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Modulation of phosphatidylinositol 3-kinase activity during *in vitro* oocyte maturation increases the production of bovine blastocysts

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Research Article

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Summary

This study aimed to evaluate the effect of regulating phosphatidylinositol 3-kinase (PI3K) activity on the kinetics of oocyte nuclear maturation and the blastocyst rate. To evaluate oocyte viability, nuclear maturation rate and *in vitro* embryo production, cumulus–oocyte complexes (COCs) were maintained for 0, 10 min, 6 h or 22 h in TCM 199 medium supplemented with 20 nM wortmannin, an inhibitor of PI3K. After each period, COCs were transferred to the same medium without wortmannin and kept under the same conditions until completion of 22 h of *in vitro* maturation (IVM). To evaluate the effect of time on progression of nuclear maturation, COCs cultivated with 20 nM wortmannin was maintained for 22, 28 or 34 h of IVM. To determine the effect of wortmannin on the activity of maturation-promoting factor (MPF), COCs were kept under IVM conditions in the presence of the inhibitor for 0, 1, 3, 6, or 8 h. Exposure of COCs to wortmannin decreased ($P < 0.05$) the percentage of oocytes that reached metaphase II (MII) up to 22 h, MPF activity and reduced PI3K activity by 30%. However, after 28 and 34 h, 70% of oocytes reached the MII stage in the presence of inhibitor Moreover, COCs matured in the presence of wortmannin showed an increase ($P < 0.05$) in the blastocyst rate. These findings suggested that the regulation of the PI3K activity during IVM of bovine COCs interfered with the meiotic progression due to control of MPF activity, positively affecting the blastocyst rate.

Introduction

Oocyte maturation involves a number of well controlled morphological and biochemical changes, leading to resumption of meiosis and its progression to metaphase of the second meiotic division (nuclear maturation), redistribution of organelles in the oocyte cytoplasm (cytoplasmic maturation), and storage of messenger RNA, proteins, and transcription factors (molecular maturation) (Sirard *et al*., 2003; Ferreira *et al*., 2009). These changes enable the oocyte to fertilization and embryo development.

Different cell signalling pathways are involved in the control of oocyte maturation in mammals. Maturation-promoting factor (MPF) and mitogen-activated protein kinase (MAPK) are the major kinases for control of this process (Bilodeau-Goeseels, 2012). MPF is involved in triggering chromatin condensation, disrupting the nuclear envelope (Heald and McKeon, 1990; Ward and Kirschner, 1990) and forming the meiotic spindle (Murray and Kirschner, 1989), whereas MAPK is involved in the dynamic regulation of microtubules, especially the maintenance of metaphase organization in oocytes (Brunet and Maro, 2005).

Among the factors involved in meiotic regulation, cyclic AMP (cAMP) stands out. This nucleotide is synthesized by mural granulosa and cumulus cells and is transferred to the oocyte through gap junctions. It is also synthesized by the oocyte itself. High intra-oocyte concentrations of cAMP maintain the oocyte in the germinal vesicle due to blocking of MPF activity (Bilodeau-Goessels, 2011). In addition, somatic cells of the follicle also provide cyclic GMP (cGMP) to the oocyte, which compete with cAMP for the same type-III phosphodiesterase (PDE3) catalytic site. As the hydrolysis rate of cGMP is 4-fold to 10-fold smaller than that of cAMP, increased cGMP levels result in less cAMP hydrolysis and the consequent increase in intracytoplasmic concentration of that nucleotide (Thompson *et al*., 2007).

The inhibition/activation of enzymes involved in meiotic resumption is efficiently controlled by protein kinases and phosphatases, which modulate cellular pathways by phosphorylation and dephosphorylation events (Bilodeau-Goessels, 2011). Phosphatidylinositol 3-kinase (PI3K) is involved in various cell functions, such as growth, proliferation, differentiation, motility,

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survival, and intracellular trafficking (Carvalheira *et al.* 2002; Yu and Cu, 2016). Several growth factors and other hormones interact with tyrosine kinase receptors (TKR) and activate PI3K. Once activated, this enzyme catalyzes the phosphorylation of the 3-hydroxyl group within the inositol ring, generating lipid products, which in turn mediate the activation of various enzymes (Falasca, 2010; Manning and Toker, 2017). PI3K acts decisively on the maturation of oocytes by activating protein kinase B (PKB or Akt), which in turn activates PDE3. This enzyme promotes a decrease in the concentration of intra-oocyte cAMP, enabling activation of MPF and the consequent meiotic resumption. Additionally, it activates the PKA pathway, which leads to MAPK activation, thereby maintaining meiotic progression (Schmitt and Nebreda, 2002; Dekel, 2005). Anas *et al.* (1997) showed for the first time the participation of the PI3K pathway in the control of MPF and MAPK activity and the resumption of meiosis in bovine oocytes.

