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Role of phosphoinositide 3-kinase/ protein kinase B/ phosphatase and tensin homologue (PI3K/AKT/PTEN) pathway inhibitors during *in vitro* maturation of mammalian oocytes on *in vitro* embryo production: A systematic review

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ABSTRACT

Modulation of phosphoinositide 3-kinase/protein kinase B/phosphatase and tensin homologue (PI3K/AKT/PTEN) pathway in mammals yields mixed results. A deep understanding of its regulation can be a powerful tool for better *in vitro* blastocyst production. This systematic review aims to map the evidence of PI3K/AKT/PTEN pathway modulation during *in vitro* maturation (IVM), to assess its effects on meiosis resumption and nuclear maturation progression of mammalian oocytes, and their impacts on embryo development and quality. A total of 1058 articles were screened in three databases, and 22 articles were included. Fifty-two IVM assessments were identified, among which 11 evaluated blastocyst yield. Three PI3K inhibitors (3-methyladenine, Wortmannin, and LY294002) and one AKT inhibitor (SH6) were investigated. The impact of this pathway modulation on meiosis resumption in swines and murines was not well established, depending on the inhibitor used, concentration, and media supplementation, while in bovines, resumption seems to be independent of PI3K/AKT/PTEN pathway. However, progression to metaphase II (MII) is highly controlled by this pathway on both bovines and swines. Studies that focused on the inhibition reversibility showed that the removal of the modulator produced MII rates similar to the control group. Experiments that aimed to temporarily block meiosis resumption or reduce PI3K activity resulted in blastocyst production equal to or even higher than control groups. Altogether, these data indicate the paramount potential of this pathway as a possible strategy to improve overall *in vitro* embryo production efficiency, by synchronizing both nuclear and cytoplasmic maturation.

1. Introduction

In vitro embryo production (IVP) has become an important industry, developing with success over the last 40 years [1]. Given that the oocyte is responsible for the future development of the embryo until embryonic genome activation, the quality of this single cell is one major factor limiting the blastocyst yield [2]. In this context, the intrinsic quality of the oocyte associated with *in vitro* maturation (IVM) conditions are both responsible for blastocyst production and, therefore, improving IVM is essential to enhance overall IVP rates [3].

A high concentration of intra oocyte cAMP is responsible for maintaining the oocyte arrested in a germinal vesicle stage by keeping maturation promoting factor (MPF) inactivated. When the oocyte is removed from the follicular environment, nuclear progression resumes spontaneously [4] due to flow reduction of cyclic nucleotides such as cAMP from the granulosa cells to the oocyte by gap junctions. This event causes a loss of synchrony between cytoplasmic and nuclear maturation, impairing the blastocyst yield [5]. In this context, modulation using either inhibitors or activators of resumption and progression of meiosis pathways is one of the possible strategies to understand the physiological cascade of such processes [6].

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Phosphoinositide 3-kinase/protein kinase B/phosphatase and tensin homologue (PI3K/AKT/PTEN) pathway is activated in response to tyrosine-kinase growth factors, protein G coupled receptors, Ras protein, or steroids [7] (Fig. 1). Class I PI3Ks catalyze the conversion of phosphatidylinositol-4,5-bisphosphate (PIP_2) to phosphatidylinositol-3,4,5-triphosphate (PIP_3). Class II PI3Ks preferentially act on the modification of phosphatidylinositol-3-phosphate (PIP) into PIP_2. Class III enzymes, conversely, produce PIP through the catalysis of phosphatidylinositol [8,9]. Phosphatidylinositol subproducts recruit phosphoinositide-dependent kinase 1 (PDK1) to the membrane, and phosphorylates AKT, a serine-threonine kinase, that in turn activates phosphodiesterase 3 (PDE3), promoting the reduction of intracellular cAMP levels and the consequent activation of MPF. This results in the resumption of meiosis [10,11]. Conversely, PTEN is the intrinsic main negative regulator of PI3K, by dephosphorylating PIP_3 [12].

In non-mammalian oocytes, such as Xenopus and Zebrafish, studies have shown that germinal vesicle breakdown (GVBD) is delayed or blocked by adding direct inhibitors of PI3K or AKT [13,14], demonstrating the paramount significance of this pathway to meiosis resumption. However, in mammals, the contrasting and variable results found in the literature demonstrate that this pathway can have singularities [15], as distinct inhibitor effects can be seen in different species. Thus, this systematic review aims to map the evidence on the modulation of the PI3K/AKT/PTEN pathway during IVM, and to assess its effects on meiosis resumption and nuclear maturation progression of mammalian oocytes, and their impacts on embryo development and quality.

