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Angiotensin II, progesterone, and prostaglandins are sequential steps in the pathway to bovine oocyte nuclear maturation

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Abstract

Oocyte meiotic resumption is triggered by the ovulatory gonadotropin surge; in cattle, angiotensin II (AngII) and prostaglandins (PG) are key mediators of this gonadotropin-induced event. Here, we tested the hypothesis that progesterone (P_4) is also involved in oocyte meiotic resumption induced by the gonadotropin surge. In Experiment I, P_4 induced nuclear maturation in a dose-dependent manner using a coculture of follicular hemisections and cumulus-oocyte complexes. In the second experiment, using an *in vivo* model, an injection of mifepristone (MIFE; P_4 receptor antagonist) at the antrum of preovulatory follicles prevented GnRH-induced oocyte meiotic resumption *in vivo*. In Experiment III (coculture system similar to that of Experiment I), MIFE prevented stimulatory effects of AngII on resumption of meiosis, but saralasin (AngII receptor antagonist) did not inhibit P_4 actions. In Experiments IV and V, fibroblast growth Factor 10 (FGF10; known to suppress steroidogenesis in granulosa cells), blocked AngII-but not P_4-induced oocyte meiotic resumption. Therefore, we inferred that AngII is upstream to P_4 in a cascade to induce meiotic resumption. Previously, we had reported that AngII acted throughout the PGs pathway to modulate nuclear progression. In Experiment V, indomethacin inhibited resumption of meiosis induced by P_4, providing further support to the AngII-P_4 sequential effect on meiotic resumption. In conclusion, we inferred that AngII, P_4 and PGs are sequential steps in the same pathway that culminates with bovine oocyte maturation.

Keywords: Ovulation; Meiotic resumption; Angiotensin; Steroid; Eicosanoid

1. Introduction

The preovulatory gonadotropin surge triggers a cascade of events that culminates with ovulation and nuclear oocyte maturation. Recently, angiotensin II (AngII) has been recognized as one of the earliest mediators of gonadotropin-induced ovulation and oocyte maturation [1–3]. The positive effect of AngII in these processes is mediated through a Type 2 receptor [1]. Furthermore, the concentration of AngII and expression of its receptors (AT2) within the follicle increased during the interval between the gonadotropin surge and ovulation (Siqueira, et al, unpublished data). Other studies provided additional evidence that AngII regulated secretion of progesterone (P_4) and prostaglandins (Pg), hormones involved in ovulation [4,5]. In granulosa cell culture, AngII upregulated expression of cyclooxygenase 2 (COX-2), the rate-limiting enzyme for PG production [3].

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During follicle development, bovine oocytes remain arrested at prophase of the first meiotic division, and resume meiosis after the preovulatory LH surge [6], or after removal from the follicular environment [7]. The presence of follicular wall fragments in a coculture system with cumulus-oocyte complexes (COCs) prevents meiotic resumption [8]. This coculture system is a good model to study the role of factors that act through follicular cells on oocyte nuclear maturation [9,10]. Using this coculture system, we reported that AngII acted through a PG pathway to mediate gonadotropin-induced oocyte meiotic resumption [2].

The cyclooxygenase pathway is a classical mediator of LH-induced ovulation and nuclear oocyte maturation in cattle [11–15]. Progesterone is another key element in the ovulatory cascade and oocyte maturation [13,14,16]. Indeed, there are indications that PGs are downstream factors to this steroid; in that regard, a gonadotropin surge stimulates an increase in intrafollicular P_4, which acts by binding to its nuclear receptor and increasing abundance of mRNA for COX2 [14]. The role of P_4 on oocyte nuclear maturation in cattle remains controversial. Nuclear and membrane progesterone receptors are present in bovine COCs, and regulated during *in vitro* maturation in the presence of FSH and LH [16]. Although Sirotkin [17] reported a stimulatory effect on oocyte meiotic resumption, more recent studies concluded that P_4 was not necessary to promote nuclear maturation, cumulus expansion, and early embryo development [18,19].

Follicular cells secrete factors that prevent oocyte meiotic resumption before the LH surge. The family of fibroblast growth factors (FGFs) is composed of more than 20 factors, largely studied for their roles in embryogenesis and oogenesis. Buratini, et al [20] reported that the bovine theca cells and oocytes expressed FGF10. Expression of FGF10 receptor (FGFR2IIIb) was identified in theca [21], granulosa [20], and cumulus cells [22]. Furthermore, FGF10 in the granulosa cell culture inhibited steroidogenesis [20] and AT2 expression [23]. Activation of FGF receptors (FGFRs) appeared to be involved in inhibition of germinal vesicle breakdown (GVBD) in mice [24]. Conversely, Zhang, et al [25] reported that FGF10 improved bovine oocyte maturation, cumulus expansion and subsequently embryo development in medium containing estradiol and in the absence of follicular cells.