However, during *in vitro* maturation (IVM), the activity of various enzymes of cumulus cells may undergo hyperactivation, and a change in gene expression may occur, caused by the environment to which the oocytes are exposed. This condition increases the percentage of apoptosis and affects the quality of oocytes (Salhab *et al.*, 2013). The *in vitro* environment also promotes alteration in the transcription pattern of oocyte genes involved in several biological processes, including metabolism, energy pathways, cell organization, biogenesis, cell maintenance, and growth, which can affect the competence of oocytes to generate embryos (Katz-Jaffe *et al.*, 2009; Salhab *et al.*, 2013).

Therefore, the regulation of PI3K activity may have a positive effect on the IVM of bovine oocytes, leading to a higher blastocyst rate. Control of PI3K activity can be achieved by using kinase inhibitors in IVM medium, such as wortmannin, a specific inhibitor of this enzyme (Anas *et al.*, 2000; Liu *et al.*, 2007).

In previous studies by our research group, the use of 20 nM wortmannin during maturation of bovine cumulus–oocyte complexes resulted in an increase in the blastocyst rate (Gomes, 2010; Mogollón-Waltero, 2013), therefore proving the importance of the regulation of the PI3K/AKT pathway in bovine oocyte maturation.

The aim of the present study was to evaluate the effect of PI3K activity regulation on MPF activity, kinetics of oocyte nuclear maturation and *in vitro* embryo production.

Materials and methods

All reagents were purchased from Sigma Chemicals Co. (St. Louis, MO, USA), except those specified otherwise.

Obtaining cumulus–oocyte complexes

Bovine ovaries were collected at slaughterhouse and transported in thermos flasks to the laboratory. Cumulus–oocyte complexes (COCs) were aspirated from follicles 3–8 mm in diameter. These were washed in medium 199 HEPES, with 20 mM sodium bicarbonate, 10% fetal bovine serum (Cultilab®, Campinas, Brazil), 100 IU/ml penicillin, and 100 µg/ml streptomycin. Subsequently, the COCs were selected and classified. Only those COCs classified as degrees I and II (De Loss *et al.*, 1989) were used.

In vitro *maturation of bovine oocytes*

Groups of 20 COCs were subjected to IVM in 100 µl of maturation medium [medium 199 with Earle's salts plus 20 mM sodium bicarbonate, 5 µg/ml luteinizing hormone (Lutropin-V; Bioniche, Belleville, Canada), 0.5 µg/ml follicle-stimulating hormone (Follitropin-V; Bioniche, Belleville, Canada), 0.2 mM pyruvate, 100 IU/ml penicillin, and 100 µg/ml streptomycin] at 38.5°C, 5% CO_2 in air, for up to 22 h (Experiment 1), or 22, 28 or 34 h (Experiment 2).

Experiment I

To evaluate oocyte viability, nuclear maturation rate and *in vitro* embryo production, COCs were maintained for 0, 10 min, 6 h, or 22 h in IVM medium supplemented with 20 nM wortmannin (Gomes, 2010). Control COCs were maintained under the same conditions without wortmannin. After each period, the COCs were washed three times (100 µl each) in IVM medium without wortmannin and then returned to the IVM medium without the inhibitor until the IVM period (22 h) was completed.

Experiment II

To verify if the nuclear maturation would happen after 22 h of IVM, COCs were maintained for 22, 28 or 34 h in IVM medium, under the same conditions described in Experiment 1. The nuclear maturation stage was determined by acetic orcein staining, and the oocytes were classified according to the meiosis stages as: germinal vesicle (GV), metaphase I (MI), anaphase/telophase I (A/TI) or metaphase II (MII). The results were obtained from five replicates.

