Theriogenology 231 (2025) 222–227

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Original Research Article

Increased *in vitro* production of bovine embryos resulting from oocyte maturation in the presence of triciribine, a specific inhibitor of AKT

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ARTICLE INFO

Keywords:
COCs
In vitro maturation
PI3K/AKT pathway

ABSTRACT

The aim of this study was to evaluate the effect of different concentrations of triciribine, a selective Akt inhibitor, on various aspects of oocyte maturation and on the IVF of bovine embryos. Cumulus-oocyte complexes (COCs) were matured *in vitro* in medium supplemented with: 0 (control), 1, 5, 10, and 20 μM of triciribine. The nuclear maturation was assessed by staining with acetic orcein, while the cytoplasmic maturation was evaluated by mitochondrial (MitoTracker® Red CMXRos) and lipid droplets distribution (LipidTOX). COCs were fertilized *in vitro* and cultured for nine days. Cleavage rates, blastocyst production, and hatching rates were determined on days three, seven, and nine of *in vitro* culture, respectively. Oocytes from COCs treated with 1 μM of triciribine were stained at 3, 6, and 9 h of IVM to determine the inhibitor's involvement in germinal vesicle breakdown. Analysis of variance (ANOVA) of the data was performed and the means were compared using the SNK test at a 5 % significance level. Exposure of COCs to 1, 5, and 10 μM of triciribine did not alter the number of matured oocytes ($P < 0.05$), a concentration of 20 μM reduced the number of oocytes in MII with a consequent increase in oocytes in MI ($P < 0.05$). This concentration markedly reduced the number of oocytes with peripheral cortical granules and the rates of cleavage and blastocysts ($P < 0.05$). On the other hand, when COCs were matured in the presence of 1 μM, there was an increase in the blastocyst rate ($P < 0.05$), but without altering the timing of meiosis resumption ($P < 0.05$). It is concluded that the Akt pathway participates in the nuclear and cytoplasmic events of *in vitro* maturation of bovine oocytes, but through mechanisms that do not interfere with germinal vesicle breakdown. Modulation of Akt activity in bovine COCs during IVM with 1 μM of triciribine increases the *in vitro* production of bovine embryos.

1. Introduction

Oocyte maturation in mammals involves several nuclear, cytoplasmic, and molecular changes that prepare the oocyte for fertilization and embryo development [1,2]. *In vivo*, the mechanisms that control this process have not yet been fully elucidated, but it is known to be process regulated by autocrine and paracrine signals originating from different follicular compartments.

The cytoplasmic alterations of the oocyte occur progressively and coordinately, initiatingbefore the LH surge when nuclear maturationis still blocked in the prophase of the first meiotic division. However, when performed *in vitro*, theremoval of the cumulus oophorus complex (COC) from the follicular environment induces the spontaneous resumption of meiosis faster than cytoplasmic changes when compared to *in vivo* maturation events. This situation has been presented as one of the factors compromising the outcomes of *in vitro* embryo production [3,4].

In order to overcome this problem, various strategiesfor delaying the resumption of meiosis in *in vitro* maturation protocolshave been evaluated. Thus, the maturation of COCs has been assessed in the presence of a) fragments of the follicular wall [5]; b) phosphodiesterase (PDE) inhibitors [6]; c) MPF inhibitors [7,8]; d) protein synthesis inhibitors [9]; and e) adenylatecyclase activators [10]. However, while some of these strategies are incompatible with *in vitro* embryo production [5], others do not result in increased blastocyst rates [7,8].