The information summarized above provided an impetus to investigate interactions between FGF10 and factors involved in triggering bovine oocyte meiotic resumption. In the present study, a combination of *in vivo* and *in vitro* experiments were conducted to test the hypothesis that P_4 plays a role in regulation of oocyte meiotic progression induced by gonadotropin surge in concert with AngII and PGs. In an *in vitro* experiment, interactions of P_4 and AngII with FGF10 (an antisteroidogenic factor recently described as an important regulator of follicular development) were studied, with regards to their roles in resumption of meiosis.

2. Materials and methods

All experimental procedures were reviewed and approved by the Federal University of Santa Maria Animal Care and Use Committee (23081.004717/2010–53 CCR/UFSM). All chemicals used were purchased from Sigma Chemical Company (St. Louis, MO, USA), unless otherwise indicated in the text.

2.1. Preparation of follicular hemisections, oocyte recovery and nuclear maturation

Bovine ovaries at various stages of the estrous cycle were obtained from an abattoir and transported to the laboratory in saline solution (0.9% NaCl) at 30 °C containing 100 IU ml^{-1} penicillin and 50 μg ml^{-1} streptomycin sulfate. Procedures for follicle dissection and culture procedures were previously validated in our laboratory [2,9,10]. Briefly, transparent follicles, 2 to 5 mm in diameter, were selected and dissected from ovarian stromal tissue, and sectioned into halves. Follicular hemi-sections were washed in TCM 199 containing 0.4% bovine serum albumin (BSA) and randomly distributed into four-well culture dishes (Nunc, Roskilde, Denmark) containing culture medium with the desired treatment. There were eight follicular halves per 200 μl of medium. Dishes were incubated for 2 h before adding COCs.

The COCs were aspirated from follicles 3 to 8 mm in diameter, recovered under a stereomicroscope, and selected according to Leibfred and First [26]. Grades 1 and 2 COCs (n = 10 to 30) were randomly distributed into treatments and cultured in an incubator at 39 °C in a saturated humidity atmosphere containing 5% CO_2 in air and 95% air, for either 7, 15, or 24 h, depending on the experiment. The culture medium used was TCM 199 containing Earle's salts and L-glutamine (Gibco BRL, Grand Island, NY, USA) supplemented with 25 mM HEPES, 0.2 mM pyruvic acid, 2.2 mg ml^{-1} sodium bicarbonate, 5.0 μg/ml LH (lutropin-V, Bioniche, ON, Canada), 0.5 μg/ml FSH (Folltropin-V, Bioniche), 0.4% fatty acid-free BSA, 100 IU ml^{-1} penicillin, and 50 μg ml^{-1} streptomycin sulfate. At the end of the

culture period, cumulus cells were removed by vortexing for 5 min and oocytes were fixed with 4% paraformldehyde for 15 min, followed by permeabilization of the nuclear membranes with 0.5% Triton X-100. After 2 h, oocytes were fixed, stained with Hoechst (33,342) and mounted under a coverslip with Vectashield (Vector Laboratories, Burlington, Ontario, L7N 3J5, Canada) for nuclear evaluation. Oocytes were classified according to their nuclear chromatin configuration using a fluorescent microscope as germinal vesicle (GV), GV breakdown (GVBD), metaphase I (MI), anaphase I (AI), telophase I (TI), and metaphase II (MII). In all experiments, all treatments were repeated three times.

2.2. Cattle, superovulation protocol, and ultrasound-guided intrafollicular injection

The superovulation protocol and intrafollicular injection procedures were previously described [2]. Five cycling cows (*Bos taurus taurus*), multiparous, with body condition scores of 3 and 4 (1 = thin to 5 = obese) were submitted to the 9-d "progesterone/FSH-based" superovulation protocol. On day 9 of the progesterone treatment, the number of follicles in each ovary was evaluated by transrectal ultrasonography, and all follicles 5 to 11 mm in diameter were aspirated using a vacuum pump, leaving no more than the three largest follicles in each ovary. On the afternoon of Day 10, after the intravaginal device had been removed, each ovary was examined with transrectal ultrasonography, a map of the follicles was prepared, and all follicles >12 mm in diameter were subjected to intrafollicular injections.

Intrafollicular injections were done with a 7.5 MHz transducer attached to a biopsy guide and a scanner (AquilaVet Scanner; Pie Medical Equipment BV, Maastricht, the Netherlands). A system with two sterile needles was used, as previously described [1]. Briefly, the ovary was manipulated to introduce the needle into the follicle via the ovarian stroma at the base of the follicle. When the ovary and follicle were in position, the outer needle was advanced until the image of its tip became visible on the screen, 3 to 5 mm from the follicle. At this moment, a second operator pushed the inner needle forward until the image of the needle tip was visible within the follicle. Treatments were then injected into the follicle. To obtain the desired final concentration inside the follicle, the dose of each treatment was calculated based on the volume of follicular fluid, estimated by the linear regression equation $V = -685.1 + 120.7\ D$, where V corresponded to the estimated follicular volume and D to the diameter of the follicle to be injected [1]. The injection volume per follicle ranged from 80 to 110 μl, approximately 10% of follicular fluid volume. Cows were excluded from the experiment if the injected follicle had a reduction in diameter >1 mm within 2 h after injection (evidence of leakage).