2. Material and methods

2.1. Search and selection of articles

The structured question to this review was: "What is the effect of the addition of PI3K/AKT/PTEN pathway modulators in the *in vitro* maturation medium of mammalian oocytes on meiosis

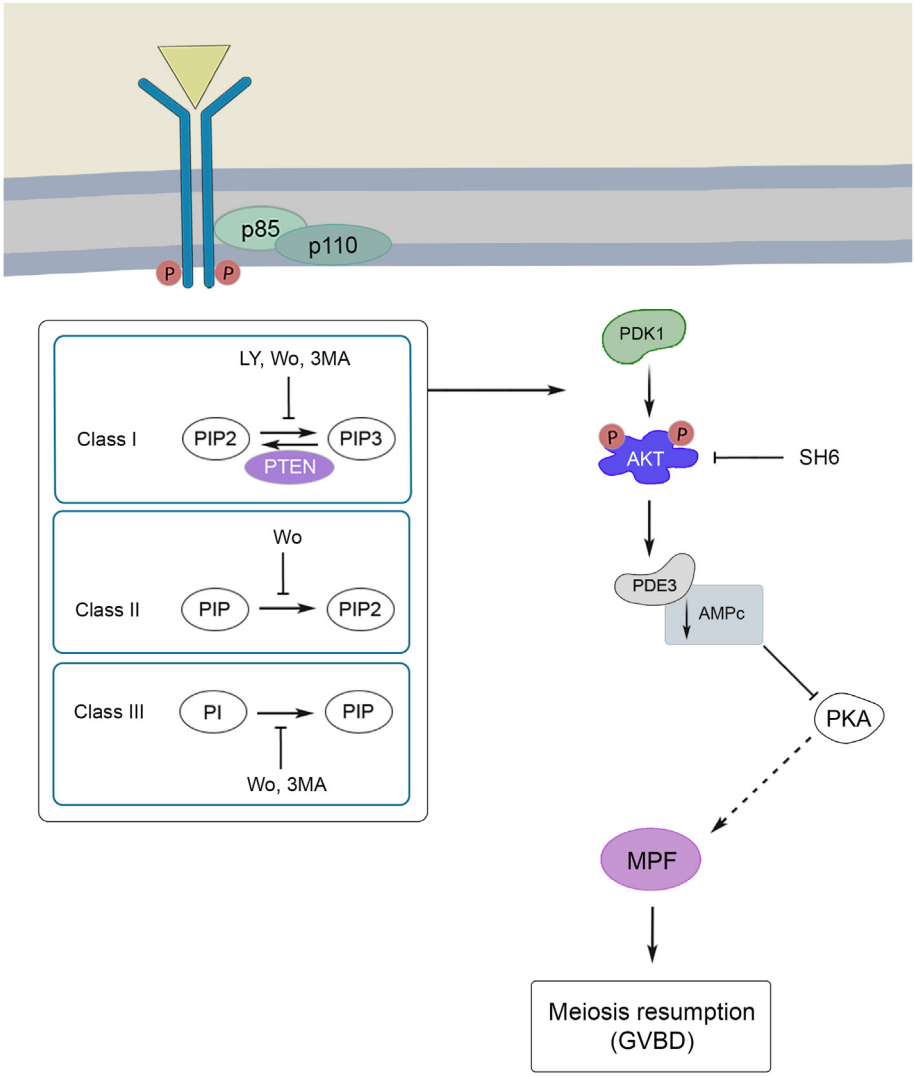

Fig. 1. The Phosphoinositide 3-kinase/protein kinase B/phosphatase and tensin homologue (PI3K/AKT/PTEN) pathway in mammals and its direct inhibitors. The PI3K is activated by insulin, steroids, or growth factors and acts on the catalyzes of phosphatidylinositol products according to the PI3K classes. Class I PI3Ks acts on the catalyzes of phosphatidylinositol-3,4,5-triphosphate (PIP_3) from phosphatidylinositol-4,5-bisphosphate (PIP_2). Along with that, class II acts on the reaction of phosphatidylinositol-3-phosphate (PIP) in PIP_2. Moreover, class III produce PIP through the catalyzes of phosphatidylinositol (PI). Specific direct inhibitors act on each class of PI3K, such as: LY294002 (LY), that only binds to class I; PTEN, an intrinsic negative regulator of the same class aforementioned; 3 MA, acts on class I and III; and Wortmannin (Wo), covalently binds to all three classes. This production of phosphatidylinositol molecules recruits PDK1 to membrane, where it phosphorylates AKT. The pathway continues by the phosphorylation of phosphodiesterase 3 (PDE3), that reduces the cAMP levels and, consequently, inhibits PKA, triggering the resumption of meiosis by MPF activation.

resumption and progression?". A Search key (Supplementary Table S1) was designed considering the PICO framework [16], a mnemonic proposed by evidence-based practice to define questions in terms of the studies problem/population, intervention, comparison, and outcome, in which: P (population) = mammalian oocytes, I (intervention) = supplementation of PI3K/AKT/PTEN pathway modulators during IVM, C (comparison) = non-modulated oocytes, and O (outcomes) = effect on meiosis resumption and progression, and, if available, subsequent embryo development rate and quality. The search was conducted in May 2021, in the electronic databases PubMed (Medline), Web of Science, (WoS), and Scopus. In the databases, no distinctions were selected in advanced search parameters regarding publication date or language.