Although it has been demonstrated that the PI3/Akt pathway is also involved in the control of oocyte maturation in different species [11–14], few studies have investigated this question for cattle. When activated, phosphatidylinositol 3-kinase (PI3K) produces

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https://doi.org/10.1016/j.theriogenology.2024.10.024
Received 3 May 2024; Received in revised form 24 October 2024; Accepted 24 October 2024
Available online 24 October 2024

phosphatidylinositol3,4,5-triphosphate (PIP3), which acts on the phosphorylation and activation of Akt [15]. Akt phosphorylates phosphodiesterase (PDE), increasing its activity and stimulating the resumption of oocyte maturation [16,17]. Studies conducted in our laboratory evaluated the participation of the PI3K/AKT pathway in oocyte maturation [18] and have shown that *in vitro* maturation of bovine COCs in the presence of wortmannin, a specific inhibitor of PI3K, resulted in an increased blastocyst rate [19]. However, few studies have evaluated the involvement of Akt during oocyte maturation in mammals, and the only study conducted in cattle demonstrated that Akt inhibition using SH6 compromised the progression of maturation between the MI-MII stages [20]. The involvement of this pathway in cytoplasmic maturation and the effect of its inhibition with triciribine during IVM in *in vitro* embryo production has not been investigated. Triciribine (Merck, Darmstadt, Germany) is a selective inhibitor of the three isoforms of Akt (Akt1, Akt2, and Akt3), which acts by preventing their phosphorylation.

Thus, we hypothesized that modulation of Akt activity improves the synchrony of nuclear and cytoplasmic maturation, with a consequent increase in the blastocyst rate.

The aim of this study was to evaluate the effect of the direct inhibition of Akt using Triciribine® during the *in vitro* maturation of bovine *cumulus oophorus* complexes in order to understand its effects on different aspects of nuclear and cytoplasmic maturation and determine the impact of the treatment on the *in vitro* production of bovine embryos.

2. Materials and methods

2.1. In vitro maturation (IVM) of cumulus oophorus complexes (COCs)

Cumulus oophorus complexes (COCs) were aspirated from ovaries obtained from local slaughterhouses. After being washed, the COCs were transferred to maturation medium (TCM-199 with Earle's salts enriched with 20 mM sodium bicarbonate, 5.0 μg/mL LH [Lutropin-V, Bioniche, Belleville, Canada], 0.5 μg/mL FSH [Folltropin-V, Bioniche, Belleville, Canada], 0.2 mM pyruvate, 0.4 mM glutamine, 100 IU/mL penicillin, and 100 μg/mL streptomycin) and covered with mineral oil. Were added to the medium 1, 5, 10, or 20 μM of the Akt inhibitor (Triciribine, Merck®), and IVM was performed in 100 μL drops,for 22 h in a humidified atmosphere of 5 % CO_2, at 38.5 °C. COCs from the control group (CG) were maintained under the same conditions, in the absence of Triciribine.

2.2. Evaluation of nuclear maturation

After 22 h of IVM, the COCs (n = 382) were mechanically denuded by successive pipetting in a solution of 0.2 % hyaluronidase (Hyalozima®, Aspen), diluted in PP medium (PBS without Ca^{2+}, supplemented with 0.1% polyvinylalcohol-PVA). Subsequently, the oocytes were placed between a slide and a cover slip and fixed in ethanol/acetic acid (3:1) (Cromoline) for 48 h. They were then kept in a 2 % acetic orcein solution (Fluka) for 3 min and observed under an optical microscope (TE 300 - NIKON) for the determination of nuclear maturation stage. Based on the chromosomal arrangement, the oocytes were classified into germinal vesicle (GV), anaphase and telophase (Anaph/Tel), metaphase I (MI), or metaphase II (MII) stages [21,22].

2.3. Evaluation of mitochondrial distribution

To evaluate the effect of the treatments on mitochondrial distribution, the COCs (n = 407) were denuded and the oocytes were placed in 100 μL drops of MitoTracker® Red CMXRos solution for 30 min at 37 °C, protected from light. They were then washed in drops of Ca^{2+} free PBS and evaluated under a fluorescence microscope (TE 300, Nikon). The oocytes were then classified into three categories according to the mitochondrial distribution: immature (I), when the mitochondria were distributed throughout the periphery of the cytoplasm, forming a peripheral halo; transition (II), when the majority of mitochondria were dispersed in the cytoplasm, but a small portion was still located in the periphery; and matured (III), when the mitochondria exhibited a more homogeneous distribution throughout the cytoplasm (Fig. 1) [23].

