Role of angiotensin in ovarian follicular development and ovulation in mammals: a review of recent advances

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Abstract

Angiotensin (Ang) II is widely known for its role in the control of systemic blood vessels. Moreover, Ang II acts on the vascular control of ovarian function, corpus luteum formation, and luteolysis. Over the past 10 years, our research group has been studying the new concept of the renin–angiotensin system (RAS) as an autocrine/paracrine factor regulating steroidogenesis and promoting different cellular responses in the ovary, beyond vascular function. We have developed and used different in vivo and in vitro experimental models to study the role of RAS in the ovary and a brief overview of our findings is presented here. It is widely accepted that there are marked species differences in RAS function in follicle development. Examples of species-specific functions of the RAS in the ovary include the involvement of Ang II in the regulation of follicle atresia in rats vs the requirement of this peptide for the dominant follicle development and ovulation in rabbits and cattle. More recently, Ang-(1–7), its receptor, and enzymes for its synthesis (ACE2, NEP, and PEP) were identified in bovine follicles, implying that Ang-(1–7) has an ovarian function. Other novel RAS components (e.g. (pro)renin receptor and renin-binding protein) recently identified in the bovine ovary show that ovarian RAS is poorly understood and more complex than previously thought. In the present review, we have highlighted the progress toward understanding the paracrine and autocrine control of ovarian antral follicle development and ovulation by ovarian tissue RAS, focusing on in vivo studies using cattle as a model.

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Introduction

In the classical definition, the renin–angiotensin system (RAS) is considered as an endocrine system. The vasoconstriction mediated by angiotensin (Ang) II on vascular smooth muscle is an example of the systemic action of RAS. However, the novel concept of a local or tissue RAS has been established more recently. These two distinct pathways (endocrine and local RAS) may be present in the control of a single physiological event. In the ovary, RAS underlies important ovarian blood pressure control (Brannstrom et al. 1998, Acosta et al. 2000) and autocrine/paracrine signaling regulating gene expression (Acosta et al. 1999, Portela et al. 2008, Ferreira et al. 2011a). A detailed discussion on the local RAS physiology has been presented by Paul et al. (2006).

Ovarian function in mammals is primarily orchestrated by endocrine factors, mainly gonadotropins (FSH and LH), their receptors (FSHR and LHR), and ovarian steroids. It is well established that follicle growth occurs in waves and that the follicular cohort development is stimulated by a transient increase in FSH; however, selection of a dominant follicle is poorly understood. Among the autocrine and paracrine factors that are known to be involved in regulating ovarian antral follicle development, the RAS peptides, especially Ang II and to a lesser extent Ang-(1–7), have recently emerged as key factors in the regulation of follicle deviation (Ferreira et al. 2011b). Follicle deviation is characterized by reduction or cessation of growth of the subordinate follicles and continuous growth of the future dominant follicle (largest follicle) (Ginther et al. 1996). In cattle, the dominant follicle acquires ovulatory capacity when it reaches a diameter of around 12 mm (Sartori et al. 2001). At the end of the follicular phase, a complex series of events culminates in ovulation, with the release of a competent egg and the initiation of luteal development. Ang II has been demonstrated to be essential in the ovulatory process (Ferreira et al. 2007) and seems to be an important mediator of LH action in the early increase in ADAM activity that induces the cascade of events leading to ovulation (Portela et al. 2011). However, the physiological function of Ang II in the ovary is not yet well established and the wide species variation makes its understanding even more challenging (Bottari et al. 1993, Nielsen et al. 1995, Yoshimura 1997, Mow et al. 1999). In the present review, the focus is to highlight the progress toward understanding the paracrine and autocrine control of the ovarian antral follicle development and ovulation by ovarian tissue RAS.
The bovine model