2.2.1. Experiment I: progesterone induced oocyte nuclear maturation in vitro

The first experiment was designed to assess the P_4 effect on nuclear maturation. Oocytes (n = 565) cultured with follicular hemisections treated were with 0, 10, 100, 1,000 or 10,000 ng/ml of P_4. After 22 h of culture, oocytes were considered mature when classified as AI, TI, or MII.

2.2.2. Experiment II: effect of progesterone antagonist on lh-induced meiotic resumption in vivo

Five cows were primed for superovulation and manipulated to have no more than three follicles >12 mm in each ovary at the time of injection. For each cow, follicles in the right ovary were treated to obtain a final concentration in follicular fluid of 1 μM of mifepristone (MIFE group; n = 10), whereas those from the left ovary were treated with 0.9% saline (control group; n = 10). Immediately after the intrafollicular injections, the cows were given 100 μg of gonadorelin acetate im (Profertil, Tortuga, Brazil), a GnRH agonist. Fifteen h after GnRH treatment, cows were ovariectomized by colpotomy. The COCs were recovered and processed as described above. Oocytes at GVBD or MI stages were considered as having resumed meiosis.

2.2.3. Experiment III: progesterone mediates AngII-induced meiotic resumption in vitro

The COCs (n = 540) were selected and distributed among the following seven groups for 15 h of culture: positive and negative controls; AngII (10^{-11} M); AngII plus MIFE (1 μM; P_4 antagonist); P_4 (100 ng/ml), P_4 plus saralasin (10^{-5} M; AngII antagonist); and AngII plus saralasin. In all groups, except the positive control, follicular hemisections and COCs were cocultured. Oocytes in MI or latter stages were considered to have normal resumption of meiosis.

To determine if there was a toxic effect of the P_4 antagonist, COCs were cultured for 22 h, without follicular hemisections, in the absence or presence of MIFE (1 μM). Oocytes were considered mature when classified as AI, TI or MII.

2.2.4. Experiment IV: effect of FGF10 on AngII-induced meiotic resumption in vitro

Control COCs were cultured in medium in the absence (positive control; n = 84) or presence (negative control; n = 88) of follicular hemisections for 7 h. Four treatment groups were established; the COCs were cultured in the presence of: a) AngII (10^{-11} M; n = 83) with follicular hemisections; b) AngII and FGF10 (100 ng/ml) with follicular hemisections (AngII+FGF10 group; n = 82); c) FGF10 with follicular hemisections (FGF10+cells group; n = 80); and d) FGF10 without follicular hemisections (FGF10 group; n = 88). Oocyte nuclear chromatin configuration was classified as GV or germinal vesicle breakdown (GVBD).

2.2.5. Experiment V: effect of FGF10 or indomethacin on progesterone-induced meiotic resumption in vitro

Control COCs were cultured for 7 h in the absence (positive control; n = 85) or presence (negative control; n = 82) of follicular hemisections. Three treatment groups were established. The COCs were cocultured with follicular cells in the presence of: a) progesterone (100 ng/ml; P_4 group; n = 84); b) P_4 plus FGF10 (100 ng/ml; P_4 + FGF10 group; n = 80) and c) P_4 plus indomethacin (a COX nonselective inhibitor; 10 μM, P_4+indo group; n = 85). Oocyte nuclear chromatin configuration was classified as GV or germinal vesicle breakdown (GVBD).

2.3. Statistical analysis

Data from Experiments I, III, IV, and V were analyzed using the ANOVA test in a statistical model for categorical data, using the PROC CATMOD (Categorical Data Analysis Procedures). All *in vitro* experiments were performed in triplicate. When there were significant differences, independent variables were compared using the contrast test. All data were analyzed using statistical analysis software (SAS; SAS Institute, Inc., Cary, NC, USA). In Experiment II, meiotic resumption was compared using the generalized linear models from JMP software (SAS Institute, Inc.).

3. Results

3.1. Experiment I: progesterone induced oocyte nuclear maturation in vitro

The hypothesis tested in this experiment was that P_4 induces nuclear maturation in bovine oocytes. Bovine COCs, recovered from abattoir-derived ovaries, were cocultured with follicular hemisections for 22 h with various concentrations of P_4. Progesterone induced nuclear maturation in bovine oocytes cultured with follicular cells in a dose-dependent manner (Fig. 1). The MII rate was greatest for oocytes cultured with follicular cells treated with 100 ng/ml of P_4 ($P < 0.01$).

3.2. Experiment II: effect of progesterone antagonist on LH-induced meiotic resumption in vivo

Once P_4 stimulated nuclear maturation *in vitro*, whether the LH-induced resumption of meiosis was mediated by progesterone was tested using an *in vivo* model. The mean initial diameter of follicles treated with progesterone antagonist (MIFE; 12.8 ± 0.4 mm) did not differ from those injected with saline (13.1 ± 0.5 mm; $P > 0.05$). From the injected follicles, 20 oocytes were recovered and evaluated (10 per group). The ability of the LH surge (induced by the GnRH agonist) to induce resumption of meiosis was impaired when follicles were treated with the progesterone receptor antagonist (MIFE; 70, 10 and 20% were GV, GVBD, and MI, respectively; $P < 0.01$; Fig. 2B). As expected, the GnRH agonist induced 90% of meiotic resumption in oocytes from saline-treated follicles (10, 10, and 80% were GV, GVBD, and MI).