2.4. Evaluation of lipid droplet distribution

For the evaluation of lipid droplet distribution in the cytoplasm, the COCs (n = 462) were denuded and the oocytes were incubated in a solution of LipidTOX (HCS LipidTOX™ Green Neutral Lipid Stain) for 1 h at room temperature, in the absence of light. The oocytes were then washed and observed under a fluorescence microscope, being classified as: immature (I) when the distribution was predominantly peripheral, and matured (II) when the distribution of lipid droplets was homogeneous throughout the cytoplasm (Fig. 2) [23].

2.5. In vitro embryo production (IVP)

After 22 h of IVM, the COCs (n = 389, approximately 20/drop) were fertilized in 50 μL drops of M199 medium, supplemented with 6.0 mg/mL fatty acid-free BSA, 2.0 mM penicillamine, 1.0 mM hypotaurine, 25 mM epinephrine, 0.2 mM sodium pyruvate, 100 IU/mL penicillin, and 100 μg/mL streptomycin. Throughout the experiment, semen straws from the same bull and from the same batch were used. The semen straws were thawed in a waterbath at 37 °C for 30 s. The spermatozoa were selected using a discontinuous Percoll® gradient (45/90 %) and incubated over the oocytes in the fertilization drops at a concentration of 2×10^6/mL in a humidified atmosphere of 5 % CO_2 at 38.5 °C for 18 h.

The *in vitro* culture (IVC) of the presumptive zygotes was carried out in a commercial SOF medium (BioKlone®) for nine days. The cleavage rates, blastocyst production, and hatching were evaluated on days 3 (D3), 8 (D8), and 9 (D9) of development, respectively. The experiment was performed with four replicates.

2.6. Evaluation of germinal vesicle (GV) breakdown kinetics

To evaluate if the increase in blastocyst rate observed in the group treated with 1 μM triciribine was due to a delay in germinal vesicle breakdown (GVBD), COCs (n = 327) were matured *in vitro* for 3, 6, or 9 h, in the presence of 1 μM inhibitor (G1) or in its absence (control - GC). After each period of IVM, the COCs were denuded, fixed, and stained with 2 % acetic orcein and observed under an optical microscope (TE 300, NIKON). The oocytes were classified according to chromosomal configurationas germinal vesicle (GV) when they displayed chromosomes enclosed by an intact nuclear membrane (characteristic of oocytes that had not initiated nuclear maturation), or as germinal vesicle breakdown (GVBD) when the oocytes had already restarted meiosis. In this latter group (GVBD), oocytes with segmented nuclear membrane and those at the anaphase/telophase and metaphase I stages were included [24].

2.7. Statistical analyses

Data on IMV, VGBD, mitochondrial distribution and lipid droplets, and *in vitro* embryo production were analyzed by analysis of variance (ANOVA) and means were compared by SNK test at the 5 % significance level. A total of 2067 COCs were used in this study. In all experiments, four replicates were performed, except in the VGBD experiment, where three replicates were performed.

3. Results

3.1. Effect of triciribine on nuclear maturation

Treatment of COCs with 1 μM, 5 μM, and 10 μM of the Akt inhibitor for 22 h during IVM did not alter the percentage of oocytes in metaphase

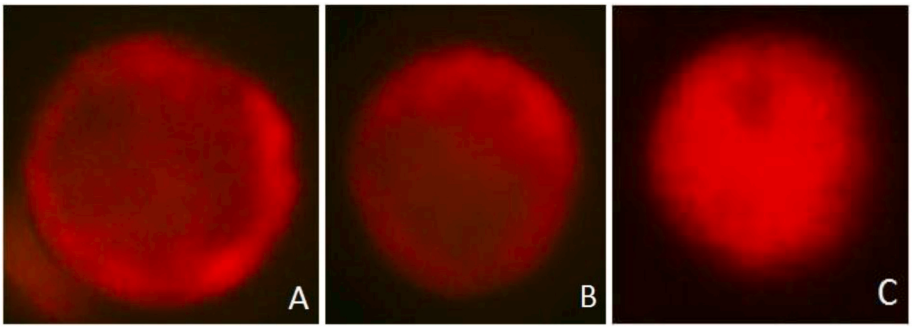

Fig. 1. Micrographs illustrating the different categories of mitochondrial distribution after IVM of bovine oocytes. The oocytes were classified as: Peripheral (A), Transition (B) or Dispersed (C).