After removal of duplicates with the Mendeley reference manager (version 1.19.8, Mendeley Ltd.), a screening was performed with the Rayyan software [17] by two independent reviewers assessing initially only the title and abstract and, subsequently, the suitable articles were fully read to confirm their eligibility by the same reviewers, with disagreements discussed and resolved by other two reviewers. Exclusion criteria included: (1) other subjects (off-topic); (2) reviews or clinical studies; (3) did not study the PI3K/AKT/PTEN pathway; (4) use of indirect inhibitors not related to IVM; (5) did not study mammalian oocytes; or (6) did not assess GVBD and/or metaphase II (MII) rates.

2.2. Critical appraisal

A critical appraisal of the quality of studies was performed based on [18], considering technical quality and adequacy to this review question, according to: (i) experimental design soundness, (ii) methodology description, (iii) article aims, and (iv) focus on the modulation of IVM. Scores were attributed for the absence (0 point) or presence (1 point) for each of these four parameters. The "technical quality" considered the detailed description of the methodology and pertinence to the study objectives. The "adequacy to the review" criteria considered if the use of inhibitors intended to directly inhibit the PI3K/AKT/PTEN pathway, and if there was any cAMP modulator added during the collection of oocytes. While no articles were excluded due to this assessment, the critical appraisal was considered in the discussion to contribute to the weight of the evidence of each article.

2.3. Data extraction

Information regarding species, modulator and concentration, oocyte maturation time, and rates of meiosis resumption and progression were extracted from all included experiments, with post-IVM assessments also compiled, if available. All experimental conditions within the same article were considered separately, except for inhibitor concentration; therefore, the total number of experiments was considered for both IVM and post-IVM (parthenogenetic or *in vitro* development) steps. Meiosis resumption was defined as the oocyte ability to undergo GVBD, while progression was characterized as their capability to undergo the transition GVBD-metaphase I-MII.

3. Results

A total of 1058 articles were obtained from the database search, and 591 remained after replicate removal (Fig. 2). A single article was added manually by reference screening in each of the included articles. Therefore, 592 articles were preliminary screened, and the 39 remainings were fully read to verify if they fitted the inclusion criteria. Twenty-two articles were selected and included in this systematic review.

The critical appraisal step indicated that 45% (10/22) of included articles exhibited all the required parameters for the objectives of the present review (Table 1), reaching a total score of 4. The study aim was the most impacting parameter for the score reduction, followed by the pre-IVM modulation, methodology description, and experimental design, accounting for seven, six, three, and two articles, respectively. Three articles scored 1 point as they exhibited: addition of milrinone in pre-IVM, sequential IVM media, and lack of statistics [11]; addition of 3-isobutyl-1-methylxanthine (IBMX) in pre-IVM, lack of statistics, and its aim was to study the distribution of PIP_3 and F-actin organization [19]; and addition of IBMX in pre-IVM, no detailed information about the cultivated structure, and its aim was to analyze the correlation between the pathway and aurora kinase A [20]. No articles were removed from this systematic review due to their quality score.

From the included articles, direct modulation of PI3K/AKT/PTEN pathway during oocyte IVM in mammals is only reported in swines (n = 10), bovines (n = 6), and murines (n = 4). Studies began in the 1990s, and the number of publications over the decades grew and then stabilized (1990: 3; 2000: 7; 2010: 8; 2020: 2, until the present moment). Due to small changes in experimental conditions within the same article, it was possible to extract 52 IVM experiments and 11 that evaluated the IVM inhibition effects in the post-IVM stages. These changes were regarding the modulator, media supplementation, cultured structure (cumulus-oocyte complex – COC – or denuded oocyte – DO), IVM duration, and exposure time to inhibitors, and activation method (either *in vitro* fertilized – IVF – or parthenogenetic activated – PA). Each experiment analyzing meiosis resumption and/or progression was categorized as positive when the analyzed data was statistically inhibited, while the absence of inhibition indicated a negative effect.