Cattle provide an excellent model for studying the role of local factors in the control of follicle development. First, cattle are a monovulatory species and the antral follicle development and deviation can be monitored on a day-to-day basis. Secondly, the timing of follicle deviation and dominance, the LH surge, and ovulation can be controlled. Finally, the follicular environment can be easily modified by ultrasound-guided i.f. injection (Kot et al. 1995, Ginther et al. 2004, Ferreira et al. 2007). Our investigations were based on two in vitro and three in vivo models. For the in vitro models, we used two serum-free culture systems of granulosa cells without spontaneous luteinization (Gutierrez et al. 1997). In one culture system, granulosa cells from medium-sized follicles (4–8 mm) were used. In the other system, granulosa cells of large follicles, in which the expression of genes in the ovulatory cascade is responsive to LH, were cultured (Portela et al. 2011). In one in vivo model, we could predict the day before, during, and after follicular deviation (Evans & Fortune 1997, Rivera et al. 2001) to evaluate RAS regulation. In another model, the largest follicle was intrafollicularly injected with a competitive inhibitor, or the second largest follicle was injected with an agonist to induce codominant follicles at the time of deviation (Gastal et al. 1995, Ginther et al. 2004). In the other model, follicles were intrafollicularly injected with a competitive inhibitor when they reached the size of 12 mm and the cows were challenged with a GNRH agonist to study ovulation (Ferreira et al. 2007, Barreta et al. 2008).

Systemic RAS

Angiotensinogen, the precursor of RAS, is encoded by a single gene, which is expressed mainly in the liver, although its mRNA expression was detected in other tissues, including the ovary (Ohkubo et al. 1986). Its cleavage to form Ang I depends on kidney-secreted renin. Catalytic activation of renin occurs after cleavage of a segment of prorenin comprising 43 amino acids (Do et al. 1987) and seems to occur only in kidneys because renin disappears from blood after bilateral nephrectomy (Sealey et al. 1977). However, noncatalytic activation of prorenin has been described and will be further discussed in the ovarian RAS section below.

Ang I is a decapeptide derived from angiotensinogen cleavage by renin at the leucine–valine bond at the N-terminal region in human or at the leucine–leucine bond in other species. Angiotensin converting enzyme (ACE) is the major enzyme capable of converting the decapeptide Ang I into octapeptide Ang II by specific cleavage at Phe8–His9 bond, releasing the dipeptide His–Leu (Skeggs et al. 1955, 1956). Early studies showed that only Ang II was capable of raising blood pressure. Nevertheless, an i.v. injection of Ang I had the same hypertensive effect, indicating abundant ACE plasma activity (Skeggs et al. 1954).

The RAS is a phylogenetically old system that is present throughout the vertebrates. In most mammalian species, the fifth amino acid of Ang II sequence is an isoleucine (Ile), while cattle and nonmammalian tetrapods have a valine present at this position (named [Val5]-Ang II). The systemic RAS is not the focus of the present review and has been widely reviewed in Yoshimura (1997), Campbell (2003), and Paul et al. (2006).

Ovarian RAS

More recently, a local RAS concept has emerged, and novel kidney-independent enzymes capable of forming Ang II have been identified. The evidence that supports the existence of an ovarian RAS includes the following: i) the concentrations of Ang II in the follicular fluid were found to be higher than those in the plasma after human chorionic gonadotropin (hCG) treatment (Husain et al. 1987), ii) high follicular fluid levels of Ang II were found after bilateral nephrectomy (Husain et al. 1987), and iii) in vitro-perfused rabbit ovaries produced Ang II when exposed to hCG (Yoshimura et al. 1994). However, renin is produced exclusively by kidneys and disappears from the plasma after bilateral nephrectomy (Do et al. 1987). Therefore, alternative pathways must be involved in the local synthesis and regulation of Ang II concentration. The contribution of this local system on ovarian physiology will be discussed.