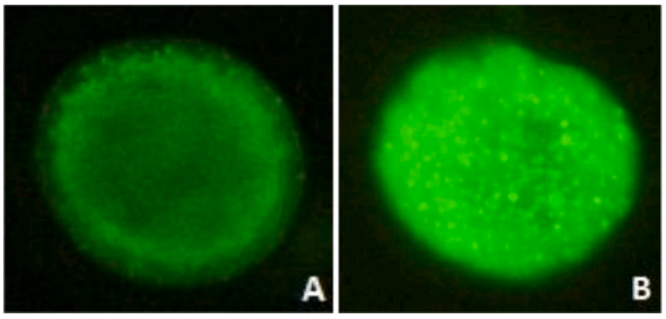

Fig. 2. Representative micrographs of the different categories of cytoplasmic distribution of lipid droplets after IVM of bovine oocytes. The oocytes were classified according to the distribution of lipid droplets in the cytoplasm as peripheral (A) and dispersed (B).

II. However, the use of triciribine at a concentration of 20 μM resulted in a reduction ($p \leq 0.05$) in the number of oocytes in MII (Table 1). No differences ($p \geq 0.05$) were observed in the percentage of oocytes that remained in the germinal vesicle stage or reached the anaphase/telophase stages. On the other hand, when the highest concentration of the inhibitor (20 μM) was used, there was an increase in the percentage of oocytes in metaphase I, compared with the control group (24.4 vs 3.7, respectively).

Interestingly, oocytes treated with 20 μM of triciribine during IVM consistently showed chromosomes in the metaphase plate forming aggregates. Additionally, the occurrence of chromosomal aberrations was observed in oocytes from this treatment (Fig. 3).

Table 1
Effects of different concentrations of triciribine on the nuclear maturation of bovine oocytes.

	n	Germinal Vesicle	Metaphase I	Anaphase/ Telophase	Metaphase II
		(%)	(%)	(%)	(%)
Control	75	9.1 ± 2.3[a]	3.7 ± 3.2[b]	1.3 ± 2.6[a]	81.97 ± 7.54[a]
1 μM	80	9.6 ± 16.0[a]	11.1 ± 11.1[ab]	3.9 ± 7.9[a]	75.38 ± 10.02[a]
5 μM	71	14.9 ± 25.2[a]	15.1 ± 11.2[ab]	0 ± 0[a]	69.86 ± 15.4[ab]
10 μM	81	9.3 ± 9.9[a]	29.1 ± 15.2[ab]	2.6 ± 5.3[a]	59.0 ± 12.4[ab]
20 μM	75	17.6 ± 18.1[a]	24.3 ± 3.7	9.5 ± 8.2[a]	48.5 ± 9.4[b]

Oocytes were evaluated after 22 h of IVM. The results represent the average percentage of each category in four replicates. Means ± standard deviation followed by the same letter do not differ statistically within columns according to the SNK test at 5 % probability.