3.3. Experiment III: progesterone mediated AngII-induced meiotic resumption in vitro

Since the role of AngII in resumption of meiosis and ovulation is well established, we tested the hypothesis that AngII is an upstream factor to P_4 in the cascade of meiotic resumption. Meiotic resumption

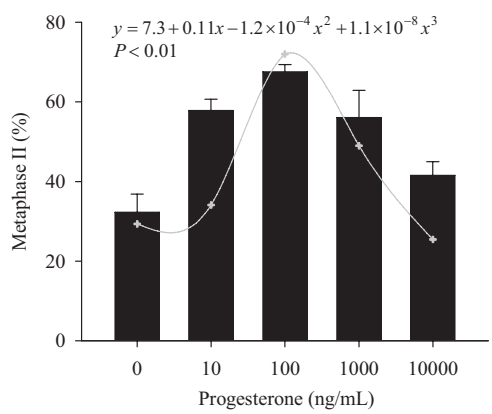

Fig. 1. Effect of progesterone on nuclear maturation of bovine oocytes (metaphase II, Anaphase I and Telophase I; Experiment I). Rates of oocyte maturation (solid bars) and predicted regression line after coculture of oocytes (n = 565) and follicular hemisections treated for 22 h with various concentrations of P_4. The experiment was performed in triplicate.

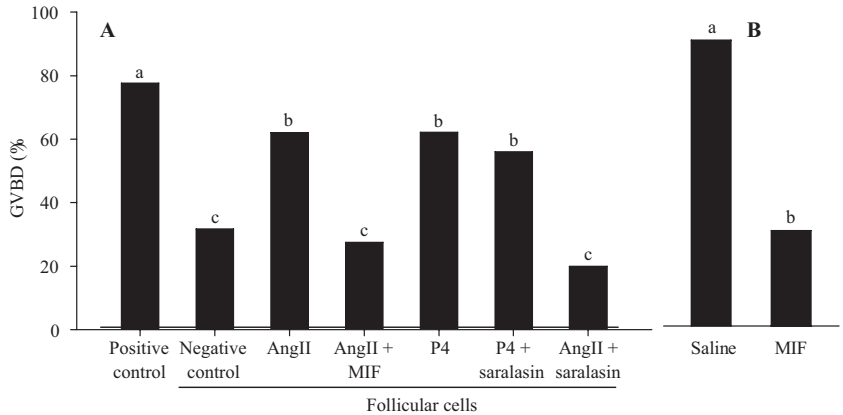

Fig. 2. Effect of angiotensin II (AngII), progesterone (P_4) or progesterone antagonist on the cascade of oocyte meiotic resumption *in vitro* (Panel A) or *in vivo* (Panel B). *in vitro*, bovine cumulus–oocyte complexes (n = 540) were cocultured for 15 h with follicular cells and AngII, AngII plus mifepristone (MIFE), P_4, P_4 plus saralasin, and AngII plus saralasin (Experiment III). *in vivo*, follicles (≥12 mm) were challenged with GnRH and intrafollicular injected with saline (n = 10) or MIFE (n = 10). After 15 h, oocytes were obtained by follicular aspiration to access the nuclear maturation stage (Experiment II). [a-c]Within a panel, columns without a common superscript differed ($P < 0.05$).

was inhibited when the COCs were cocultured with follicular hemisections (Fig. 2A; positive vs. negative controls). With this model, AngII or P_4 induced meiotic resumption (61 and 66%, respectively, compared with 32% of the negative control; $P < 0.01$). However, AngII did not induce resumption of meiosis when saralasin or MIFE was present in the maturation medium. Independent of the presence of saralasin, most oocytes reached MI in the presence of P_4. A further experiment was done, culturing COCs without follicular hemisections for 22 h, with or without MIFE, to exclude a detrimental effect on oocyte maturation. Oocytes treated with MIFE reached a similar rate of nuclear maturation (88%) to that of oocytes cultured in the control medium (85%).

3.4. Experiment IV: effect of FGF10 on AngII-induced meiotic resumption in vitro

Our hypothesis was that FGF10 has a negative role in the resumption of meiosis induced by AngII. In the absence of follicular cells, the rate of meiotic resumption rate was not different between the positive control and FGF10-treated COCs after 7 h of culture (Fig. 3A). Also, FGF10 did not affect the ability of follicular cells to prevent oocytes from resuming meiosis. However, FGF10 inhibited the AngII effect in follicular cells. When oocytes were cultured simultaneously with AngII and FGF10, 32% achieved GVBD, whereas 62% of those cultured only with AngII achieved GVBD ($P < 0.01$; Fig. 3A).

