PGF_{2\alpha}$ from the uterus. $PGF_{2\alpha}$ then mediates the luteolytic mechanism via counter-current exchange between the uterine vein and the ovarian artery, inducing regression of the CL (Forde et al., 2009). This reduces circulating P4 concentrations, E2 concentrations increase and GnRH in the hypothalamus is stimulated as the animal enters the follicular phase of the estrous cycle (Forde et al., 2011).

P4 must be maintained at sufficiently high levels so that embryogenesis and attachment of the developing conceptus to the endometrium can take place. The embryo enters the uterus between days 2 and 5 after ovulation, depending on the species. The critical series of events by which the conceptus initially signals its presence to the dam and enables pregnancy to continue is referred to as maternal recognition of pregnancy (Senger, 1997). If an adequate signal is not delivered in a timely manner, the dam will experience luteolysis, P4 concentrations will decline and pregnancy will be terminated (Roberts et al., 1996). The CL of ruminants produces oxytocin which stimulates endometrial cells to synthesize $PGF_{2\alpha}$. The production of $PGF_{2\alpha}$ is dependent upon a threshold number of oxytocin receptors (OR) that are synthesized by endometrial cells at a critical time during the estrous cycles. When these OR are available in sufficient numbers, pulsatile secretion of $PGF_{2\alpha}$ occurs in response to luteal oxytocin secretion and luteolysis follows (Senger, 1997). Clearly, this mechanism must be prevented if a successful pregnancy is to proceed (Spencer et al., 2004). Interferon tau (INF-τ) is produced by the trophoblastic cells of blastocyst and is present in the uterus from about day 13 to 21 after ovulation (Bazer et al., 2009). INF-τ binds to the endometrium and inhibits OR synthesis by endometrial cells. In addition to blocking OR synthesis, INF-τ also binds to the apical portion of the uterine glands and promotes protein synthesis believed to be critical to preimplantation embryonic survival (Senger, 1997; Imakawa et al., 2004).

2.6.2 Corpus luteum dysfunction

During the preimplantation period, the uterus undergoes important developmental changes stimulated by P4; hence, disorders related to its secretion are

likely to affect the pregnancy outcome (Inskeep et al., 1988). Inadequate P4 production causes delayed or otherwise abnormal pattern of endometrial development which leads to disorder classically described as luteal phase deficiency (Usadi et al., 2003). Luteal phase defect is a relatively uncommon but important cause of infertility and/or habitual abortion (Hommeida et al., 2004). Approximately one-half of all luteal phase defects are due to improper function of the GnRH pulse generator, namely, following ovulation the increased serum P4 levels over suppress the GnRH pulse generator, resulting in improper luteal function (Santos et al., 2004). In cases where the CL is LH-responsive, such as the hypothalamic CL insufficiency and the large luteal cell defect, treatment with GnRH (Howard et al., 2006) or hCG is advisable (Hanlon et al., 2005; De Rensis et al., 2010).

In modern dairy cattle, increased genetic capability for milk production, coupled with changes in nutritional management and herd size, have been associated with decreased fertility (Lucy et al., 1992). Sub-functional CL, having reduced life span and/or lower P4 production, has been reported following spontaneous and induced ovulation in domestic ruminants (Hunter, 1991). In cows, reduced fertility was associated with low P4 during the early luteal phase (Larson et al., 1997). P4 concentrations 5 to 10 days after insemination were higher in pregnant than non-pregnant cows (Larson et al., 1997). Insufficient or delayed P4 production (or both) comprised 13 of 15 abnormal cycles (Hommeida et al., 2004). In ovariectomized cows treated with P4 and E2, subnormal P4 concentrations resulted in a stronger luteolytic signal (Mann and Lamming, 2001), which might predispose to a higher incidence of embryo loss. Delayed CL formation was associated with a marked and progressive reduction in pregnancy rate in cattle (Santos et al., 2004), perhaps due to asynchrony between the uterus and the embryo. In contrast, cows with an early postovulatory increase in P4 and high luteal phase P4 concentrations had larger embryos 15 to 17 days after AI; these embryos produced larger amounts of INF-τ and therefore the pregnancy was more likely to be maintained (Hommeida et al., 2004).