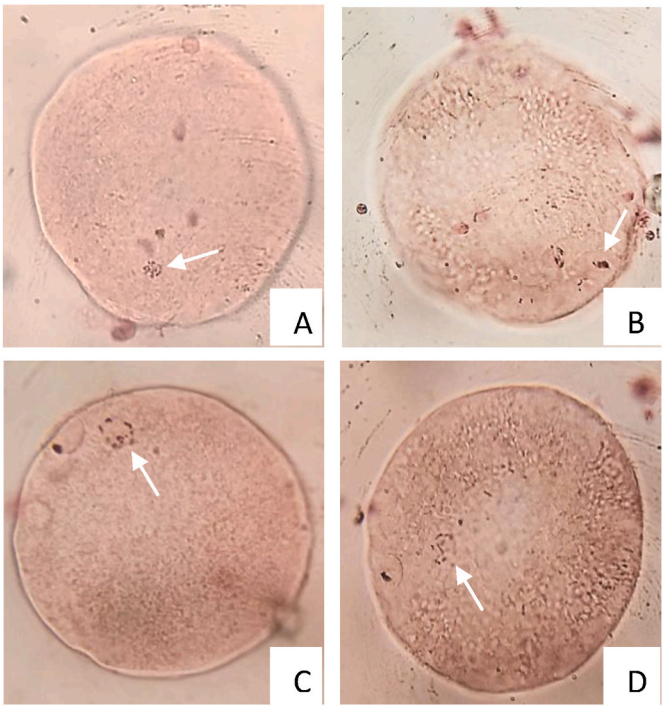

Fig. 3. Bovine oocytes matured *in vitro* for 22 h, stained with 2 % acetic orcein. A: Control group showing a metaphase plate with well-defined and organized chromosomes; B: Group treated with 20 μM of triciribine showing a metaphase plate with condensed chromosomes; C and D: Aberrant metaphase plate appearance. Arrow – Metaphase plate.

3.2. *Effect of triciribine on mitochondrial distribution*

Treatment of COCs with 1 μM triciribine during IVM resulted in a reduction in the percentage of oocytes with peripheral mitochondria and an increase ($p \geq 0.05$) in the percentage of oocytes with transitioning mitochondria compared with the control. Treatment with 10 μM triciribine caused a reduction ($p \geq 0.05$) in the percentage of oocytes with peripherally distributed mitochondria compared with the control. Furthermore, treatment of COCs with 20 μM of the Akt inhibitor led to a reduction ($p \geq 0.05$) in the percentage of oocytes with dispersed mitochondria, when compared with the other groups (Table 2).

3.3. *Effect of Akt inhibition on the distribution of cytoplasmic lipids after IVM*

The use of 5 μM triciribine during IVM reduced the percentage of oocytes that showed lipid droplets in the periphery of the cytoplasm

Table 2
Distribution of mitochondria in the cytoplasm of bovine oocytes matured in the presence of different concentrations of triciribine.

	n	Peripheral (%)	Transition (%)	Dispersed (%)
Control	88	37.8 ± 12.1[a]	31.5 ± 17.2	30.7 ± 6.6[a]
1 μM	83	17.3 ± 4.9[b]	54.8 ± 8.2	32.0 ± 6.0[a]
5 μM	70	23.7 ± 1.6[ab]	39.8 ± 7.5	36.8 ± 8.3[a]
10 μM	82	8.4 ± 6.6[b]	49.3 ± 6.3	42.3 ± 3.9[a]
20 μM	84	39.1 ± 8.7[a]	41.8 ± 5.4	19.1 ± 4.4[b]

The evaluations were performed after 22 h of IVM. The results represent the average percentage of each category in four replicates. Means ± standard deviation followed by the same letter do not differ statistically within columns according to the SNK test at 5 % probability.

compared with the control group. However, the inhibitor was not able to alter the percentage of oocytes with dispersed cytoplasmic lipids at any of the concentrations used (Table 3).

3.4. Effect of triciribine on in vitro embryo production

COCs matured *in vitro* in the presence of 1 μM triciribine and subsequently fertilized *in vitro* showed a increase ($p \geq 0.05$) in the blastocyst rate compared with the control group. In contrast, COCs treated with 5 μM or 10 μM of triciribine did not show different blastocyst rates ($p \geq 0.05$) from those observed in the control group. However, the treatment of COCs with 20 μM of triciribine resulted in a lower blastocyst rate ($p \geq 0.05$) compared with the control group (Table 4). The treatment of COCs with triciribine did not influence the hatching rate.