Three direct PI3K inhibitors – 3-methyladenine (3 MA), Wortmannin (Wo), LY294002 (LY) – and one AKT inhibitor (SH6) were used during IVM in the studies, accounting for four (8%), 14 (27%), 26 (50%) and eight (15%) experiments, respectively. No articles were found using PTEN activators. Meiosis resumption rate was analyzed in 81% (42/52) of the experiments, showing a positive effect in 40% of those that analyzed GVBD. Progression to MII was analyzed in 52% (27/52) of the experiments exhibiting negative effects in five (18%) of them. The effect of inhibitors on both meiosis resumption and progression was verified in 33% (17/52) of the experiments.

Evaluation of the impacts of inhibitors supplementation on post-IVM steps was investigated in seven articles (35%), providing 11 experiments on swines and bovines. Therefore, no publication on murine on this matter was identified as published within the timeline of the search, ending May 2021. Out of those, 64% of the experiments reported performing traditional IVF, while 36% induced PA. In all experiments, embryo development rates were assessed, while in 46% (5/11) embryonic quality was also evaluated.

3.1. Swines

Within the ten articles assessing the swine species, 21 experiments were extracted (Table 2), that evaluated the effect of LY (52%), Wo (14%), 3 MA (19%), and SH6 (14%) during IVM. Under five experimental conditions, post-IVM outcomes were described in four articles, using LY, Wo, and 3 MA in 40%, 20%, and 40% of the experiments, respectively (Table 3).

Of the nine experiments (Table 2) analyzing meiosis resumption after swine oocyte IVM supplemented with LY, 44% (4/9) showed positive effects. Regarding meiosis progression, all experiments exhibited positive effects. Inhibitor concentration supplemented during IVM of COCs ranged from 10^{-7} to 10^{-4} M. Positive effect of the inhibitor on meiosis resumption was observed when the

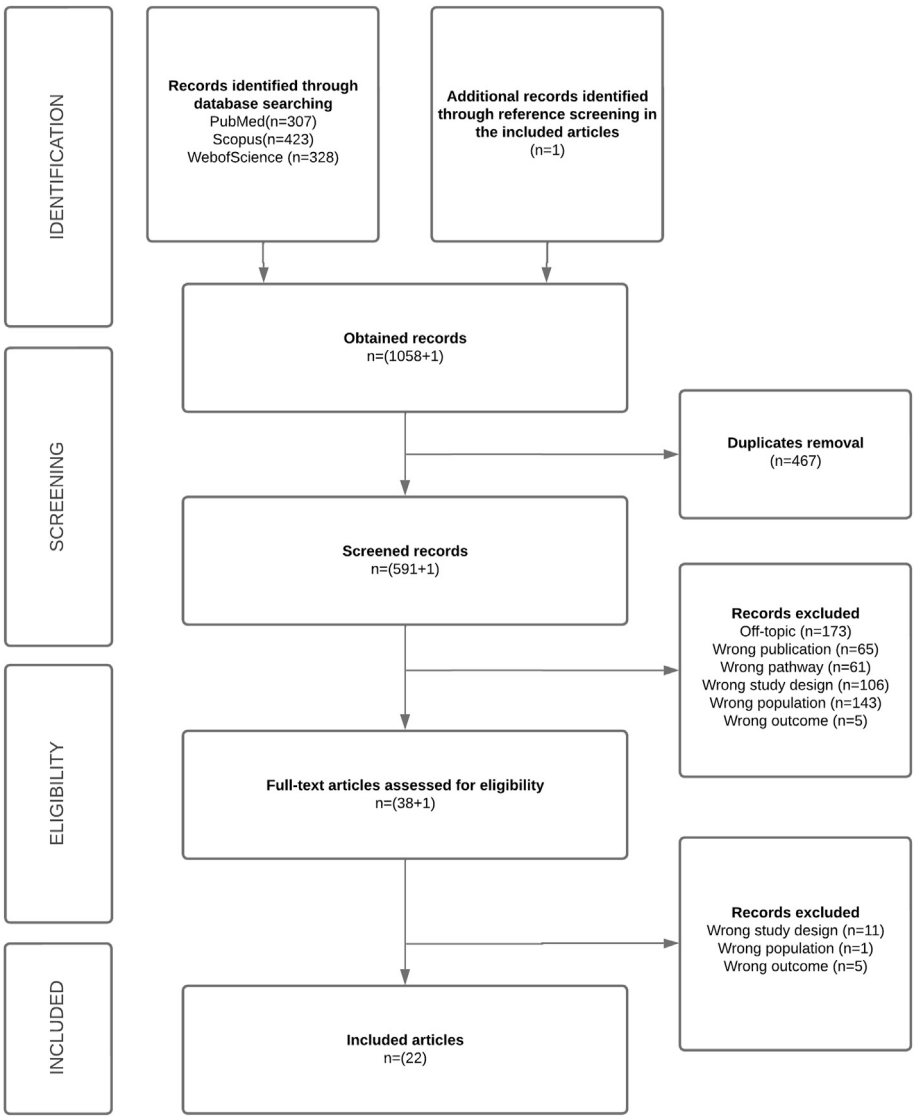

Fig. 2. PRISMA flow chart of identification, screening, eligibility, and included articles considered in this systematic review of Phosphoinositide 3-kinase/protein kinase B/phosphatase and tensin homologue pathway on meiosis resumption and progression of mammalian oocytes according to Moher et al. (2009) [40].