2.7 Regulatory proteins

2.7.1 B cell lymphoma-2

In the ovary, apoptosis occurs mainly at the early antral follicle stage, and > 99% of ovarian follicles undergo atresia during reproductive life (Hsueh et al., 1996). Morphological and biochemical studies have demonstrated that the death of both somatic and germ cells in the ovary is mediated by apoptosis (Billig et al., 1993). There are two major pathways of apoptosis: the death receptor pathway and the mitochondrial pathway (Borner, 2003). The death receptor pathway is initiated at the cell surface through the death receptor (Fas/TNF-R1 family protein; Ashkenazi and Dixit, 1998). The clustering of the death domains in the intracellular portion of the receptors recruits the adapter molecule which then recruits pro-caspase 8 (initiator). Activation of pro-caspase 8 through self-cleavage leads to a series of downstream events, including activation of pro-caspase 3 (effector), cleavage of multiple caspase substrates and induction of mitochondrial damages (Xiao-Ming, 2000). The mitochondrial pathway is regulated by the pro- and anti-apoptotic members of the Bcl-2 family of proteins and leads to the cytosolic realization of mitochondrial intermembrane space proteins that can trigger caspase-dependent death pathways (Arnoult et al., 2003).

Bcl-2 family proteins are key regulators of the apoptotic process. These proteins exert their function at the mitochondrial level by regulating the permeability of the outer mitochondrial membrane (Jurisicova et al., 1998). It has been found that the Bcl-2 protein prevents apoptosis induced by a variety of stimuli and maintains cell survival by influencing the release of cytochrome c from mitochondria rather than by altering proliferation (Yang et al., 1997). Incidentally, most of the Bcl-2 family proteins possess a transmembrane C-terminal domain, allowing their anchorage to mitochondrial membranes as well as to the membranes of other cellular organelles, such as the endoplasmic reticulum and nuclear envelope (Zhai et al., 2008). Several members of the Bcl-2 family have been found to be expressed in mammalian oocytes and early embryos (Boumela et al., 2011). Bcl-2 mRNA and protein are expressed at high levels in adult human oocytes (Grondahl et al., 2010). Interestingly, Bcl-2 mRNA abundance was positively correlated with oocyte quality in cows (Melka et al.,

2009). These findings suggest that Bcl-2 could play important roles in controlling oocyte survival (Boumela et al., 2011).

During the early cleavage stages preceding embryonic genome activation, embryonic functions are largely controlled by maternally inherited mRNAs, proteins and other molecules (Boumela et al., 2011). Interestingly, the expression of Bcl-2 was high in good quality oocytes and embryos, low in fragmented embryos, and hardly detectable in denuded oocytes (Yang and Rajamahendran, 2002). Previous study reported that preimplantation embryos are very sensitive to conditions that cause oxidative stress and that their glutathione levels change dramatically during development (Gardiner and Reed, 1994). The glutathione has many physiological roles; for example, it serves as a storage and transport form of cysteine, protects cells from oxidative stress, and guards against ROS generated as a result of normal oxidative metabolism (Gardiner and Reed, 1994). The localization of abundant Bcl-2 protein to the mitochondria of cells suggests that the mechanism of Bcl-2 may be linked to respiratory metabolism and reduction oxidation reactions (Kane et al., 1993). Bcl-2 family of proteins has been shown previously to regulate ATP/ADP exchange across the mitochondrial membranes and to prevent the loss of coupled mitochondrial respiration during apoptosis (Manfredi et al., 2003).

2.7.2 Connexin43

The coordinated function of the two compartments of the follicle, oocyte and granulosa/cumulus cells is mediated by humoral (contact independent) as well as gap junctional (contact dependent) interactions (Grazul-Bilskaet al., 1997). Gap junctions occur at sites of close cell apposition; they are an array of intercellular membrane channels that allow inorganic ions, second messengers and metabolites to pass from cell to cell (Granot and Dekel, 2002). Gap junctions are composed of hemi channels (connexons) that consist of six subunit proteins that are termed connexins (Cx; Grazul-Bilska et al., 1997).

Gap junctions in granulosa/cumulus cells of ovarian follicles may contain several different connexins – including Cx26, Cx32, Cx37, Cx43 and/or Cx45 – depending on the species (Grazul-Bilska et al., 1997). Cx43 is one of the major gap junctional proteins expressed in the cumulus oocyte complex (COC) and granulosa cells of several species (Kidder and Mhawi, 2002). Models of targeted mutation of

Cx43 clearly demonstrated that gap junctional coupling mediated by Cx43 channels plays an indispensable role in both germ line development and postnatal folliculogenesis (Juneja et al., 1999). Normal oocyte growth and differentiation depend on an intimate association between follicular (somatic) cells and the developing germ cells (Eppig et al., 1997). This somatic cell – oocyte interactions via gap junctions are essential for oocyte growth, provision of substrates for energy metabolism, cytoplasmic maturation of the oocyte, inhibition of maternal genes and maintenance of meiotic arrest (Larsen et al., 1996). The expression of Cx43 and gap junctional intercellular communication in the ovaries is affected by several factors including: stage of follicular development; hormones such as FSH, LH or E2; and second messengers (Pant et al., 2005).