3.5. Evaluation of the effect of Akt inhibitor on germinal vesicle breakdown kinetics

No differences in the timing of germinal vesicle breakdown were detectedin COCs treated with 0 μM (control) or 1 μM of triciribine during the IVM (Table 5).

4. Discussion

Despite Akt's involvement in important biological processes, little is known about its role during the *in vitro* maturation of mammalian oocytes. Therefore, the present study aimed to understand the effect of regulating its activity on different aspects of *in vitro* maturation of bovine oocytes and its impact on early embryonic development using triciribine, a selective Akt inhibitor.

Triciribine is a potent selective inhibitor of all three isoforms of Akt (Akt1, Akt2, and Akt3), and its use for the treatment of neoplasms has been studied due to the enzyme's involvement in the control of cell proliferation and survival. In humans, concentrations of 50–75 μM have significantly reduced the growth of insulinoma and intestinal endocrine cell tumors [23]. In our study, lower concentrations were evaluated compared with those used for neoplasm treatment in an effort to achieve a modulation effect on Akt activity rather than complete inhibition.

Table 3
Distribution of lipids in the cytoplasm of bovine oocytes matured in the presence of different concentrations of triciribine.

	n	Peripheral (%)	Dispersed (%)
Control	102	33.6 ± 2.6[a]	66.4 ± 2.6[a]
1 μM	95	24.8 ± 5.1[ab]	74.3 ± 5.9[a]
5 μM	94	17.2 ± 8.1[b]	79.6 ± 10.5[a]
10 μM	95	27.5 ± 11.1[ab]	72.6 ± 11.1[a]
20 μM	78	28.2 ± 2.2[ab]	71.7 ± 2.2[a]

The evaluations were performed after 22 h of IVM. The results represent the average percentage of each category in four replicates. Means ± standard deviation followed by the same letter do not differ statistically within columns according to the SNK test at 5 % probability.

Table 4
Effect of supplementation of the IVM medium with 0 (control), 1, 5, 10, 20 μM of triciribine on *in vitro* embryo production.

	n	Cleavage rate(%)	Blastocyst rate(%)	Hatching rate(%)
Control	81	92.5 ± 3.0[a]	27.3 ± 4.1[b]	67.3 ± 23.9[a]
1 μM	76	94.4 ± 5.1[a]	47.1 ± 11.1[a]	60.2 ± 23.6[a]
5 μM	82	97.6 ± 4.8[a]	45.8 ± 5.7[ab]	59.0 ± 20.3[a]
10 μM	80	93.7 ± 4.8[a]	30.1 ± 8.3[b]	65.6 ± 19.9[a]
20 μM	70	70.4 ± 7.7[b]	12.8 ± 6.2[c]	33.3 ± 33.3[a]

The results represent the mean percentage of each treatment with four replicates. Means ± standard deviation followed by the same letter do not differ statistically within columns according to the SNK test at 5 % probability.

The maturation of COCs in the presence of 1, 5, or 10 μM of triciribine did not influence the percentage of oocytes in metaphase II. On the other hand, when triciribine was used at a concentration of 20 μM, the percentage of oocytes progressing to the metaphase II stage decreased, while there was a significant increase in the percentage of oocytes in metaphase I, indicating a dose-dependent effect of triciribine on meiotic progression. A similar result was reported by Tomek and Smiljakovic (2005) [20], who obtained an increase in the percentage of bovine oocytes in the metaphase I stage when 50 μM SH6, another Akt inhibitor, were used during the 22 h *in vitro* maturation period.

