Control of ovulation in mammals

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Abstract

The ovulatory LH surge induces dramatic molecular and structural changes in follicular environment, culminating with follicle rupture and release of a mature oocyte. This review addresses the pre-LH surge events associated with the ovulatory process, such as dominant follicle differentiation and acquisition of ovulatory capacity, as well as autocrine and paracrine factors induced after the LH surge. Over the last few years, our research group has focused on studying the contribution of renin-angiotensin system in ovulation and the participation of Ang II and Ang-(1-7) and their interactions with factors essential for ovulation, which are also discussed.

Keywords: angiotensin, follicle, LH, ovulation, renin.

Introduction

Ovulation is the apex of follicular development, which begins with the activation of primordial follicles and culminates with the release of a mature oocyte capable of being fertilized and with the formation of a corpus luteum. Ovulation is triggered by the pituitary LH surge, which initiates a series of ovarian events, generating a cascade of paracrine/autocrine factors, enzymes and transcription factors responsible for the rupture of the apical follicle wall, remodeling of extracellular matrix (ECM) and cell differentiation. The understanding of the ovulatory process is essential to elucidate some of the problems associated to female fertility, and to improve technologies applied to animal breeding.

In the present review, we briefly discuss the final stage of dominant follicle differentiation, acquisition of ovulatory capacity and the molecular signals in response to LH, inducing the expression of paracrine and autocrine factors that constitute the preovulatory cascade. We then focus on our recent results showing the importance of Angiotensin II (Ang II) and Angiotensin-(1-7) [Ang-(1-7)] in the control of ovulation in cattle.

Dominant follicle differentiation and acquisition of ovulatory capacity

After bovine follicular deviation, a dominant follicle continues its growth and differentiation and acquires ovulatory capacity at about 12 mm diameter in Bos taurus taurus cows, as indicated by the response to LH treatment (Sartori et al., 2001). Ovulation of smaller follicles (10 mm) can also be induced, but higher doses of LH are necessary (Sartori et al., 2001). The main events associated with final follicle differentiation are acquisition of LH receptors in granulosa cells (Xu et al., 1995) and an acute increase in mRNA expression of steroidogenic enzymes HSD3B1 and CYP11A1 in both theca and granulosa cells and CYP17A1 in theca cells (Tian et al., 1995). Estradiol synthesis is dramatically increased in the dominant follicle after deviation; however, upregulation of CYP19 mRNA is not observed in granulosa cells (Ferreira et al., 2011), suggesting that an increase in aromatizable substrate accounts for the increased steroidogenesis (Tian et al., 1995).

Although the exact mechanism leading to final follicular differentiation and ovulatory capacity is not fully understood, several lines of evidences point to a pivotal role for oocyte-secreted factors bone morphogenetic protein 15 (BMP15) and growth and differentiation factor 9 (GDF9). Naturally occurring mutation in ovine BMP15 and GDF9 genes were identified and based on observed phenotypes, it was postulated that the inactivation of one gene and consequently reduced availability of BMP15 or GDF9 affects the differentiation of granulosa cells, inducing precocious differentiation of small antral follicles with fewer granulosa cells (Otsuka et al., 2001). In fact, granulosa cells from ewes heterozygous for BMP15 mutation are more responsive to LH, as assessed by cAMP production after hCG treatment (McNatty et al., 2009). A mutation in BMPR1B (also known as Alk6) involved in BMP2, 4 and 15 signaling, was also identified in ewes resulting in increased ovulation rate (Mulsant et al., 2001; Souza et al., 2001). In cattle, Juengel et al. (2009) induced multiple ovulations in 6/10 cows immunized against GDF9 and BMP15 peptides, suggesting a function for these proteins in the regulation of follicle differentiation and ovulation rate in this species. Similar results were observed in ewes, in which a significant increase in ovulation rate was induced following BMP15 and GDF9 immunization (McNatty et al., 2007). These data point to a role of BMP system in granulosa cell differentiation, but a direct effect of these factors on ovulation process has not been described in monovular species.