During the preimplantation stage, Lonergan et al. (2003a) suggested that such embryos with altered transcript abundance survive to implantation because of the contribution of one or more additional connexin genes that are normally expressed along with Cx43 in preimplantation development. Moreover, mouse conceptuses lacking Cx43 develop to birth despite severely reduced gap junctional coupling during their preimplantation development (De Sousa et al., 1998). Bovine embryos developed in vitro with a higher abundance of Cx43 mRNA are more similar to those developed in vivo (Lonergan et al., 2003b). In cattle, previous study demonstrated that the relative abundance of Cx43 contributes to the more physiological characteristics in in vitro produced bovine embryos and can serve as a marker of oocyte developmental competence (Nemcova et al., 2006).

2.8 Animal model of reproductive biology

A research model is a general pattern that scientists use as a tool to investigate a general phenomenon. Research models usually imply the use of specific techniques and experimental units (e.g., animals) with which data were originally gathered and patterns were originally defined (Ginther et al., 1989). Research models offer a conceptual framework upon which specific hypotheses may be formed and tested, and they permit the extension of a concept into new areas (e.g., different species). Good research models are readily accessible (i.e., abundant and inexpensive), malleable

(i.e., easy to work with and adaptable), of broad applicability, and lend themselves to quantitative assessment (Adams and Pierson, 1995).

2.8.1 Bovine model of ovarian function

Ovarian function is perhaps most studied and best understood in the bovine species (Ginther et al., 1989). Studies in the bovine species have served as a template for elucidating physiologic mechanisms related to ovarian function and for characterizing reproductive events in many other species, including humans (Yapura et al., 2011). The following is intended as an overview of the bovine model for studying ovarian function and its remarkable impact on our understanding of the reproductive biology of many other domestic (Baerwald et al., 2003) species. For instance, profound similarities in the dynamics of follicle development exist between the menstrual cycle in humans and the estrous cycle in cattle and horses (Ginther et al., 1989). The wave pattern of follicle development appears to be a widely preserved, fundamental biologic phenomenon. The model, derived from studies in mature nonpregnant cows, has been extended to other reproductive statuses and other species (Adams and Pierson, 1995). Using the model as a foundation the wave pattern has been recognized and characterized during pregnancy (Ginther et al., 1989), the postpartum period (Savio et al., 1990), and in prepubertal calves (Adams et al., 1993). The same approach has subsequently been used to document follicular dynamics in horses (Bergfelt and Ginther, 1993), camelids (Adams et al., 1990), sheep (Ravindra et al., 1994), and goats (Ginther ant Kot, 1994). A bovine model to study ovarian function in women has been proposed (Adams and Pierson, 1995), and was the basis of the discovery of follicular waves in women. Follicular and endocrine events of the normal reproductive cycle; i.e. follicular wave emergence, follicle selection, and ovulation, were fundamentally similar between cattle and women (Baerwald et al., 2003).

2.8.2 Goat model of ovarian function

The small ruminants, such as goats, represent a valuable model system for the elucidation of endocrine and local mechanisms that control both the early and final stages of follicle development in monovulatory species (Simoes et al., 2006). In humans, limited availability of suitable ovarian tissue is a major constraint to research in this area, and goats represent a physiologically relevant model to elucidate basic

mechanisms before moving on to more focused clinical investigations (Saraiva et al., 2012). However, to address common causes of infertility and to devise innovative strategies to increase the efficiency of assisted reproduction technologies, it is necessary to understand the basic physiology underlying the complex process of folliculogenesis (Wu et al., 2000). Among the main substances that regulate the complex mechanisms of folliculogenesis are the pituitary gonadotropins FSH and LH, key hormones that regulate gametogenesis and steroidogenesis in the ovary (Baird, 2006). In vivo, preantral follicles are considered to be gonadotropin independent because animal and human preantral follicles can develop to the antral stage in conditions of minimal circulating gonadotropins (Santos et al., 2006). In addition, goats are gaining acceptance as an established model for biomedical research and for surgical tanning and teaching (Santos et al., 2009; Xu et al., 2009).