Determination of oocyte viability

The effect of exposure time to wortmannin (0 h, 10 min, 6 h, or 22 h) on oocyte viability was evaluated by calcein AM fluorescence staining. The results were obtained from four replicates. After denudation, oocytes were exposed to a calcein solution [10 µg/ml calcein + 0.1% PVA in phosphate-buffered saline (PBS)] for 10 min, washed with PBS, and evaluated under an optical fluorescence microscope (Eclipse TE300 Nikon – Tokyo, Japan) at 400 × magnification. Oocytes that emitted green fluorescence were considered viable. The entire procedure was carried out in a dark room.

Determination of nuclear maturation stage

The denuded oocytes were held between a slide and a cover slip for 24–48 h in ethanol/acetic acid (3:1, v:v). The results were obtained from three replicates. Then, the oocytes were stained with 2% acetic orcein and observed under an optical microscope (Eclipse TE300 Nikon – Tokyo, Japan) at 400 × magnification to determine the nuclear maturity stages. Only oocytes that showed the metaphase plate (MII) were considered mature.

Quantification of PI3K activity

Quantification of PI3K activity was determined by western blotting. The oocytes matured *in vitro* for 22 h were subjected to rapid incubation in liquid nitrogen, followed by immersion in warm water and then incubation for 5 min in 0.1% Triton X-100 (diluted in Ca^{2+}- and Mg^{2+}-free PBS) for cell membrane lysis. Samples were centrifuged, and the sediment was discarded. The material was further centrifuged for 1 min at 14,000 *g* at 4°C; the supernatant was then discarded and the pellet was washed three times with PBS. Subsequently, the pellet was resuspended with sample buffer and boiled for 10 min. Next, the samples were subjected to electrophoresis in polyacrylamide gel under denaturing conditions (10% SDS-PAGE).

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The proteins were then transferred onto a polyvinylidene difluoride (PVDF) membrane, which was kept in a blocking solution (TBS-Tween buffer with 3% skimmed milk) for 3 h and then incubated with anti-PI3-kinase p85α rabbit primary antibody (sc-423; 1:500; Santa Cruz Biotechnology, Santa Cruz, USA) overnight. Afterward, the membrane was incubated with anti-rabbit peroxidase-conjugated secondary antibody (sc-2030; 1:2000; Santa Cruz Biotechnology). Finally, the membrane was incubated with the developing solution (DAB). The relative amount of proteins transferred was measured using Gel Perfect software as described by Bozzo and Retamal (1991).

Determination of MPF activity

To verify if wortmannin could interfere with the resumption of meiosis, MPF activity in COCs was determined using a CycLex cdc^2-cyclin B kinase assay kit (MBL International Corp, Woburn, Massachusetts, USA) according to the manufacturer's instructions. In summary, after the maturation period corresponding to each treatment (0, 1, 3, 6 and 8 h), the COCs were washed in extraction medium and then subjected to three cycles of freezing and thawing for cell lysis.

Subsequently, the supernatant was transferred to microcentrifuge tubes and centrifuged at 13,500 *g* for 5 min. The supernatant was diluted (1:5) in Qbuffer and stored on ice for 15 min. Then, another centrifugation was carried out at 13,500 *g* for 15 min. All the supernatant was stored in microcentrifuge tubes at −20°C.

The reaction was initiated with the addition of 10 µl of the sample with 90 µl of the reaction buffer. Later 30 min, wells were washed three times, and the monoclonal anti-phosphoserine primary antibody cdc7 T3T6 solution was added. Another incubation was carried out under the same conditions. Then, another sequence of washes was performed as previously described. Later, secondary HRP-conjugated anti-rat IgG antibody was added to each well, and another incubation was carried out with successive well washes. Absorbance was measured in triplicate with a spectrophotometer at 450 nm and was related to quantity of cdc^2-cyclin B activity in the sample.

In vitro *embryo production*

After the maturation period, the COCs were fertilized in 50-µl droplets of Fert-TALP medium (medium 199 supplemented with 6 mg/ml fatty acid-free BSA, 2 mM penicillamine, 1 mM hypotaurine, 25 mM epinephrine, 0.2 mM sodium pyruvate, 100 IU/ml penicillin and 100 µg/ml streptomycin). Drops with 20 presumptive zygotes were covered with mineral oil and kept in a CO_2 incubator.