Follicular size at ovulation

A wide range of diameter (9-30 mm) is observed in bovine follicles undergoing spontaneous

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Received: June 13, 2012
Accepted: August 1, 2012
ovulation (Perry et al., 2005; Echternkamp et al., 2009). The effect of follicle diameter at ovulation on pregnancy rates and endocrine profiles has been extensively studied. It seems that induced but not spontaneous ovulation of smaller follicles is accompanied by reduced pregnancy and embryo viability rates (Perry et al., 2005). However, in cattle selected for multiple ovulations, spontaneous ovulation of follicles between 8 and 10 mm is also associated with reduced fertility (Echternkamp et al., 2009). It is unknown whether this is due to oocyte or follicular factors.

The main difference between cows ovulating small or large follicles is the pattern of progesterone secretion. Animals experiencing multiple ovulations present smaller oocyte diameter at ovulation induction (Atkins et al., 2010a, b), but not after spontaneous ovulation (Busch et al., 2008). Based on the aforementioned studies, follicle diameter does not seem to influence fertility and progesterone profile in fully differentiated follicles that ovulate spontaneously. Nevertheless, management practices that optimize ovulatory follicle size and differentiation may improve fertility when ovulation is pharmacologically induced.

**The effects of LH surge in the early stages of ovulation**

At the end of the estrous cycle, the shift in the progesterone/estrogen ratio induces an increase in the GnRH/LH pulse frequency that results in the preovulatory LH surge (Kesner et al., 1982). Luteinizing hormone binds to receptors present in the dominant follicle stimulating the classical route of cAMP and protein kinase A (PKA) that induce the expression of paracrine and autocrine factors that trigger the preovulatory cascade (Marsh, 1976; Richards, 1994). The LH receptor (LHCGR) is expressed in granulosa and theca cells, but not in cumulus cells and oocytes (Peng et al., 1991, van Tol et al., 1996). In rat theca cells, LH stimulates the production of interleukin 1β (IL-1β) produced by leukocytes; Kol et al., 1999), which is important for cumulus expansion, and induces Insulin-like 3 (Ins-l-3; Bathgate et al., 1999; Kawamura et al., 2004). InsIl-3 seems to attenuate adenylate cyclase activity, decreasing cAMP in the oocyte, which is essential for meiosis resumption. In granulosa cells LH activates ERK1/2, probably mediated by PKA, resulting in phosphorylation of proteins involved in ovulation, including cAMP response element-binding protein (CREB) and stimulatory proteins 1 and 3 (Sp1/3), which stimulate progesterone receptor (PR), EGF-like factors (EGF-L: amphiregulin, betacellulin and epiregulin), Early growth response-1 (Egr-1), the disintegrin and metalloproteinase with thrombospondin repeats (ADAMTS-1), cathepsin L and versican (Russell and Robker, 2007).

Steroidogenesis is immediately affected and follicular fluid progesterone levels increase 4-5 fold about 1.5 h after the LH surge (Fortune et al., 2009), while a gradual decrease in estradiol secretion is observed from 3 h post-GnRH (Santos et al., 2011). Progesterone is essential for ovulation (Bridges et al., 2006) and oocyte maturation (Siqueira et al., 2012a), probably by stimulating the expression of prostaglandin-endoperoxide synthase 2 (PTGS2). Progesterone receptor (PR) antagonists as well as the suppression of the expression of PR or nuclear receptor interacting protein 1 (NRIP1) blocks ovulation and decreases proteases cathepsin L and ADAMTS-1, EGF-L, hyaluronan synthase-2, PTGS2, pentraxin-3(3-PTX) and tumor necrosis factor stimulator gene-6(TSG-6) (Russell and Robker, 2007, Robker et al., 2000).