Prorenin is produced and secreted mainly by kidneys, but extrarenal sources have also been described. Prorenin concentration in follicular fluid is 12 times higher than that found in the systemic circulation and is immunologically identical to kidney and plasma prorenin (Glorioso et al. 1986). In the former studies, prorenin was not identified as a catalytic enzyme; however, in the early 1990s, the ovarian concentration of prorenin was found to be associated with follicular atresia (Schultz et al. 1989, Mukhopadhyay et al. 1991). Controversially, renin secretion seems to be regulated by gonadotropins. An increase in both plasma renin (Itskovitz et al. 1987) and follicular fluid renin activity (Yoshimura et al. 1994) has been observed after hCG treatment.

For many years, prorenin was considered to be the only inactive precursor of renin (Do et al. 1987). However, the nonproteolytic activation of prorenin has now been identified (Suzuki et al. 2003). The renin and prorenin receptor (named (pro)renin receptor) is able to not only bind renin and prorenin but also activate prorenin by inducing a conformational change in protein folding (Nabi et al. 2006). In vivo, (pro)renin receptor binds mainly to prorenin due to its higher affinity to prorenin than to renin and its high concentration in tissues that produce it locally, i.e. in the ovary (Batenburg et al. 2007). Moreover, prorenin also binds to (pro)renin receptor and induces signaling-promoting
angiotensin-like effects, independent of Ang II synthesis (Kaneshiro et al. 2007). Recently, we have demonstrated the presence of mRNA-encoding (pro)renin receptor in the ovary and its differential mRNA expression in dominant and subordinate follicles (Ferreira et al. 2011b), suggesting a role for this receptor either by prorenin nonproteolytic activation or by mediating Ang II-independent effects.

The role of ACE in ovarian physiology is not fully understood. Captopril (an ACE inhibitor) did not inhibit ovulation in rats (Dauda et al. 1990). In contrast, the Ang II type 2 receptor (AGTR2) blocker inhibited ovulation in the rabbit and bovine (Yoshimura et al. 1992, Ferreira et al. 2007), suggesting the presence of an alternative enzymatic route to the synthesis of ovarian Ang II. The members of plasminogen activator (PA) family, cathepsin D and chymase, are enzyme candidates to convert Ang I into the octapeptide Ang II (for review see Paul et al. 2006). Yoshimura et al. (1996a) used streptokinase, an exogenous PA, to stimulate the intrafollicular Ang II content and follicular growth in the rabbit. The same authors have shown using an in vitro-perfused rabbit ovary model that IGF1 stimulates both follicular growth and PA activity in the follicular fluid. Recently, we have demonstrated that the expression of ACE mRNA in bovine granulosa cells is negatively correlated with follicular growth (Ferreira et al. 2011b). Therefore, additional studies are required to characterize the enzyme responsible for cleaving Ang I into Ang II in the ovary.

**Ang II and follicle development**

**Ang II receptors in follicular cells**

The role of Ang II in the regulation of follicular development has mainly been studied in antral follicles. However, the Ang II type 1 and 2 receptors (AGTR1 and AGTR2) have been identified in porcine granulosa cells of earlier stages of follicle development (Shuttleworth et al. 2002b). A positive action of Ang II in estradiol synthesis and growth of preantral follicles in culture were also reported in swine (Shuttleworth et al. 2002a). The hypothesis that RAS is involved in early follicle development was recently reinforced. Pountain et al. (2010) detected both AGTR1 and AGTR2 proteins in the granulosa cells of primordial, primary, and secondary follicles of pig ovaries from fetuses of at least 45 days of gestation. They also detected mRNA-encoding prorenin and angiotensinogen in preantral follicles, suggesting Ang II synthesis during early follicle growth.

During antral follicle development, there are considerable differences between species regarding Ang II receptor functions. In rats, AGTR2 was found to be involved in follicle atresia through apoptosis (Tanaka et al. 1995, Kotani et al. 1999). In fact, AGTR1 receptor has been localized in healthy follicles, while the mRNA expression of AGTR2 receptor has been identified in small- to medium-sized atretic follicles in rats (de Gooyer et al. 2004). Moreover, AGTR2 protein expression has been found to be inhibited by FSH (Pucell et al. 1988), and FSH-induced estradiol production was inhibited by Ang II and that effect was reversed by an Ang II receptor antagonist (PD123,319) (Kotani et al. 1999).