Frozen semen was used for insemination of the COCs. The semen was prepared according to the mini-Percoll technique, and an insemination dose of 2×10^6/ml spermatozoa was used. The gametes were kept for 18 h at 38.5°C and 5% CO_2.

After this period, the putative zygotes were washed and then co-cultured with their cumulus cells in 100-µl drops (20/drop) of synthetic oviductal fluid (SOF) medium. They were kept in an incubator for 7 days at 38.5°C and 5% CO_2 in air. Three days after fertilization, 50% of the culture medium was changed (feeding), and the specific cleavage rate was determined. The blastocyst rate was determined on day 7 after fertilization. This assay was performed in five replicates.

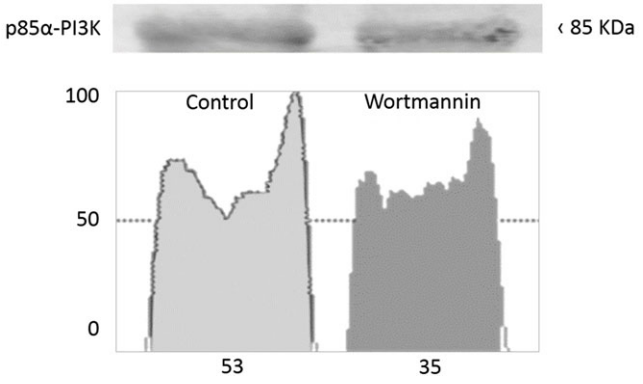

Figure 1. Densitometric analysis of the protein bands of the p85 subunit of bovine oocytes matured *in vitro* in the absence (left) or presence (right) of 20 nM of wortmannin. The x-axis of the diagram represents the area occupied by each phosphorylated protein band, in the absence (53) or presence (35) of wortmannin.

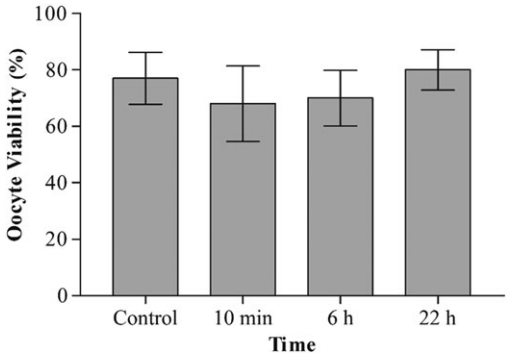

Figure 2. Effect of exposure time of bovine COCs to wortmannin on the viability of *in vitro* matured oocytes.

Statistical analysis

The data were evaluated by analysis of variance (ANOVA) followed by Student–Newman–Keuls (SNK) test for comparison of means at 5% level of significance, and presented in percentage ± standard deviation. For two-to-two comparisons, the data were evaluated using *t*-test, at a 5% level of significance (SAS, 2009).

Results

Experiment I

Effect of wortmannin on PI3K activity

The PI3K activity was determined in oocytes matured *in vitro* for 22 h by densitometric analysis of the protein bands of the p85 subunit (PI3K-activating subunit), which showed a decrease of 30% in the oocytes treated with wortmannin compared with the controls (Fig. 1).

Evaluation of cell viability

Exposure of COCs to wortmannin for 0 min, 10 min, 6 h and 22 h did not interfere with oocyte viability, as determined by calcein AM fluorescence staining (Fig. 2).

Effect of wortmannin exposure time on nuclear maturation

Treatment of oocytes with wortmannin during IVM influenced the meiotic progression. Regardless of the exposure time to the

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Table 1. Effect of exposure time of bovine COCs to wortmannin on the progression of meiosis of *in vitro* matured oocytes

Time	*n*	MI (% ± SD)	AI/TI (% ± SD)	MII (% ± SD)
Control	50	12 ± 1.00^b	6 ± 1.00^a	82 ± 2.08^a
10 min	46	26 ± 1.00^a	10.8 ± 0.57^a	63.2 ± 0.57^b
6 h	49	36.7 ± 1.0^a	6.1 ± 1.15^a	57.1 ± 1.52^b
22 h	49	36.7 ± 1.15^a	4.08 ± 0.57^a	59.2 ± 1.52^b

MI, metaphase I; A/TI, anaphase/telophase I; MII, metaphase II. Equal letters in the same column indicate not different at 5% significance. In all treatments, the total time of the *in vitro* maturation was 22 h, regardless of the exposure time to 20 nM wortmannin.