The upregulation of PTGS2, characteristic of the ovulation process, suggests that prostanooids are important mediators of LH-induced changes in the ovulatory follicle environment. In fact, intrafollicular injection of an inhibitor of prostanooids synthesis (indomethacin; a potent nonsteroidal anti-inflammatory) blocks the LH-induced increase in PGE2 (Li et al., 2006) and inhibits the LH-induced upregulation of amphiregulin (AREg) in theca and granulosa cells, while it negatively affects ADAM17 (a disintegrin and metalloprotease 17) protease in theca cells (Li et al., 2009). These results demonstrate that LH-induced AREG and ADAM17 expression in theca cells is prostanoid-dependent. However, the precise function of ADAM17 in theca cells is not clear. ADAMs have been identified as the main sheddases involved in activation and release of EGF-L extracellularly from the cell membrane (Sahin et al., 2004). Prostaglandins also seem to increase collagenolytic activity of follicular tissue (as assessed by coincubation with radiolabeled collagen) and proteolytic enzymes such as tissue plasminogen activator (tPA) and plasmin (Li et al., 2006; Fortune et al., 2009). We must emphasize that these enzymes are essential to digest components of basement membranes, such as collagen type-IV and laminin. Other factors have been identified as being involved in ovulation as phosphodiesterase (PDE), EGF-L, Ang II, plasminogen activators (tPA and uPA), plasmin, and proteases of the extracellular matrix (Robker et al., 2000; Curry et al., 2001; Park et al., 2004; Yang et al., 2004; Ferreira et al., 2007; Portela et al., 2011; Siqueira et al., 2012b).

**Extracellular matrix remodeling in ovulation**

The follicular cells, the basement membrane, the tunica albuginea and the ovarian surface epithelium of the follicle form an organized and stable structure until the preovulatory LH surge. During the process of ovulation, the extracellular matrix of these structures undergoes intense remodeling and revascularization to form the corpus luteum. Enzymes such as matrix...
metalloproteinases (MMPs), ADAMTS proteases, plasmin and plasminogen activators (tPA and uPA) are involved (although not all are essential) in remodeling of the extracellular matrix, degrading of the basement membrane and rupturing of the follicular apex (Mittaz et al., 2004; Og iwara et al., 2005). Tissue inhibitors of MMPs (TIMPs) are also involved in the ovulatory process and the pattern of in vivo expression suggests that decreased TIMPs levels and increased MMPs and PAs are involved in follicle rupture (Li et al., 2006). The extracellular matrix remodeling is completed by a process similar to the inflammatory process, with the participation of macrophages, neutrophils, cytokines produced by leukocytes, platelet activating factor and free radicals (Murdoch et al., 1999; Wu et al., 2004).

Angiotensin II and ovulation

A role for Ang II in ovulation was first postulated based on the increase of the concentration of this peptide and its precursors in the follicular fluid after the LH surge (Yoshimura et al., 1994; Acosta et al., 2000). The demonstration of the involvement of Ang II in the control of ovulation has been troubled by differences between species and experimental models utilized in different studies (Husain et al., 1987; Pelllicer et al., 1988; Daud et al., 1989; Yoshimura et al., 1992; Kuji et al., 1996). Using in vitro and in vivo models, we performed a series of experiments to characterize the role of Ang II in bovine ovulation. Two Ang II receptor subtypes have been identified and characterized. The type 1 receptor (AGTR1) mediates a number of well-known Ang II effects on smooth muscle contraction and blood pressure regulation, while the type 2 (AGTR2) receptor has been shown to mediate reproductive functions (Kuji et al., 1996; Yoshimura et al., 1996). We conducted an experiment to demonstrate that the Ang II signaling participates in the ovulatory cascade. For our surprise, the inhibition of Ang II receptors efficiently blocked ovulation when the receptor blocker was administered before estrus onset (Fig. 1).