In monovular species, the expression of AGTR2 receptor was first identified in theca cells from bovine dominant follicles (Schauser et al. 2001). These data were further confirmed by Berisha et al. (2002) who demonstrated mRNA-encoding AGTR1 and AGTR2 receptors in both theca and granulosa cells from bovine and observed a slight increase in AGTR2 mRNA in theca cells from highly estrogenic follicles (>180 ng/ml) in comparison with less estrogenic follicles (20–180 ng/ml). Recently, our group showed that the expression of AGTR2 mRNA in granulosa cells was significantly higher in healthy than in atretic follicles (Portela et al. 2008). Furthermore, we also found that FSH, IGF1, and bone morphogenetic protein-7, which augmented estradiol secretion, increased AGTR2 expression at both mRNA and protein levels (Portela et al. 2008). However, fibroblast growth factors-7 and -10, which decreased estradiol secretion, reduced AGTR2 protein levels in primary cell cultures of granulosa cells (Portela et al. 2008). Collectively, these data suggest that rodent and bovine models are different regarding Ang II signaling during antral follicle development. The detection of high AGTR2 expression in bovine dominant follicles suggests a role for Ang II signaling during follicle deviation and selection of dominant follicle in monovular species.

**Ang II signaling: studies in vivo and in vitro**

In cattle, i.f. injection of saralasin (an octapeptide competitive antagonist of Ang II) inhibited dominant follicle growth, decreased estradiol concentration in follicular fluid, and downregulated CYP19A1 mRNA expression in granulosa cells (Ferreira et al. 2011a). However, the follicle regression induced by saralasin was reversed with systemic FSH and the dominant follicle reached ovulation (Fig. 1).

Analysis of follicular cells isolated from saralasin-treated follicles revealed that the expression of AGTR2 was downregulated in theca cells, and mRNAs encoding CYP19A1, LHR, cyclin D2, and 3βHSD were severely downregulated in granulosa cells (Ferreira et al. 2011a; Fig. 1). These are key genes for dominant follicle development. There is strong evidence that Ang II is required for and plays an important role in upregulating essential genes to stimulate high levels of estradiol secretion for maintaining follicle development when FSH levels decline during follicular deviation (Adams et al. 1992).

In another experiment conducted by our group, i.f. injection of Ang II peptide or AGTR2 receptor-specific
agonist (CGP42112A) temporarily prevented the expected regression of the second largest follicle (subordinate follicle) at deviation (Ferreira et al. 2011a; Fig. 1). In isolated perfused rabbit ovaries, IGF1 stimulated ovarian Ang II production (Yoshimura et al. 1996a), and in cattle, IGF1 induced an increase in AGTR2 mRNA and protein (Portela et al. 2008). This interaction between Ang II and IGF1 suggests that Ang II is required for antral follicle development and may explain the temporary ‘rescue’ of the larger subordinate follicle from atresia.

On the other hand, a series of independent studies conducted with rodents have demonstrated that Ang II is involved in the regulation of follicle atresia caused by apoptosis of granulosa cells (Pucell et al. 1988, Daud et al. 1990, Tanaka et al. 1995, Kotani et al. 1999, de Gooyer et al. 2004). Transgenic rats that overexpress renin and angiotensin in extrarenal tissues, including ovarian cells, had a reduced number of large antral follicles and small litter size (de Gooyer et al. 2004). In the same study, similar negative effects on follicle development were observed after Ang II infusion in wild-type females. In swine, the concentrations of Ang II in follicular fluid were found to be negatively correlated with follicular diameter (Li et al. 2004). These studies indicate the existence of marked species differences of the RAS function in follicle development and atresia.