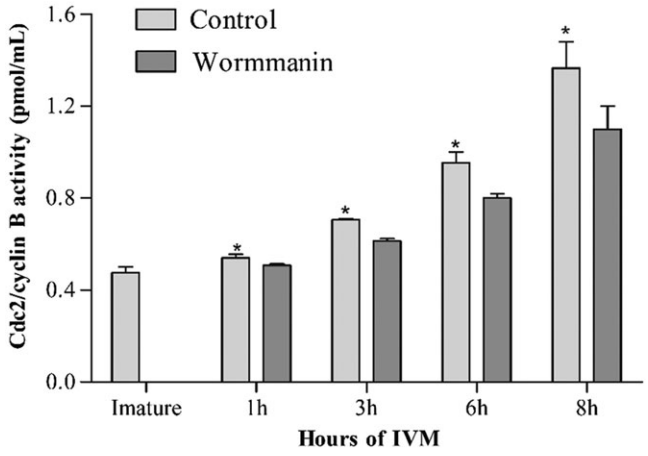

Figure 3. Effect of wortmannin on MPF activity in bovine COCs during the first 8 h of IVM. Results represent the mean ± SD of three replicates, using 50 COCs per replicate. The data were evaluated by *t*-test. The asterisk (*) indicates a significant difference ($P < 0.05$) in the time evaluated.

inhibitor (0 min, 10 min, 6 h or 22 h), wortmannin significantly decreased ($P < 0.05$) the percentage of oocytes in MII at 22 h of IVM, compared with the control. However, there was no difference in the percentage of oocytes in MII ($P > 0.05$) between different times of exposure to wortmannin (Table 1).

Evaluation of cdc2/cyclin B concentrations

The activity of MPF, as determined by the formation of the cdc2/cyclin B complex, increased gradually over the maturation time evaluated (8 h) for both treatments. The oocytes exposed to wortmannin showed lower MPF activity ($P < 0.05$) when compared with the control, in the analyses carried out at 1 h (0.51 ± 0.01 and 0.54 ± 0.02 pmol/ml), 3 h (0.61 ± 0.01 and 0.7 ± 0.01 pmol/ml), 6 h (0.8 ± 0.02 and 0.95 ± 0.05 pmol/ml), and 8 h (1.1 ± 0.1 and 1.37 ± 0.12 pmol/ml), respectively (Fig. 3).

Evaluation of in vitro *bovine embryo production*

The exposure time of the COCs to wortmannin influenced the cleavage and blastocyst rates. The COCs matured for 22 h in the presence of the PI3K inhibitor showed a higher ($P < 0.05$) cleavage rate than the control group and the other treatments, but the COCs matured with wortmannin for 10 min or 6 h did not differ from the control.

The COCs exposed to wortmannin for 22 h also showed an increase ($P < 0.05$) in the blastocyst rate compared with the COCs from the control group and those treated with wortmannin

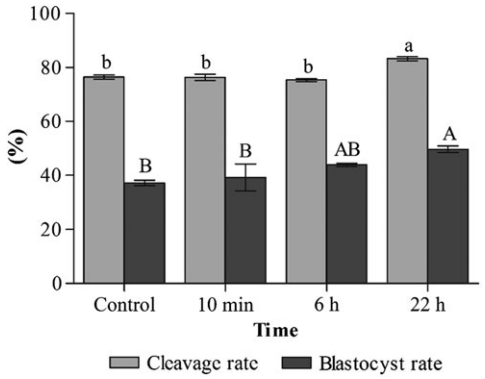

Figure 4. Effect of exposure time of bovine COCs to wortmannin on the cleavage and blastocyst rates *in vitro* produced. Lowercase and uppercase letters correspond to cleavage and blastocyst rates, respectively. Equal lowercase or uppercase letters indicate no difference statistically at 5% significance. In all treatments, the total time of the *in vitro* maturation was 22 h, regardless of the exposure time to 20 nM wortmannin. The blastocyst rate was calculated in relation to the number of oocytes.

for 10 min. However, no difference was observed in the COCs exposed to wortmannin for 6 h (Fig. 4).