Based on the fact that the inhibition of Ang II blocks ovulation only when performed before estrus onset, we hypothesize that Ang II acts at the beginning of the ovulatory cascade in cattle. When an Ang II receptor antagonist was injected in preovulatory follicles, the ovulation was inhibited only in the first 6 h after GnRH-analogue injection (Fig. 2A). Then, we investigated the receptor type involved in ovulation using an in vivo model with intrafollicular injection of specific antagonists. We demonstrated that the effect of Ang II on ovulation is mediated by AGTR2 receptor (Fig. 2B; Ferreira et al., 2007).
Figure 2. A) Ovulation rate following ultrasound-mediated intrafollicular injection of saralasin at 0, 6, or 12 h after GnRH agonist challenge. B) Effect of angiotensin II type 1 (AGTR1) and type 2 (AGTR2) receptor antagonists on ovulation rate in cow. The animals received an ultrasound-mediated intrafollicular injection of 0.9% NaCl (saline, n = 6), losartan (LO, 10 µl; AGTR1 receptor antagonist; n = 6), PD123319 (PD, 10 µl; AGTR2 receptor antagonist; n = 6), or 10 µl LO+10 µm PD (n = 6). Follicle diameter was at least 12 mm at intrafollicular injection. Different letters above bars indicate statistical difference between groups (P < 0.05). Figure adapted from Ferreira et al., 2007.

Angiotensin II levels in preovulatory follicle

In the early 90s, an increase in follicular fluid Ang II levels after LH surge was reported in rodents (Yoshimura et al., 1994). Recently, we characterized the changes in Ang II, Ang receptors and Ang II-synthesis related factors during preovulatory period (0, 3, 6, 12 and 24 h after GnRH). The first significant increase in the concentration of Ang II in follicular fluid occurred at 6 h and a dramatic increase at 24 h, after the GnRH injection. Angiotensinogen and angiotensin converting enzyme (factors related to Ang II synthesis) mRNAs were significantly upregulated in granulosa cells (24 h after GnRH) and theca cells (6 h after GnRH), respectively. Immediately after the expected LH surge (3 h after GnRH), an acute increase in AGTR2 mRNA was observed (Siqueira et al., 2012b). Taken together these data further confirm that Ang II and renin-angiotensin system (RAS) components are regulated during ovulation.

Angiotensin II in the ovulatory cascade

Angiotensin II has a crucial role in the early events of ovulation, acting through AGTR2 receptors. The challenge is to understand how Ang II interacts with other ovulatory factors and where it is located in the ovulatory cascade. Evidence that Ang II interacts with prostaglandins (PG) was observed when Ang II antagonist blocked the PG synthesis and ovulation in rabbit (Yoshimura et al., 1993). The role of PGs in ovulation was first described by Labhsetwar (Labhsetwar, 1971, 1972) and extensively studied during the last decades. Prostaglandins (mainly PGE2) induce vasodilatation and tissue changes in the apical region of ovulatory follicle (Kitai et al., 1985; Yoshimura et al., 1988). Intravenous administration of indomethacin during the ovulatory process decreased PGE2 and PGF2α levels and inhibited ovulation (Espey et al., 1986). PTGS2 is upregulated by the LH surge promoting an increase in prostaglandins synthesis (Sirois et al., 1992). In PTGS2 knockout mice (Lim et al., 1997) and cows submitted to intrafollicular injections of a PTGS2-specific inhibitor (Peters et al., 2004) ovulation did not occur normally. In vitro, Ang II added to a culture medium containing LH stimulated granulosa cells to secrete 3 to 5 fold more P4, PGE2 and PGF2α compared with controls with or without LH (Siqueira et al., 2012b). Taken together, these results suggest that Ang II mediates and enhances the gonadotropin-induced ovulatory cascade at least in part by stimulating the production of prostaglandins.