**Regulation of RAS members during follicle development**

Estrus synchronization and follicular dynamics followed by ovariectomy as described previously by Rivera et al. (2001) allowed the characterization of novel RAS components near follicular deviation. We recently demonstrated that RAS is regulated and involved in follicular deviation in monovular species (Ferreira et al. 2011b). Renin-binding protein (RnBP, an inhibitor of renin activity) mRNA was found to be upregulated in bovine subordinate follicles at the expected moment of follicular deviation (day 3 of the follicular wave emergence) and the day after deviation (day 4). This correlation between increased RnBP mRNA expression and atresia was further confirmed by i.f. injection of fulvestrant (an estradiol receptor antagonist) (Ferreira et al. 2011b). Prorenin activity was previously observed to be negatively correlated with estradiol levels in bovine follicular fluid, leading to atresia of large follicles (Schultze et al. 1989, Mukhopadhyay et al. 1991). Nevertheless, we observed that Ang II levels do not increase in the subordinate follicle after deviation. Therefore, we can hypothesize that factors other than RAS peptides are regulating prorenin and renin activities in the dominant and subordinate follicles. In contrast to the data obtained in cattle, renin activity and estradiol levels were positively correlated in the follicular fluid from women who underwent ovarian stimulation (Fernandez et al. 1985). However, there are not enough studies to support the participation of RnBP in follicle development and atresia.

Ang II production in follicular cells seems to be regulated by gonadotropins. The concentrations of Ang II in the follicular fluid of hCG-treated rodents after bilateral nephrectomy were higher than those in the plasma (Husain et al. 1987). Near ovulation, the levels of Ang II also increased in the ovary after treating rabbits with hCG (Yoshimura et al. 1994), cattle with LH (Acosta et al. 2000, Shimizu et al. 2007), and women with human menopausal gonadotrophin (hMG) and hCG (Lightman et al. 1987). Similarly, Ang II and Ang III were found to be positively correlated with estradiol levels and follicle diameter in women stimulated with hMG (Jarry et al. 1988).

Despite the evidence that Ang II synthesis is regulated by gonadotropins, the pattern of Ang II levels in the follicular fluid around the time of follicle deviation was unknown until recently. One of the most recent findings regarding the involvement of RAS in follicular development is the increase in Ang II levels in the follicular fluid of the dominant follicle at the expected moment of deviation (day 3), which coincides with the acute
increase in CYP19A1 mRNA expression and estradiol levels (Ferreira et al. 2011a, b). Also, granulosa cells treated with Ang II showed a positive correlation with CYP19A1 mRNA expression in vitro. However, the inter- 
regulation between Ang II and steroidogenesis in the follicular cells is still poorly understood.

**Ang II and ovulation**

**Ang II signaling and receptors near ovulation**

Several studies on cattle demonstrated that Ang II levels increase in follicular fluid after administration of an ovulatory dose of gonadotropin (Acosta et al. 2000, Shimizu et al. 2007). Schauser et al. (2001) observed intense AGTR2 receptor binding in cow periovulatory follicles, mainly in the theca cells. Using an in vivo model, our group demonstrated that i.m. injection of GNRH regulates the mRNA-encoding AGTR2 receptor in theca cells from follicles ≥12 mm in diameter.

Ang II receptor antagonists inhibit ovulation and have been used to study the role of RAS during the ovulatory process in rodents, rabbits, and cattle (Pellicer et al. 1988, Kuji et al. 1996, Ferreira et al. 2007). i.p. injection of saralasin blocked gonadotropin-induced ovulation in immature rats (Pellicer et al. 1988). However, a selective nonpeptide antagonist for AGTR2 receptors (PD123,319) had no effects on the ovulation rate in in vitro-perfused rat ovaries (Mikuni et al. 1998). Furthermore, the administration of PD123,319 diminished but did not completely block ovulation in rats (Mitsub et al. 2003). Taken together, these results indicate that Ang II is essential for ovulation in rats, but this effect is not exclusively regulated via the AGTR2.