Experiment II

Effect of wortmannin on time to complete the nuclear maturation

The effect of wortmannin on formation time of metaphase plate was evaluated at 22, 28 and 34 h of IVM. No significant difference ($P > 0.05$) on percentage of control oocytes that reached MII stage was found among the three analyzed times. However, treatment with wortmannin reduced the percentage of MII oocytes at 22 h of IVM, compared with control oocytes (61.6 ± 2.4 × 79.9 ± 5.3, respectively). After 28 and 34 h of IVM, the percentage of mature oocytes (presented metaphase plate) treated with wortmannin increased, reaching values similar ($P > 0.05$) to those found in the control group, at 22 h of IVM (Table 2).

Discussion

In bovines, wortmannin has been used to show the participation of the PI3K/AKT signalling pathway in oocyte maturation (Anas *et al.*, 1997, 2000). However, few studies have shown the effect of this inhibitor on nuclear maturation kinetics and the competence of oocyte development (Carnero and Lacal, 1998; Das *et al.*, 2013).

In the present study, the use of wortmannin during IVM led to a significant decrease ($P < 0.05$) in the percentage of oocytes that reached MII, determined at 22 h of maturation, irrespective of the time of exposure of the COCs to the PI3K inhibitor (10 min, 6 h or 22 h). These findings confirmed the importance of the PI3K/AKT pathway in the progression of meiosis in bovine oocytes, in addition to demonstrating that wortmannin has a fast effect, even after incubation of only 10 min. These results support those of Anas *et al.* (2000), who found a marked decrease in the percentage of oocytes that reached MII after incubation in medium supplemented with 100 nM of wortmannin, if compared with control (15.6 ± 1.6 and 85.2 ± 1.8, respectively), and also reported the irreversibility of oocyte treatment with the PI3K inhibitor. This decrease was not as intense in our study as that previously reported (Anas *et al.*, 1997, 2000), possibly because of the dose–response effect showed by wortmannin (Anas *et al.*, 1997). At the

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Table 2. Effect of wortmannin on the meiotic stage of bovine oocytes matured *in vitro* for 22, 28 or 34 h

Time	Treatment	*n*	VG	MI	AI/TI	MII
22 h	Control	84	1.1 ± 2.5^{A}	15.4 ± 1.4^{A}	3.6 ± 5.1^{A}	$79.9 \pm 5.3^{A*}$
	Wm	91	1.0 ± 2.2^{a}	$33.0 \pm 5.0^{a*}$	4.5 ± 4.5^{a}	61.6 ± 2.4^{b}
28 h	Control	90	0 ± 0^{A}	17.0 ± 5.0^{A}	4.3 ± 2.4^{A}	78.7 ± 5.1^{A}
	Wm	82	0 ± 0^{a}	19.0 ± 5.8^{b}	6.4 ± 4.8^{a}	74.6 ± 2.5^{a}
34 h	Control	99	0.9 ± 1.9^{A}	17.0 ± 4.1^{A}	0 ± 0^{A}	$82.1 \pm 3.4^{A*}$
	Wm	92	0 ± 0^{a}	$27.4 \pm 2.7^{a*}$	0 ± 0^{a}	72.6 ± 2.7^{a}

Means followed by equal letters (uppercase = control, lower case = 20 nM wortmannin [Wm]) do not differ in analysis of variance (ANOVA) among the different cultivation times, according to the SNK test at 5% probability. Means followed by * differ from each other in the analysis between treatments at different times of IVM, at each maturation stage evaluated, according to the *t*-test at 5% probability.

concentration used in our study (20 nM), oocytes incubated with wortmannin for 22 h during IVM, presented a decrease of 30% in PI3K activity (Fig. 1). This decreased PI3K activity can cause regulation of the activity of downstream enzymes of this cascade, which may influence the resumption of meiosis. Temporal inhibition of meiosis resumption has been used as a strategy to improve the synchronization between nuclear and cytoplasmic maturation, with the aim of increasing the potential of *in vitro* oocyte development (Albuz *et al.*, 2010). In general, these studies are based on the elevation of the intra-oocyte cAMP (Luciano *et al.*, 1999), the inhibition of protein synthesis (Lonergan *et al.*, 1997, 1998), or the inhibition of MPF activity (Mermillod *et al.*, 2000). Some of these studies did not report satisfactory results for the blastocyst rate, due to the adverse effects of these inhibitors on the COCs (Bilodeau-Goeseels, 2012).