supplementation was from 5×10^{-6} M to 7.5×10^{-5} M [15,21–23], and negative with 10^{-7}, 1 and 5×10^{-6}, and 1 to 5×10^{-5} M [21–24]. As for progression, the concentrations of 1 and 5×10^{-6}, and 1 to 5×10^{-5} M generated positive effects [21,25,26], while 5×10^{-6}, 1 and 2×10^{-5}, and 10^{-4} M resulted in negative effects [21,26]. In DOs, the concentrations of 5×10^{-6} and 5×10^{-5} M were unable to inhibit GVBD [15,22]. Two experiments (Table 3) analyzed the effect of LY supplementation during IVM on *in vitro* culture (IVC). Of the studied concentrations (10^{-6} to 10^{-4} M), 2 and 5×10^{-5} M were able to reduce cleavage rates [26]. The blastocyst rate was reduced with the addition of 10^{-6}, 5×10^{-5}, and 10^{-4} M [25,26]. Also, one experiment analyzed embryonic quality, indicating a reduction in the blastomere/embryo rate [26].

All three experiments assessing the use of Wo (Table 2) exhibited a positive effect, being two of them on resumption rates and one on meiosis progression [15,27]. All concentrations used to study meiosis resumption inhibited GVBD in COCs (10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} M) and DOs (10^{-8} and 10^{-7} M) [15]. Regarding meiosis progression, 10^{-5} M dose exhibited a positive effect, while 1 and 5×10^{-6} M presented a negative effect [27]. Quality and embryonic development rates were analyzed in a single experiment (Table 3), in which the concentrations of 5×10^{-6} and 10^{-5} M were able to reduce blastocyst rate, while not altering the apoptosis rate with the addition of the inhibitor [27].

Four experiments (Table 2) evaluated meiosis resumption and two assessed its progression using 3 MA presenting a positive effect, analyzing the 10^{-2} M concentration in both COCs and DOs [28]. One experiment analyzed the effects of the inhibitor on IVF, demonstrating that 10^{-2} M 3 MA supplementation during IVM did not impact mono- and polyspermy rates. The use of the inhibitor was evaluated in two other experiments on embryo quality and development (Table 3), one being parthenogenetic activated and the other fertilized *in vitro*. In both experiments, rates were similar to the control [28].

The use of SH6 was tested in one article (Table 2), which used a fixed concentration of 5×10^{-5} M. Three experiments were performed in DOs, and only the time of IVM varied (10, 24, or 44 h). At 24 and 44 h, the inhibitor did not impact the meiosis resumption rate, but in the 10 h IVM, resumption was promoted. However, when meiosis progression was evaluated, the inhibitor generated

Table 1
Quality assessment of the articles included in the systematic review regarding the modulation of the PI3K/AKT/PTEN pathway during *in vitro* maturation of mammalian oocytes.

Reference		Technical quality		Adequacy to the review's question		Total
		Experimental Design	Methodology description	Objective	Pre-IVM modulation	
[11]	Kalous et al., 2006	0	0	1	0	1
[15]	Shimada et al., 1998	1	1	1	1	4
[19]	Zheng et al., 2013	1	0	0	0	1
[20]	Saskova et al., 2008	1	0	0	0	1
[21]	Prochazka et al., 2012	1	1	0	1	3
[22]	Li et al., 2008	1	1	1	0	3
[23]	Shimada et al., 2003	1	1	1	1	4
[24]	Shimada et al., 1999	1	1	1	1	4
[25]	Shen et al., 2018	1	1	0	1	3
[26]	Jiao et al., 2020	1	1	1	1	4
[27]	Song et al., 2018	0	1	1	1	3
[28]	Park et al., 2010	1	1	1	1	4
[29]	Kalous et al., 2009	1	1	1	0	3
[30]	Anas et al., 1998	1	1	1	1	4
[31]	De Souza et al., 2018	1	1	1	1	4
[32]	Anas et al., 2000	1	1	1	1	4
[33]	Pereira et al., 2020	1	1	1	1	4
[34]	Sheikh et al., 2019	1	1	0	1	3
[35]	Tomek & Smiljakovi, 2005	1	1	1	1	4
[36]	Hoshino et al., 2004	1	1	1	0	3
[37]	Liu et al., 2019	1	1	0	1	3
[38]	Liu et al., 2018	1	1	0	1	3

Each article was scored in a binary system, with technical quality being assessed by the presence (1) or absence (0) of a detailed description of the methodology and whether it was relevant (1) to the objective of the article or not (0). For the adequacy to the review criteria, the objective of each article was analyzed and verified whether the use of inhibitors intended directly to inhibit pathway (1) or not (0), while it was also analyzed if there was any addition of any cAMP modulator (0) during oocyte collection or not (1).

positive impacts, when observed in 24 or 44 h, by reducing the MII rate [29].