In our study, the highest concentration of triciribine (20 μM) resulted in the appearance of aberrant chromosomal configurations. The nuclear events of meiosis depend on the dynamics of microtubules and microfilaments, which must act together for the correct segregation of chromosomes when preparing the oocyte for fertilization. In mice, Akt participates in the proper arrangement of microtubules and chromosomes during IVM [25]. The use of specific antibodies for pAkt-Ser473 during mouse oocyte IVM compromised the extrusion of the second polar body, and when anti-pAktThr^{308} was used, the distribution of microtubules was affected and the chromosomes showed aberrant alignment [26]. Therefore, the use of triciribine appears to be correlated with the incorrect segregation of chromosomes during bovine oocyte IVM by dose-dependently inhibiting Akt activity during the dynamics of microtubule and microfilament polymerization and depolymerization. This finding demonstrates the importance of Akt in meiotic spindle stabilization, chromosome segregation and meiosis progretion in the maturation process of bovine oocytes, similar to what has been demonstrated in other species [26–28].

In the present study, mitochondrial distribution was used as one of the indicators of cytoplasmic maturation. Considering that there is no synthesis of new organelles until the blastocyst stage, the energy supply for transcription and traslation during oocyte maturation and embryonic development must be guaranteed and provided by the oocyte mitochondria [29]. In immature bovine oocytes, mitochondria are small, largely inactive, and distributed in clusters in the peripheral oocyte region [30]. During *in vitro* maturation, mitochondria move from the peripheral region of the oocytes and are redistributed throughout the cytoplasm. Mitochondrial redistribution failureis related to incomplete cytoplasmic maturation and correlated with low competence for embryonic development [31–35].

In our study, 30 % of the oocytes in the control group showed dispersed distribution of mitochondria in the cytoplasm, 31.5 % were in a transitional region, and approximately 38 % continued to exhibit a peripheral distribution characteristic of immature oocytes. Similarly, Del Collado (2015) [23] reported that after *in vitro* maturation, 32.3 % of bovine oocytes showed dispersed mitochondria, 29.2 % were in a transitional region, and 38.5 % of bovine oocytes exhibited peripheral mitochondria. Liu et al. (2010) [36] evaluated the mitochondrial distribution in human oocytes after *in vitro* and *in vivo* maturation. According to these authors, only 13.2 % showed a diffuse distribution of mitochondria after IVM among oocytes in metaphase II, while 75.5 % exhibited a semi-peripheral distribution and 11 % showed a peripheral distribution. In contrast, 80 % had mitochondria distributed in the

Table 5
Effect of triciribine on meiotic resumption.

		3 h			6 h			9 h	
		GV	GVBD		GV	GVBD		GV	GVBD
	n	(mean ± SD)	(mean ± SD)	n	(mean ± SD)	(mean ± SD)	n	(mean ± SD)	(mean ± SD)
Control	56	(84.4 ± 7.2)	(15.6 ± 7.2)		(55.6 ± 27.4)	(44.4 ± 7.4)		(3.7 ± 6.4)	(96,30 ± 6,4)
1 μM		(83.3 ± 8.4)	(16.6 ± 8.4)		(40.5 ± 19.8)	(57.5 ± 16.4)		(2.8 ± 4.8)	(97,23 ± 4,8)

The evaluations were performed at 3, 6, and 9 h of IVM. The results represent the mean percentage of each category in four replicates. Means ± standard deviation followed by the same letter do not differ statistically within the column by the SNK test at 5 % probability. VG – germinal vesicle, GVBD - germinal vesicle breakdown.

central region of the oocyte among those matured *in vivo*. These findings indicate alterations in the cytoplasmic redistribution of mitochondria during *in vitro* maturation, while approximately 40 % of the oocytes appear to maintain peripherally distributed mitochondria.

However, when 1 μM and 10 μM of triciribine were used during IVM, there was a significant reduction in the percentage of oocytes with peripheral mitochondria compared with the control group, and when 1 μM of triciribine was used, there was an increase in these organelles in the transitional region, which may indicate an improvement in cytoplasmic maturation in the presence of the inhibitor. After *in vitro* maturation of oocytes, mitochondrial activity increases and its distribution becomes diffuse throughout the cytoplasm in regions with higher ATP and calcium requirements [31,34]. This change is essential for the oocyte to achieve nuclear and cytoplasmic maturation and meiotic competence and could be one of the reasons for the increased blastocyst rate in the group treated with 1.0 μM of triciribine. On the other hand, there was a significant reduction in the number of oocytes exhibiting mitochondria uniformly distributed in the cytoplasm compared with the other groups when 20 μM of the inhibitor was used. Along with the findings regarding nuclear maturation, this result underscores the cytotoxic effects of the inhibitor when used at higher concentrations.