Phosphorylation of PI3K has been shown to activate Akt, which activates PDE3, the principal enzyme responsible for the degradation of cAMP in bovine oocytes (Conti *et al.*, 2002; Han *et al.*, 2006; Richard, 2007), which contributes to the decrease of the intra-oocyte concentration of this nucleotide. High concentrations of cAMP are involved in the maintenance of meiotic blockage (Mehlmann, 2005). Decrease in the intracellular concentration of this nucleotide is related to the resumption of meiosis, mediated by activation of MPF (Conti *et al.*, 2012).

In the present study, MPF activity was quantified as a way to evaluate the effect of PI3K regulation on resumption of meiosis. In bovines, the GV is present from 0 to 6 h and germinal vesicle breakdown (GVBD) occurs at 6.6–8.0 h (Sirard *et al.*, 1989). Our results showed that COCs treated or untreated (control) with wortmannin presented a progressive increase in the activity of the cdc^2/cyclin B complex, when the analyses were carried out at 1, 3, 6 and 8 h of maturation. Compared with the control group, COCs matured in the presence of PI3K inhibitor showed lower cdc^2/cyclin B activity ($P < 0.05$) at 3, 6, and 8 h. This result is in line with the lower percentage of oocytes that reached the MII stage at 22 h of IVM, after treatment with wortmannin (Table 1). Likewise, Anas *et al.* (1997) reported a decrease in the percentage of oocytes in MII after treatment of COCs with 100 nM of wortmannin (55.9 ± 2.9), compared with the control (80.9 ± 1.1%).

In our study, exposure of COCs to PI3K inhibitor for 10 min or 6 h during IVM did not influence the blastocyst rate. However, when the COCs were kept in the presence of wortmannin during the entire IVM period, the blastocyst rate increased ($P < 0.05$) compared with the control group and the COCs treated for 10 min. This improvement in the blastocyst rate might be associated with slower nuclear maturation resulting from the lower MPF activity.

It has been shown that the lack of synchrony between nuclear and cytoplasmic maturation is detrimental to the oocyte, and this fact may negatively influence embryonic development (Rizos *et al.*, 2002). Conversely, prolonging *in vitro* meiotic arrest by temporary blockage or delayed nuclear maturation can stimulate this synchronization. This block may be essential for structural and biochemical changes to occur, which can make the oocyte able to be fertilized and develop into an embryo (Chaves *et al.*, 2010). Enzymatic inhibitors, such as wortmannin (PI3K), Org9935 (PDE) and roscovitine (MPF/CDK) manipulate intra-oocyte cAMP concentrations by direct or indirect inhibition of PDE in the oocyte cytoplasm, temporarily exercising a function of nuclear maturation control (Lonergan *et al.*, 2003; Nogueira *et al.*, 2003; Mogollón-Waltero *et al.*, 2015). In our study, the increase in blastocyst rate, considered together with the lower percentage of oocytes in MII of the matured group in the presence of wortmannin for 22 h of IVM, and equal percentages of MII after 28 and 34 h (Table 2), in both oocytes matured in the presence of the inhibitor and in the control condition, demonstrates that wortmannin caused a reduction in meiosis progression rate, with part of the oocytes reaching MII during the IVF stage. Therefore, our study suggests that the progression delay in nuclear maturation, promoted by the reduction of PI3K activity, may be able to synchronize nuclear and cytoplasmic events during IVM, resulting in better blastocyst production rates.

In conclusion, the results of the present study provide evidence that the use of wortmannin during IVM of COCs bovine promotes regulation of the PI3K activity and positively affects the blastocyst rate by control of the meiotic progression due to regulation of MPF activity.

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Conflicts of interest. None.

Ethical Standards. Not applicable.

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