3.5. Experiment V: effect of FGF10 or indomethacin on P_4-induced meiotic resumption in vitro

The role of FGF10 in the oocyte meiotic resumption induced by P_4 was examined. Using a coculture system of oocytes and follicular hemisections, the P4 effect on the meiosis resumption was not affected by FGF10 ($P > 0.05$). However, when indomethacin (a nonselective PG antagonist) was included in the coculture system of oocytes and follicular hemisections, the P_4 effect was inhibited, implicating prostaglandins in P4-induced meiosis resumption (Fig. 3B).

4. Discussion

In the present study, we tested the hypothesis that P_4 is an intermediate factor between AngII and PGs in the meiotic resumption stimulatory cascade. The main findings were 1) progesterone induced meiosis resumption, in a concentration-dependent manner, of bovine cumulus-enclosed oocytes cultured with follicle walls; 2) MIFE inhibited GnRH-induced oocyte meiotic resumption *in vivo*; 3) MIFE inhibited oocyte meiotic resumption induced by AngII *in vitro*, whereas an AngII receptor antagonist did not interfere with the P_4 stimulatory effect; 4) P_4-induced oocyte meiotic resumption was blocked by indomethacin (cox non-selective inhibitor) *in vitro*; and 5) FGF10 inhibited AngII-but not P_4-induced oocyte meiotic resumption *in vitro*. Previously, using the same *in vitro* coculture system of bovine cumulus-enclosed oocytes and follicular hemisections, we reported that AngII acted through

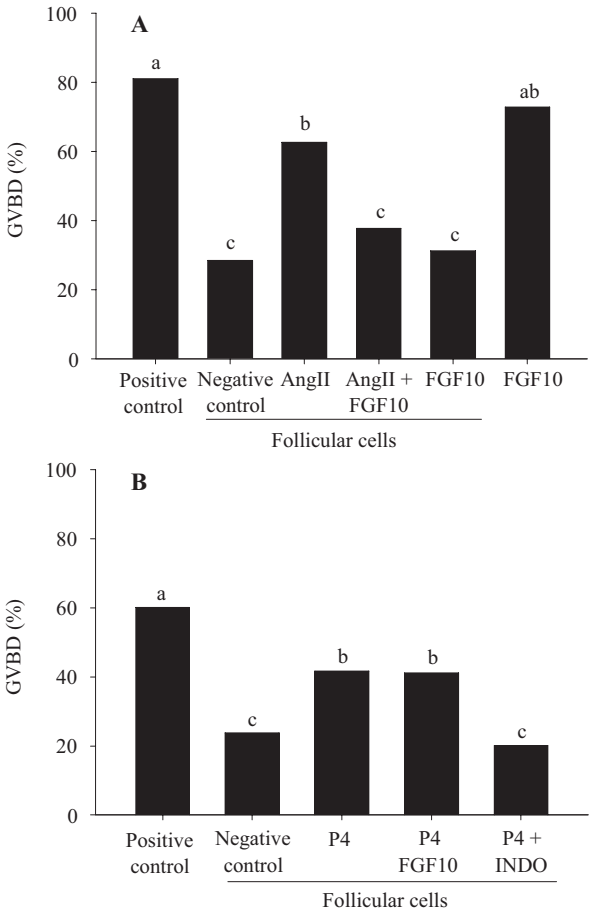

Fig. 3. Effect of fibroblast growth Factor 10 (FGF10) or indomethacin (INDO-) on AngII- or P4-induced meiotic resumption. Bovine cumulus-oocyte complexes were cultured for 7 h, with or without follicular hemisections. Both experiments were performed in triplicate. [a-c]Within a panel, columns without a common superscript differed ($P < 0.05$).

PGs to mediate LH-induced oocyte meiotic resumption [2] and that AngII, in synergism with LH, induced P_4 and PG synthesis in the bovine dominant follicle (Siqueira, et al unpublished). Based on all of these data, we inferred that AngII is upstream to P_4 in a pathway to induce oocyte nuclear maturation that is initiated by a gonadotropin surge and stimulates production of PGs.

In this study, we used two experimental models already established. In the first approach, spontaneous meiotic progression was inhibited in a coculture system with oocyte and follicular hemisections [8,9]. With this model, P_4 stimulated oocyte nuclear maturation in a dose-dependent manner. In the second model, cows were primed for superovulation and, after a GnRH challenge, intrafollicular injections guided by ultrasongraphy were performed in the right (treatment) and left (control) ovaries [1,2]. In this experiment, MIFE inhibited oocyte meiotic resumption. Progesterone also participates in the oocyte nuclear maturation in primates and swine [27,28]. In monkeys, inhibition of follicular progesterone production by trilostane (steroid synthesis inhibitor) did not reduce gonadotropin-induced oocyte maturation, but increased the percentage of degenerated oocytes [27]. In pigs, treatment of COCs with MIFE modified the pattern of expression of P_4 receptors in cumulus and reduced progesterone synthesis [28].