In rabbit ovaries perfused in vitro, it was demonstrated that Ang II signals through AGTR2 receptor subtype (Kuji et al. 1996). Furthermore, ovulation induced by Ang II and prostaglandins (PGE and PGF$_{2\alpha}$) synthesis induced by hCG were inhibited by PD123,319 (Yoshimura et al. 1996b). We recently demonstrated that the ovulation is inhibited when AGTR2 receptor antagonists (saralasin or PD123,319) are intrafollicularly injected at the moment of GNRH injection in cows (Ferreira et al. 2007). Moreover, the LH-induced prostaglandin synthase 2 (PTGS2) mRNA expression is increased by Ang II and inhibited by PD123,319 in granulosa cell cultures from large dominant bovine follicles (Portela et al. 2011). In both bovine and rabbit models, the AGTR1 blockade did not affect gonadotropin-induced ovulation (Kuji et al. 1996, Ferreira et al. 2007). As discussed above, these in vitro and in vivo studies suggest that Ang II plays an important role in the early mechanism of ovulation via the AGTR2 receptor subtype in rabbits and cattle.

Ang II is capable of inducing ovulation in rabbits even in the absence of gonadotropins (Kuo et al. 1991, Yoshimura et al. 1992). The effect of Ang II during ovulation is mediated, at least in part, by PGs. In rabbit ovaries perfused in vitro, Ang II stimulated the synthesis of PGE$_2$ and PGF$_{2\alpha}$ in the absence of gonadotropins (Yoshimura et al. 1993). The same authors inhibited Ang II-induced PGs synthesis and ovulation using indomethacin (an inhibitor of cyclooxygenases). The fact that PGs mediate Ang II functions in the periovulatory period was further confirmed in other studies assessing meiotic resumption of bovine oocytes (Barreta et al. 2008).

Factors from the EGF family, known as EGF-like growth factors, are immediately upregulated by LH surge in vivo and also after LH treatment in follicular cell cultures (Park et al. 2004, Li et al. 2009). Amphiregulin (AREG) and epiregulin (EREG) are two of the LH-induced genes mediating the gonadotropin-induced PTGS2 expression (Park et al. 2004, Andric & Ascoli 2008, Li et al. 2009). The hypothesis that Ang II is a mediator of LH action in the cascade of events leading to ovulation was confirmed by our group using bovine granulosa cell cultures derived from >10 mm follicles. Using this in vitro system, Ang II significantly amplified the positive effect of LH on PTGS2 (mRNA and protein expression), AREG, EREG, and plasminogen activators (Portela et al. 2011). A dramatic upregulation of ADAM17, an essential factor for the initial ovulatory process (Yamashita et al. 2007), was also observed 1 h post-treatment with Ang II. This early effect of Ang II preceded the induction of LH-induced genes, such as AREG, EREG, and PTGS2 by 2–5 h. Galardin, a broad-spectrum matrix metalloproteinase inhibitor, completely blocked the effects of LH + Ang II, demonstrating that sheddase activity is the direct target of Ang II. Collectively, the results obtained in vivo using Ang II receptors blockade and in vitro with granulosa cell cultures suggest that Ang II is a mediator of LH surge during the early mechanism of bovine ovulation (Fig. 2).