2.8.3 Sheep model of placental angiogenesis

The study of the development of the fetal membranes is an ancient one, and the importance of placental vascular development to placental function has long been recognized. Study of the human placenta, or of human embryos for that matter, was confined to anatomical descriptions until the advent of biochemical and tissue culture methods early in the 20th century (Wulff et al., 2003). Thus, animal models have been central to the study of the placenta since the earliest times, and much of our knowledge of placental anatomy and physiology continues to be derived from comparative studies in animals (Reynolds et al., 2005). Using sheep model of placental angiogenesis, the major factors regulating placental angiogenesis have been identified. These major factors include VEGF, bFGF, ANG, and their receptors (Reynolds and Redmer, 2002). With these methods and models now in place, we should soon be able to establish the mechanisms involved in both normal and abnormal placental angiogenesis (Reynolds et al., 2005).

2.9 Oocyte and follicle cell interactions

Complex bidirectional communication between the oocyte and its surrounding somatic cells is essential for the coordinated development of both germ cell and somatic cell compartments (Epigg, 2001). The oocyte plays an active role in cumulus expansion (Mtango et al., 2008). The growth and development of the oocyte and the

somatic components of the follicle occur in a highly coordinated manner. Previous study found that fully grown oocytes removed from antral follicles underwent a spontaneous, gonadotropin-independent resumption of meiosis in culture, and concluded that follicular somatic cells (granulosa and theca cells) maintain oocytes in meiotic arrest (Buccione et al., 1990). Oocytes regulate their own maturation and also affect the functions of the neighboring somatic cells (e.g., cumulus cell expansion) and ovulation rate (Juengel and McNatty, 2005). Follicular somatic cells in turn regulate oocyte transcription (De La Fuente and Eppig, 2001) and promote oocyte competence to undergo fertilization and preimplantation embryogenesis (cytoplasmic maturation; Buccione et al., 1990). Granulosa cells participate in the global suppression of transcription in oocytes that occurs before nuclear maturation (Mtango et al., 2008). In antral follicles, oocyte-derived factors, such as growth differentiation factor 9 (GDF9), promote the development of the cumulus cell phenotype by suppressing expression of the mural granulosa cell phenotype (McNatty et al., 2003). Oocytes also secrete a potent mitogenic factor that promotes mural granulosa and cumulus cell DNA synthesis and cell proliferation (Gilchrist et al., 2003). Oocytes modulate follicle FSH-induced P4 and E2 synthesis by mural and cumulus granulosa cells (Li et al., 2000) and suppress FSH-induced luteinizing hormone receptor mRNA expression (Eppig et al., 1997).

The development of the oocyte and the somatic cells occurs simultaneously and this is responsible for ensuring an ovulated oocyte ready for fertilization (Mtango et al., 2008). Disruption of this communication will result in oocyte developmental failure. Oocytes can regulate the rate of development of the somatic cells (Eppig, 2001). Follicles are also regulated by extraovarian factors such as gonadotropin hormones (require FSH and LH) and receptor to become large antral preovulatory follicles (Fortune et al., 2004). Changes in oocyte development are normally coordinated with follicular differentiation. This coordination, however, can be changed abruptly by accelerating follicular development with exogenous gonadotropins, or other factors (Mtango et al., 2008). Early recruitment of small antral follicles into the pool of preovulatory follicles results in the ovulation of oocytes capable of maturation, fertilization, or embryogenesis (Hunter, 1998).

It has long been recognized that nutrition and other environmental factors have a profound influence on reproductive performance of females in domestic ruminants (Gong, 2002). Metabolic hormones can act either directly to control gonadotrophin-independent stages of follicle development (Gong, 2002), or in synergy with gonadotrophins to modulate follicular recruitment and final development and maturation of preovulatory follicles (Armstrong et al., 2002). These observations have led to the more recent investigations into the possibility that nutritional influence on reproductive function is mediated at the ovarian level (Robinson, 1996). These have included the direct effects on ovarian follicle growth, oocyte maturation, and early embryo development of specific nutrients and substrates, and metabolic hormones such as growth hormone, insulin, and IGF (Boland et al., 2010)

2.10 Summary

The research focused on the vascularity quantitation (capillary area density, capillary number density, and area per capillary) in bovine follicles using immunofluorescence localization of Factor VIII protein and investigated the relationships among vascularization, eNOS expression, and mitotic activity in antral follicles to use of relationships among these variables as an indicator of follicular growth and development in bovine ovaries. Furthermore, there were studies on and investigated the application of assisted reproductive technology (ART) to induce multi-follicular growth and development using FSH regime and to produce a large number of healthy oocytes and healthy embryos suitable for in vitro production (IVP). Additionally, there were studies on the effects of GnRH replacement with hCG in TAI protocol on ovulation and conception rates, accessory CL, and subsequent plasma P4 concentrations in dairy cows. Knowledge of this study can be applied to control the ovarian follicular and CL functions and to improve the reproductive management in ruminants.