The reason of the lower positive effect at higher doses of progesterone was unclear. A similar progesterone dose–response effect was observed in oxytocin secretion in bovine granulosa cells cultured *in vitro* [29]. Nevertheless, 1,000 and 10,000 ng/ml are supraphysiologic doses; therefore, reduced support for oocyte maturation with these doses may not be physiologically relevant. Previous studies demonstrated that progesterone concentrations in follicular fluid *in vivo* increased between Time 0 and 3.5 h after GnRH, decreased between 6 and 18 h, with a second increase in progesterone evident at 24 h [14]. These increases were concomitant to the upregulation of progesterone receptor mRNA expression in follicular wall [4]. Nevertheless, the maximum concentration of progesterone in follicular fluid between the LH surge and ovulation in cattle is 250 ng/ml [14].

Previously, others and we reported that P_4 (mediated by AngII) [2] and PGs [30,31] are in the pathway of oocyte meiotic resumption. Herein, we confirmed the hypothesis that AngII is upstream to P_4 in the cascade of resumption of meiosis. In Experiment III, a P_4 receptor antagonist prevented AngII stimulatory effects on resumption of meiosis, but saralasin did not inhibit P_4 actions. There are indications that the stimulatory effects of AngII on oocyte nuclear maturation are mediated by PGs [2]. In Experiment V, indomethacin inhibited resumption of meiosis induced by P_4, suggesting that PGs also mediate this steroid actions. Progesterone is essential to induce PG secretion during the ovulatory process [5] and we recently demonstrated that AngII has a synergistic action with LH to induce production of P_4 and PGs by granulosa cells from large dominant follicles (Siqueira, et al, unpublished). Based on these data, we inferred that AngII, P_4 and PGs are sequential steps from the same pathway.

We previously demonstrated that Ang II has no effect on meiotic resumption *in vitro* in the absence of follicular cells [9]. Nevertheless, we also demonstrated that Ang II is indispensable for bovine oocyte meiotic

resumption *in vivo* [2]. Cumulus-oocyte complexes matured *in vitro* in media supplemented with BSA and gonadotrophins, (in similar concentrations to those used in the present experiments) can synthesize progesterone, reaching concentrations of 40 ng/ml after 16 h of culture [32]. Perhaps these concentrations are not enough to overcome the negative effect of follicular cells. Unfortunately, progesterone secretion by COCs cocultured with follicular hemisections was not measured.

Oocytes remain arrested in germinal vesicle during follicle development and are able to reinitiate meiosis after the LH surge. The coculture of oocytes and follicular hemisections efficiently inhibits oocyte meiotic resumption, probably because during the culture period, cells from 3 to 8 mm follicles produce inhibitory factors. Using the coculture system, we can reproduce the inhibitory effect of the follicle environment and test if LH-induced factors, e.g. Ang II, progesterone and prostaglandins, are able to overcome the negative effect of follicular cells-secreted factor on meiotic resumption.

There were no indications that toxicity was responsible for the inhibitory effects of the antagonists used. Saralasin, MIFE, and indomethacin are safe for cell viability and function [2,5]. Indeed, in the present study, saralasin did not affected P_4-induced meiotic resumption. Also, MIFE (1 μM) in the absence of follicular hemisections did not impair bovine oocyte nuclear maturation (Experminent III), nor did it affect subsequent embryo development [16]. Recently, it was demonstrated that progesterone signaling is not essential for bovine oocyte meiotic resumption *in vitro* using trilostane [16]. Based on these data, we inferred that the progesterone positive effect on oocyte meiotic resumption is mediated through follicular cells *in vivo*.

In the present study, FGF10 inhibited the positive effect of AngII, but not of P_4 on oocyte meiotic resumption. Although cumulus cells also express FGF10 receptors [22], FGF10 did not affect meiotic resumption rate in the absence of follicular cells. Therefore, we inferred that FGF10 inhibited meiotic progression by acting on the follicular wall. Indeed, FGF10 may be acting on AngII-induced meiotic resumption by modulating steroid production in follicular cells. Type II receptors for AngII (AT2) are responsible for transducing AngII positive signal for resumption of meiosis in oocytes and ovulation [1]. Furthermore, FGF10 downregulates the expression of AT2 receptors in follicular cells [33] and inhibits steroidogenesis [20]. Activation of FGFR2IIIb (FGF10 receptor) inhibits gonadotropin-induced progesterone secretion in granulosa cells [34]. Therefore, FGF10 could be exerting its negative effect through downregulation of AT2 expression, and consequently, decreasing AngII-stimulated progesterone synthesis or directly inhibiting follicular cell steroidogenesis. The discrepancy between our results and those recently reported by Zhang, et al [25] could be due to differences in culture conditions, such as the presence of estradiol and the absence of follicular cells in the system. Nevertheless, further studies are necessary to elucidate the role of FGF10 on bovine oocyte nuclear maturation.

Taken together with other studies from our group, it is possible to propose a model (Fig. 4) in which the gonadotropin surge stimulates a single cascade of events to induce ovulation and nuclear oocyte maturation. In this model, gonadotropin surge stimulates AngII secretion and upregulates AT2 expression in follicular cells, whereas AngII increases follicular cells secretion of, P_4 that stimulates PGs. Ultimately, this sequence of events culminates with ovulation of a fertile oocyte.