Lipids represent an important source of energy through β-oxidation and are stored in droplets that are relocated during the maturation process. Similar to mitochondria, lipids are structures that tend to migrate from the periphery towards the nucleus to ensure the supply of energy. It is possible that the correlation between these organelles is favorable for β-oxidation [37]. In our study, the use of 5 μM of the inhibitor led to an increase in the percentage of oocytes with lipid droplets distributed more centrally, which indicates an improvement in cytoplasmic maturation.

Taken together, the results regarding mitochondrial distribution and lipid droplets during IVM in the presence of the inhibitor demonstrated that the use of triciribine at doses of 1 and 5 μM can lead to enhanced cytoplasmic maturation.

When the lowest concentration (1 μM) of the inhibitor was used during IVM, there was an increase in the number of embryos produced *in vitro*. On the other hand, when COCs matured in the presence of 20 μM of triciribine were fertilized *in vitro*, early embryonic development was compromised and there was a significant reduction in cleavage rate and blastocyst production. These results confirm the dose-dependent effects of the inhibitor, but it remains unclear how modulation of Akt activity during IVM can increase the *in vitro* production of embryos. It is known that Akt regulates PDE, an enzyme involved in the control of intraoocytic cAMP levels [20,38]. It is possible that the modulation of Akt activity promotes a reduction in PDE activity, leading to a less abrupt decrease in cAMP concentration in oocytes during the resumption and progression of meiosis. This may have contributed to enhanced synchrony between nuclear and cytoplasmic events during maturation, thus bolstering oocyte competence for fertilization and embryonic development.

Oocytes matured in the presence of 1 μM of triciribine were evaluated at 3, 6, and 9 h post-IVM in an attempt to elucidate whether the increase in the number of blastocysts produced in this group was due to an alteration in the kinetics of germinal vesicle breakdown and a consequent improvement in the synchrony between nuclear and cytoplasmic maturation events in the early hours of IVM. Interestingly, there was no significant difference between the treated group and the control group. Anas et al. (2000) [39] also found no significant difference in the timing of germinal vesicle breakdown in the presence of the PI3K inhibitor wortmannin. Therefore, it is suggested that the use of 1 μM of triciribine during IVM does not alter the kinetics of resumption of maturation at the analyzed time points, but rather the progression of maturation, especially between the stages of metaphase I and II.

Since Akt is also involved in other cellular processes such as energy metabolism, cell survival, and protein synthesis, among others, it is possible that the modulationof its activity can influence oocyte maturation and early embryonic development through mechanisms beyond those analyzed in this study.

5. Conclusion

The supplementation of *in vitro* maturation medium with triciribine, a selective inhibitor of Akt, affected nuclear maturation, the distribution of mitochondria and lipid droplets without compromising meiotic resumption. Treatment of COCs with 1 μM oftriciribine resulted in an increased rate of blastocyst production. These results offer important insights for the development of potential enhancement techniques for the production of bovine embryos through the use of Akt inhibitors.

CRediT authorship contribution statement

A.G. Curcio: Writing – original draft, Methodology, Formal analysis, Data curation. **T.I.S. Ribeiro:** Writing – original draft, Methodology. **H. F. Gomes:** Writing – original draft, Formal analysis, Conceptualization. **C.S. Paes de Carvalho:** Writing – original draft, Methodology, Formal analysis. **M.C.C. Bussiere:** Writing – original draft, Supervision, Formal analysis. **A.J.B. Dias:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was supported by the FAPERJ (process no. E-26/112.123/2012).

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