3.2. Bovines

Six articles described the use of pathway modulators during bovine oocyte IVM, enabling the extraction of 17 experiments (Table 4). Four (23%) experiments analyzed only the resumption of meiosis, six (35%) the progression, and seven (41%) both rates. Four (23%), 11 (65%), and two (12%) analyzed the addition of LY, Wo, and SH6, respectively. Moreover, six experiments investigated the effects of inhibitors in post-IVM steps (Table 5), being 33% (2/6) of them performed with LY, 50% (3/6) with Wo, and 17% (1/6) with SH6.

Four experiments (Table 4) supplemented the LY Inhibitor during bovine oocyte IVM. Two of them analyzed the effects on the meiosis resumption, exhibiting a negative effect [30]. When the effects on progression to MII were studied, three and one experiments showed a positive [30,31] and negative effect [31], respectively. LY concentrations of 10^{-4} M and 2.5, 5, and 7.5 $\times$ 10^{-5} M were used in COCs and 2.5, 5, and 7.5 $\times$ 10^{-5} M in DOs. At all concentrations tested, the effects on meiosis resumption rates were negative, while for progression to MII most concentrations used were able to promote a positive effect, apart from 2.5 $\times$ 10^{-5} M [30] and 10^{-4} M when the COCs were cultured without FSH [31]. Two experiments analyzed the effects of adding LY to IVM medium over embryonic development rates (Table 5). The concentration of 10^{-4} M was able to reduce cleavage and blastocyst rates, when FSH was added to IVM media. However, the same concentration without the FSH supplementation yielded the same rates as the control group [31].

All of the 11 experiments (Table 4) that investigated the Wo inhibitor effects on meiosis resumption rates exhibited a negative effect and concentrations used ranged from 10^{-8} to 10^{-6} M [30,32,33]. Furthermore, progression to MII was categorized as positive in 71% (5/7) of the experiments. The studied concentrations ranged from 10^{-8} to 10^{-6} M, and the exposure time to Wo and duration of IVM also varied from 10 min to 34 h and from 22 h to 34 h, respectively. Most concentration/times were able to inhibit the rates of MII in COCs and DOs [30,32,33], however, the addition of 2 $\times$ 10^{-8} M Wo during 28 and 34 h of IVM presented similar MII rates as the control group [33]. Post-IVM rates (Table 5) were analyzed in three experiments, which used a fixed concentration of 2 $\times$ 10^{-8} M of Wo and evaluated inhibitor exposure (10 min, 6 h, or 22 h). An increase in the cleavage and blastocyst rate was described in the group exposed to the inhibitor for 22 h [33].

The addition of SH6 (Table 4) during bovine oocyte IVM was studied in two experiments, which only estimated the effects on the meiosis progression rate. In both experiments, rates were reduced with the addition of the inhibitor, characterizing a positive effect. Supplementation of 1–7.5 $\times$ 10^{-5} M of SH6 was explored in these experiments. While the concentrations of 5 and 7.5 $\times$ 10^{-5} M were able to inhibit the MII progression, 2.5 and 1 $\times$ 10^{-5} M resulted in negative effects [34,35]. One experiment analyzed SH6 addition to embryo quality and development (Table 5). Supplementation of 2.5 $\times$ 10^{-5} M did not change cleavage rate, however, 5 and 7.5 $\times$ 10^{-5} M doses were able to reduce this rate in a dose-dependent manner. Blastocyst rates, similarly, decreased according to applied dose. Furthermore, with the addition of 5 $\times$ 10^{-5} M, it was possible to observe an increase in apoptosis rates, while the blastocyst/embryo and mitochondrial activity rates were reduced [34].

3.3. Murines

Six articles evaluated the effects of PI3K/AKT/PTEN pathway inhibitors on murine oocyte IVM from which 14 experiments were extracted (Table 6), and two inhibitors were used: LY (79%) and SH6 (21%). No article was found addressing post-IVM analysis.