**Ang-(1–7) and follicle development and ovulation**

Studies regarding Ang-(1–7) functions in ovarian physiology are more recent and scarcer than those regarding Ang II. Ang-(1–7) was first described in rat ovaries and was shown to be regulated during the estrous cycle (Costa et al. 2003). Recently, Ang-(1–7), MAS receptor, and ACE2 were identified in all the stages of follicular development in humans (Reis et al. 2011). Ang-(1–7) g-protein-coupled receptor (MAS) and ACE2, an enzyme capable of converting Ang I or Ang II into Ang-(1–7), are expressed in the rat ovary (Pereira et al. 2009). Many enzymes present in several tissues are involved in Ang-(1–7) synthesis from either Ang I or Ang II. Neutral endopeptidase (NEP) converts Ang I into Ang-(1–7). Prolyl endopeptidase (PEP) and ACE2 are involved in the conversion of Ang I or Ang II into Ang-(1–7). ACE and aminopeptidases (AMPs) inactivate Ang-(1–7) through its cleavage into smaller fragments. The pathways of Ang II and Ang-(1–7) synthesis, their receptors, and interactions between RAS components were recently revised by Santos et al. (2008).
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Figure 2 Proposed model of the renin–angiotensin system in the regulatory mechanism of ovulation. Angiotensin II (Ang II) amplified the positive effect of LH on PTGS2, EREG, AREG, and ADAM17 mRNA expression. The metalloproteinase inhibitor Galardin blocked the effect of Ang II plus LH on AREG, EREG and PTGS2 mRNA expression. These findings, taken together with the data of the literature that ADAM17 is the major sheddase in granulosa cells, suggest that epidermal growth factor-like peptide precursors (Pro-EGF(P)) are proteolytically cleaved by ADAM17, resulting in amphiregulin (AREG) or epiregulin (ERG), which binds to EGF receptor (EGFR). In short, Ang II seems to be a mediator of LH action in promoting the activation/expression of disintegrin and metalloproteinase (ADAM)-17 in the induction of events leading to ovulation in cattle.

Functional studies evidenced a role for the RAS branch composed of ACE2, MAS receptor, and Ang-(1–7) in follicle development in the rodent model. In ovarian homogenates from immature rats, Ang-(1–7) was shown to increase during proestrus and estrus and after equine chorionic gonadotropin (eCG) treatment (Costa et al. 2003). Immunolocalization studies demonstrated that eCG-induced Ang-(1–7) synthesis occurs mainly in the theca and interstitial cells (Pereira et al. 2009). Interestingly, MAS receptor immunostaining, which was not observed in immature ovaries, appeared in the theca and interstitial cells from antral and preovulatory follicles after eCG treatment (Pereira et al. 2009). A positive effect of gonadotropins on Ang-(1–7) concentration was also observed in the plasma from women who underwent ovarian stimulation (Reis et al. 2011).

Perfusion of Ang-(1–7) or Ang II in immature rat ovaries induced an increase in estradiol and progesterone synthesis (Costa et al. 2003). The positive effect of Ang-(1–7) on steroidogenesis was inhibited by the specific MAS receptor antagonist A-779. In contrast, A-779 disrupted Ang II effect on progesterone, but not estradiol synthesis, suggesting that Ang II effect on progesterone may be a result of its conversion to Ang-(1–7) (Costa et al. 2003, Simoes et al. 2004).

Ang-(1–7) protein is also present in rat oocytes, while MAS and Ang-(1–7) are absent in rat granulosa cells (Pereira et al. 2009). ACE2 was observed in stroma and granulosa cells from immature ovaries and in all follicular compartments, including the oocyte, from eCG-treated ovaries (Pereira et al. 2009). In accordance with the protein levels and mRNA expression, MAS receptor and ACE2 increase after eCG treatment in rat ovaries homogenates (Pereira et al. 2009). In cattle, ACE2 mRNA expression was upregulated in the dominant follicle at the expected time of follicular deviation, coinciding with an acute increase in intrafollicular estradiol levels (MH Barreta, R Ferreira & PB Goncalves 2011, unpublished data). The regulation of ACE2 by gonadotropins and its differential mRNA expression in dominant and subordinate follicles reinforce the theory of local Ang-(1–7) production.