We also carried out a series of experiments to investigate the involvement of Ang II in the regulation of events upstream of prostaglandins. In vitro experiments showed that Ang II can increase the abundance of mRNA encoding disintegrin and ADAM17, AREG and EREG, in the presence of LH. Moreover, the inhibition of ADAM17 sheddase activity abolished the stimulatory effect of Ang II on AREG, EREG and PTGS2 mRNA, suggesting that ADAM17 mediates Ang II action at the beginning of the ovulatory cascade (Portela et al., 2011).
Angiotensin-(1-7) and ovulation

Ang-(1-7), an active heptapeptide of the renin-angiotensin system (RAS), was recently identified in the rodent, human and bovine ovaries (Costa et al., 2003; Reis et al., 2011 Santos et al., 2011). This peptide is formed from the cleavage of Ang I and Ang II by angiotensin converting enzyme 2 (ACE2) and prolyl endopeptidase (PEP). In another pathway, Ang-(1-7) is produced from Ang I, which is cleaved by neutral endopeptidase (NEP; Santos et al., 1992, Donoghue et al., 2000). Ang-(1-7) is cleaved into smaller fragments by ACE and other aminopeptidases, which probably constitutes a mechanism to control its functions (Yamada et al., 1998, Chappell et al., 2000). Ang-(1-7) actions are mediated by a specific receptor coupled to G protein: MAS (Santos et al., 2003, Dilauro and Burns, 2009).

Little is known about the functions of Ang-(1-7) during the ovulation process. Increased expression of MAS and ACE2 mRNA in ovarian homogenates, and immunolocalization of Ang-(1-7) and MAS in theca and interstitial cells in response to gonadotropins were reported (Pereira et al., 2009). In bovine follicles ≥12 mm, the presence of mRNA for MAS, ACE2 NEP and PEP was observed in theca and granulosa cells (Santos et al., 2011) and MAS receptor was located to granulosa cells (unpublished data). Moreover, levels of Ang-(1-7) increased 12 h after treatment with GnRH in the follicular fluid (Santos et al., 2011), which was probably a consequence of the concomitant increase in ACE2, NEP and PEP mRNA expression in granulosa cells (Santos et al., 2011).

Disruption of MAS receptor signaling inhibited gonadotropin induced ovulation in the ovarian perfusion model in rabbits. In addition, Ang-(1-7) was able to induce ovulation and estradiol production in the absence of gonadotropins (Viana et al., 2011). However, ovulation was not blocked when MAS receptor signaling was inhibited with intrafollicular injections of A-779 in cows challenged with GnRH (unpublished data). This is different from the results obtained with Ang II receptor blocker (Ferreira et al., 2007), suggesting that Ang-(1-7) has a distinct role during the ovulatory process. Interestingly, Ang-(1-7) stimulated arachidonic acid (AA) and prostaglandin in rabbit aortic vascular smooth muscle cells (Muthalif et al., 1998) and it is well known that prostaglandins are essential for ovulation (Yoshimura et al., 1988; Sirois et al., 2004; Fortune et al., 2009). Therefore, the results obtained so far suggest that Ang-(1-7) is involved in the regulation of the ovulatory process, although more studies are needed to understand its specific roles.

Final considerations

The ovulation process initiates with LH binding to its receptor and activating ERK1/2 in granulosa cells, probably mediated by PKA and EGF-L, resulting in phosphorylation of proteins important for cell proliferation and differentiation and ECM remodeling. These initial LH effects induce a gradual decrease in estradiol synthesis, increase in progesterone, induction of cumulus expansion, oocyte maturation and follicular rupture. The molecular control of ovulation after the LH surge involves transcription factors, peptides and enzymes that form a cascade resulting in the release of an oocyte capable of being fertilized. We have focused our research on the role of renin-angiotensin system (RAS) in follicular development, ovulation and oocyte maturation using the cow as the animal model as it is a monovulatory species in which ovulation time can be predicted and follicular environment modified in vivo. We observed that intrafollicular injection of a competitive antagonist of Ang II impairs ovulation in cows and that Ang II acts via the AGTR2 receptor. We also found that Ang II enhances the LH-induced increase in P4, PGE2 and PGF2α, which are essential for ovulation. Furthermore, we showed that Ang II stimulated the expression/activity of ADAM17, which resulted in upregulation of EGF-L and PTG2S. Taken together, these results demonstrate that RAS is essential for ovulation in cattle.

References


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