The inhibitor LY was evaluated in 11 experiments, and meiosis resumption was assessed in ten of them, while progression was verified in five (Table 6). The effects over GVBD and MII rates were

Table 2
List of articles and experiments that evaluated the effects of PI3K/AKT/PTEN inhibition during *in vitro* maturation on meiosis resumption and/or progression in swine species. Except for the inhibitor concentration, different experimental conditions within the same article allowed their allocation into experiments.

Reference	Experiment	Inhibitor	COC/DO	Dose (M)	Exp/Dur (h)	IVM Media (protein source and hormones)	Effects on*	
							Resumption	Progression
[28]	1	3 MA	COC	10^{-2}	22	TCM-199; 10% PFF; 0.5 μg/mL FSH; 10 ng/mL EGF; 1 μg/mL E2	Positive	–
	2			10^{-2}	42		Positive	Positive
	3		DO	10^{-2}	22		Positive	–
	4			10^{-2}	42		Positive	Positive
[24]	5	LY	COC	5×10^{-5}	12	NCSU37; 0.3% PVP	Negative	–
[22]	6			10^{-7}	24	TCM-199; 3 mg/mL BSA; 10 ng/mL EGF	Negative	–
				10^{-6}			Negative	–
				10^{-5}			Positive	–
				5×10^{-5}			Positive	–
[15]	7			5×10^{-6}	24	TCM-199; 10% FCS; 10 IU/mL ECG; 1 μg/mL E2; 10 IU/mL HCG	Positive	–
				5×10^{-5}			Positive	–
				7.5×10^{-5}			Positive	–
[23]	8			5×10^{-6}	24	NCSU37; 20 ng/mL FSH	Positive	–
				2×10^{-5}			Positive	–
	9			5×10^{-6}		NCSU37; 2 mM Hx	Negative	–
				2×10^{-5}			Negative	–
[26]	10			10^{-5}	42–44	TCM-199; 10% FBS; 10% PFF; 10 IU/mL EGF; 10 IU/mL HCG	–	Negative
				2×10^{-5}			–	Negative
				5×10^{-5}			–	Positive
				10^{-4}			–	Negative
[21]	11			5×10^{-6}	42	M-199; 5% FCS; 100 ng/mL AREG	Positive	Positive
				10^{-5}			Positive	Positive
				2×10^{-5}			Negative	Positive
				5×10^{-5}			Negative	Positive
	12			5×10^{-6}		M-199; 5% FCS; 1 UI/mL FSH	Negative	Negative
				10^{-5}			Negative	Positive
				2×10^{-5}			Negative	Positive
				5×10^{-5}			Negative	Positive
[25]	13			10^{-6}	44	TCM-199; 10 ng/mL EGF; 10% PFF; 10 IU/mL FSH; 10 IU/mL LH	–	Positive
[22]	14		DO	5×10^{-5}	28	TCM-199; 3 mg/mL BSA; 10 ng/mL EGF	Negative	–
[15]	15			5×10^{-6}	24	TCM-199; 10% FCS; 10 IU/mL ECG; 1 μg/mL E2; 10 IU/mL HCG	Negative	–
				5×10^{-5}			Negative	–
[29]	16	SH6	DO	5×10^{-5}	10	TCM-199; 5% BSA	Negative	–
	17			5×10^{-5}	24		Negative	Positive
	18			5×10^{-5}	44		Negative	Positive
[15]	19	Wo	COC	10^{-9}	24	TCM-199; 10% FCS; 10 IU/mL ECG; 1 μg/mL E2; 10 IU/mL HCG	Positive	–
				10^{-8}			Positive	–
				10^{-7}			Positive	–
				10^{-6}			Positive	–
[27]	20			10^{-6}	44	Sequential: (1) TCM-199; 10% PFF; 10 IU/mL ECG; 10 IU/mL HCG and (2) with no hormones/inhibitor	–	Negative
				5×10^{-6}			–	Negative
				10^{-5}			–	Positive
[15]	21		DO	10^{-8}	24	TCM-199; 10% FCS; 10 IU/mL ECG; 1 μg/mL E2; 10 IU/mL HCG	Positive	–
				10^{-7}			Positive	–

* A positive effect was characterized when the inhibitor supplementation led to the inhibition, while the absence of inhibition was considered as negative effect. Abbreviations: COC: *cumulus*-oocyte complex; DO: denuded oocytes; Exp/Dur: Inhibitor exposure and *in vitro* maturation duration; PFF: porcine follicular fluid; FSH: follicle-stimulating hormone; Wo: wortmannin; LY: LY294002; 3 MA: 3-metiladenina EGF: epidermal growth factor; E2: estradiol; BSA: bovine serum albumin; FCS: fetal calf serum; ECG: equine chorionic gonadotropin; HCG: human chorionic gonadotropin; Hx: hypoxanthine; AREG: amphiregulin; LH: luteal hormone.