Regarding the enzymatic activity, ACE and NEP activities in the rat ovaries were reduced after eCG treatment (Pereira et al. 2009). In the bovine model, ACE mRNA was more expressed in the subordinate follicles entering atresia and was severely suppressed in the estrogenic dominant follicle (Ferreira et al. 2011b). Collectively, these data suggest that mRNA expression and activity of ACE are negatively correlated with the estradiol levels and moreover indicate a reduction in Ang-(1–7) cleavage when estradiol levels are elevated. The increased availability of Ang-(1–7) may positively regulate estradiol synthesis as demonstrated previously in rat ovaries perfused in vitro (Costa et al. 2003). Regarding PEP enzyme, conflicting results were observed in different models. In rodents, PEP activity seems to be positively correlated with the estradiol levels (Ohta et al. 1992, Pereira et al. 2009). On the other hand, our preliminary results demonstrate that PEP mRNA expression is upregulated during subordinate follicle atresia in cattle.

In the ovulation process, Ang-(1–7) enhanced the ovulatory efficiency in in vitro-perfused rabbit ovaries, which was antagonized by A-779 (Reis et al. 2009). Using a bovine model in which cows are synchronized to obtain preovulatory follicles of at least 12 mm, Ang-(1–7) levels increased in the follicular fluid between 12 and 24 h after GNRH injection in vivo (Fig. 3). The increase in Ang-(1–7) just before ovulation is probably a consequence of ACE2, NEP, and PEP activities, since mRNAs that encode these three enzymes are upregulated concomitantly to Ang-(1–7) increase (Santos et al. 2011). Collectively, these results and the fact that A-779 reduces hCG-induced ovulation in rabbits (Reis et al. 2009) suggest that Ang-(1–7) is a mediator of gonadotropin functions in the ovulatory cascade.

**Summary and conclusion**

It is increasingly evident that RAS is involved in the local control of ovarian physiology. However, the intracellular mechanism of action triggered by angiotensin is not yet known in the ovary. New enzymes and receptors have been described and seem to modulate the local synthesis of angiotensin. It has been recently demonstrated that prorenin, which is thought to be an inactive peptide, can
bind to (pro)renin receptor and trigger local action independently of Ang II. The RAS is extremely complex and difficult to study, given that most of the active peptides are post-translationally processed. For many years, it was thought that Ang II was the only active peptide of the RAS; however, it is now known that Ang-(1–7), Ang III, and Ang IV play important roles in various tissues. Based on recent findings, we propose that these peptides are acting in the ovary. However, additional studies are still necessary to better characterize the cell and molecular pathways.

There is consensus that RAS functions in the ovary are species specific. We have focused our research in the cow model because it is a monovulatory species in which we could monitor the follicle deviation, accurately predict ovulation time, and modify the follicular environment. We have shown cattle to be a reliable in vivo animal model to study the RAS in ovarian function. Moreover, a serum-free culture system of granulosa cells without spontaneous luteinization and a culture system of large follicles were used to further investigate RAS on specific regulatory factors of follicular cells. Based on these models, we found that i) Ang II concentration increases in the follicular fluid of the dominant follicle during and after deviation; ii) follicular development was completely blocked with i.f. injection of a competitive antagonist of Ang II or a selective antagonist for AGTR2 receptors; iii) i.f. injection of Ang II or AGTR2 agonist prevented the expected regression of the second largest follicle at deviation; iv) Ang II antagonist downregulated the mRNA expression of genes involved in granulosa cell proliferation and differentiation (LHR, CYP19A1, and HSD3β) during follicular deviation; v) i.f. injection of a competitive antagonist of Ang II decreased ovulation rate in cows induced with a GNRH agonist; and finally vi) Ang II acts via the AGTR2 receptor, increasing the expression/activity of ADAM17 and resulting in the upregulation of mRNA-encoding genes involved in the ovulation cascade, such as AREG, EREG, and PTGS2. Based on these findings and studies by other groups, we conclude that RAS plays fundamental roles in follicular development, deviation, atresia, and ovulation in a species-specific manner.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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