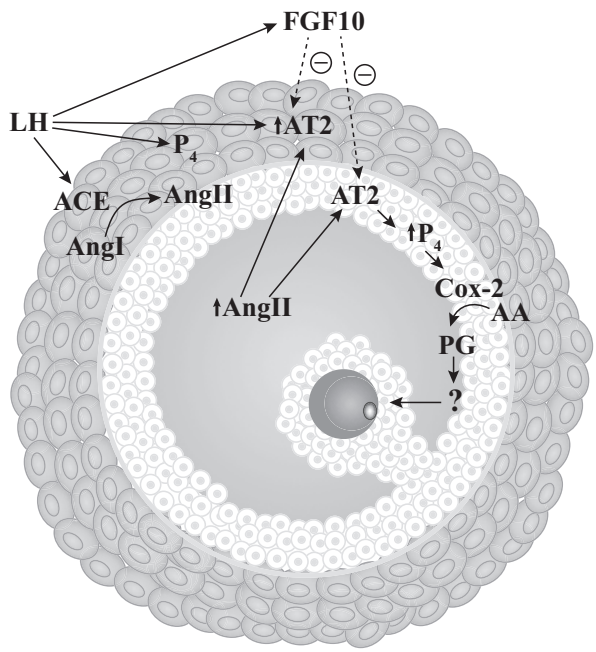

Fig. 4. Proposed model for a single cascade of events to induce ovulation and nuclear oocyte maturation in cattle. Preovulatory gonadotropin surge induces an up regulation of Type II angiotensin receptors (AT_2) and follicular Angiotensin converting enzyme expression (ACE). The FGF10 inhibitory effect to AT_2 expression is overcome by the gonadotropin surge. Upegulation of ACE induces an increase in follicular Angiotensin II (AngII) synthesis, which will bind to AT_2 to stimulate synthesis of progesterone (P_4) and PG.

In summary, based on the present work, we concluded that P_4 in cattle, similar to AngII and PGs, mediated the resumption of meiotic progression induced by gonadotropin surge. Indeed, based on our study, we speculated that AngII, P_4 and PGs are sequential steps in the same pathway that culminates with oocyte maturation.

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References

[1] Ferreira R, Oliveira JF, Fernandes R, Moraes JF, Gonçalves PB. The role of angiotensin II in the early stages of bovine ovulation. Reproduction 2007;134:713–19.
[2] Barreta MH, Oliveira JF, Ferreira R, Antoniazzi AQ, Gasperin BG, Sandri LR, et al. Evidence that the effect of angiotensin II on bovine oocyte nuclear maturation is mediated by prostaglandins E2 and F2alpha. Reproduction 2008;136:733–40.
[3] Portela VM, Zamberlam G, Gonçalves PB, de Oliveira JF, Price CA. Role of angiotensin II in the periovulatory epidermal growth factor-like cascade in bovine granulosa cells in vitro. Biol Reprod 2011;85:1167–74.
[4] Jo M, Komar CM, Fortune JE. Gonadotropin surge induces two separate increases in messenger RNA for progesterone receptor in bovine preovulatory follicles. Biol Reprod 2002;67:1981–88.
[5] Bridges PJ, Komar CM, Fortune JE. Gonadotropin-induced expression of messenger ribonucleic acid for cyclooxygenase-2 and production of prostaglandins E and F2alpha in bovine preovulatory follicles are regulated by the progesterone receptor. Endocrinology 2006;147:4713–22.
[6] Ayalon D, Tsafriri A, Lindner HR, Cordova T, Harell A. Serum gonadotrophin levels in pro-oestrous rats in relation to the resumption of meiosis by the oocytes. J Reprod Fertil 1972;31:51–8.
[7] Pincus G, Enzmann EV. The comparative behavior of mammalian eggs in vivo and in vitro: I. The activation of ovarian eggs. J Exp Med 1935;62:665–75.
[8] Richard FJ, Sirard MA. Effects of follicular cells on oocyte maturation. II: Theca cell inhibition of bovine oocyte maturation in vitro. Biol Reprod 1996;54:22–8.
[9] Giometti IC, Bertagnolli AC, Ornes RC, da Costa LF, Carambula SF, Reis AM, et al. Angiotensin II reverses the inhibitory action produced by theca cells on bovine oocyte nuclear maturation. Theriogenology 2005;63:1014–25.
[10] Stefanello JR, Barreta MH, Porciuncula PM, Arruda JN, Oliveira JF, Oliveira MA, et al. Effect of angiotensin II with follicle cells and insulin-like growth factor-I or insulin on bovine oocyte maturation and embryo development. Theriogenology 2006;66:2068–76.
[11] Algire JE, Srikandakumar A, Guilbault LA, Downey BR. Preovulatory changes in follicular prostaglandins and their role in ovulation in cattle. Can J Vet Res 1992;56:67–9.
[12] Bridges PJ, Fortune JE. Regulation, action and transport of prostaglandins during the periovulatory period in cattle. Mol Cell Endocrinol 2007;263:1–9.
[13] Nuttinck F, Marquant-Le Guienne B, Clément L, Reinaud P, Charpigny G, Grimard B. Expression of genes involved in prostaglandin E2 and progesterone production in bovine cumulus-oocyte complexes during in vitro maturation and fertilization. Reproduction 2008;135:593–603.
[14] Fortune JE, Willis EL, Bridges PJ, Yang CS. The periovulatory period in cattle: Progesterone, prostaglandins, oxytocin and ADAMTS proteases. Anim Reprod 2009;6:60–71.
[15] Nuttinck F, Gall L, Ruffini S, Laffont L, Clement L, Reinaud P, et al. PTGS2-related PGE2 affects oocyte MAPK phosphorylation and meiosis progression in cattle: Late effects on early embryonic development. Biol Reprod 2011;84:1248–57.
[16] Aparicio IM, Garcia-Herreros M, O'Shea LC, Hensey C, Lonergan P, Fair T. Expression, regulation, and function of progesterone receptors in bovine cumulus oocyte complexes during in vitro maturation. Biol Reprod 2011;84:910–21.
[17] Sirotkin AV. Involvement of steroid hormones in bovine oocytes maturation in vitro. J Steroid Biochem Mol Biol 1992;41:855–58.
[18] Silva CC, Knight PG. Effects of androgens, progesterone and their antagonists on the developmental competence of in vitro matured bovine oocytes. J Reprod Fertil 2000;119:261–69.
[19] Wang HF, Isobe N, Kumamoto K, Yamashiro H, Yamashita Y, Terada T. Studies of the role of steroid hormone in the regulation of oocyte maturation in cattle. Reprod Biol Endocrinol 2006;4:4.
[20] Buratini J Jr., Pinto MG, Castilho AC, Amorim RL, Giometti IC, Portela VM, et al. Expression and function of fibroblast growth factor 10 and its receptor, fibroblast growth factor receptor 2b, in bovine follicles. Biol Reprod 2007;77:743–50.
[21] Berisha B, Sinowatz F, Schams D. Expression and localization of fibroblast growth factor (FGF) family members during the final growth of bovine ovarian follicles. Mol Reprod Dev 2004;67:162–71.
[22] Cho JH, Itoh T, Sendai Y, Hoshi H. Fibroblast growth factor 7 stimulates in vitro growth of oocytes originating from bovine early antral follicles. Mol Reprod Dev 2008;75:1736–43.
[23] Portela VM, Gonçalves PB, Veiga AM, Nicola E, Buratini J Jr., Price CA. Regulation of angiotensin type 2 receptor in bovine granulosa cells. Endocrinology 2008;149:5004–11.
[24] Peluso JJ. N-cadherin mediated cell contact inhibits germinal vesicle breakdown in mouse oocytes maintained in vitro. Reproduction 2006;131:429–37.
[25] Zhang K, Hansen PJ, Ealy AD. Fibroblast growth factor 10 enhances bovine oocyte maturation and developmental competence in vitro. Reproduction 2010;140:815–26.
[26] Leibfried L, First NL. Characterization of bovine follicular oocytes and their ability to mature in vitro. J Anim Sci 1979;48:76–86.
[27] Borman SM, Chaffin CL, Schwinof KM, Stouffer RL, Zelinski-Wooten MB. Progesterone promotes oocyte maturation, but not ovulation, in nonhuman primate follicles without a gonadotropin surge. Biol Reprod 2004;71:366–73.
[28] Shimada M, Yamashita Y, Ito J, Okazaki T, Kawahata K, Nishibori M. Expression of two progesterone receptor isoforms in cumulus cells and their roles during meiotic resumption of porcine oocytes. J Mol Endocrinol 2004;33:209–25.

[29] Voss AK, Fortune JE. Estradiol-17 beta has a biphasic effect on oxytocin secretion by bovine granulosa cells. Biol Reprod 1993; 48:1404–09.
[30] Murdoch WJ, Peterson TA, Van Kirk EA, Vincent DL, Inskeep EK. Interactive roles of progesterone, prostaglandins, and collagenase in the ovulatory mechanism of the ewe. Biol Reprod 1986;35:1187–94.
[31] Murdoch WJ. Differential effects of indomethacin on the sheep ovary: Prostaglandin biosynthesis, intracellular calcium, apoptosis, and ovulation. Prostaglandins 1996;52:497–506.
[32] Mingoti GZ, Garcia JM, Rosa-e-Silva AA. Steroidogenesis in cumulus cells of bovine cumulus-oocyte-complexes matured in vitro with BSA and different concentrations of steroids. Anim Reprod Sci 2002;69:175–86.
[33] Portela VM, Gonçalves PBD, Veiga AM, Nicola E, Buratini J Jr., Price CA. Regulation of angiotensin type 2 receptor in bovine granulosa cells. Endocrinology 2008;149:5004–11.
[34] Parrott JA, Skinner MK. Developmental and hormonal regulation of keratinocyte growth factor expression and action in the ovarian follicle. Endocrinology 1998;139:228–35.