characterized. However, three resumption [11] and one progression [19] experiments did not exhibit reliable statistical analyzes, so it was not possible to assume its effect in this systematic review. However, it is noticeable that the authors of both articles indicate that LY was able to inhibit: GVBD during a 55 min IVM [11] using a 10^{-4} M dose; and MII in a 16 h IVM with 2×10^{-5} M LY [19]. Therefore, considering the available data, rates were positive in 57% (4/7) and 50% (2/4), respectively. Inhibitor concentrations supplemented ranged from 10^{-5} to 10^{-3} M in resumption studies, and 10^{-5} and 10^{-4} M in progression. Experiments that used COCs analyzed the effects of 10^{-5} and 10^{-4} M, with addition of FSH and Hx (induced IVM) or not (spontaneous IVM) after Hx pre-IVM supplemented media culture. In spontaneous conditions, supplementation of 10^{-5} M and 10^{-4} M were not able to inhibit GVBD or MII. Alternatively, in induced IVM, the addition of 10^{-5} M did not affect the resumption rate, but inhibited meiosis progression. Also, 10^{-4} M of LY not only inhibited meiosis resumption but also the meiosis progression in FSH-induced IVM [36]. In DOs, in both spontaneous and induced IVM the resumption was not inhibited with 10^{-5} M and 10^{-4} M LY, however, only induced IVM was able to inhibit the MII rate [36]. When IVM did not proceed Hx preculture, meiosis resumption was inhibited with 10^{-3} and 5×10^{-4} M doses [37]. Two other experiments analyzed the meiosis resumption by adding 10^{-4} M LY and exhibited positive effects. However, it was not clear if it was performed in COC or DO.

Three experiments investigated SH6 (Table 6) supplementation in murines IVM, and only meiosis resumption was evaluated, showing a positive effect in all of them. The concentrations used ranged from 10^{-6} to 10^{-4} M. Except for the 10^{-6} M concentration, the doses tested (1 and 5×10^{-5}, and 10^{-4} M) resulted in GVBD inhibition [20,38]. The concentrations of 10^{-6}, 10^{-5}, and 10^{-4} M were studied in DO [38], while in Saskova et al. [20], a 5×10^{-5} M dose was used, but in the described methodology it was not possible to determine which structure was cultivated, limiting the usefulness of the results in the comparisons.

Table 3
List of articles and experiments that evaluated the effects of PI3K/AKT/PTEN inhibition during *in vitro* maturation of complex *cumulus*-oocyte on *in vitro* culture (IVC) of swine embryos. Except for the inhibitor concentration, different experimental conditions within the same article allowed their allocation into experiments. The effects were registered based on the statistical analysis of each article and its control group.

Reference	Experiment	Inhibitor	Dose (M)	IVF/PA	Exp (h)	Dur (h)	Effects on the rates:			
							Cleavage	Blastocyst	Blast/embryo	Apoptosis
[26]	1	LY	10^{-5}	PA	42–44	42–44	Similar	Similar	–	–
			2×10^{-5}				Lower	Similar	–	–
			5×10^{-5}				Lower	Lower	Lower	–
			10^{-4}				Similar	Lower	–	–
[25]	2		10^{-6}		44	44	Similar	Lower	–	–
[27]	3	Wo	10^{-6}		22		–	Similar	Similar	Similar
			5×10^{-6}				–	Lower	Similar	Similar
			10^{-5}				–	Lower	Lower	Similar
[28]	4	3 MA	10^{-2}				Similar	Similar	Similar	–
	5		10^{-2}	IVF			Similar	Similar	Similar	–

Abbreviations: LY: LY294002; Wo: wortmannin; 3 MA: 3-metiladenina; IVF: *in vitro* fertilized; PA: parthenogenetic; Exp: duration of inhibitor exposure; Dur: *in vitro* maturation duration; Blast: blastomere; Similar: similar to the control; Lower: lower than the control.

4. Discussion

According to the overall data retrieved in the present review, the PI3K/AKT/PTEN pathway does not regulate meiosis resumption and progression in mammals in a uniform fashion. The results reported in the selected papers are mainly dependent on species, followed by the inhibitor used, and IVM medium components (Fig. 3). Most of the included articles in this systematic review aimed at the complete pathway inhibition for a basic understanding of the pathway's role on IVM. Yet, a few have explored strategies to temporarily block or delay meiosis resumption, which demonstrated the modulation potential during IVM to improve blastocyst production efficiency.

It is important to note that, although no article was excluded from this systematic review due to its quality assessment score, the described methodology in the three murine articles was insufficient, as did not detail enough to determine with certainty whether COC or DO were cultivated [20], or the effects over GVBD and MII rates, due to lack of statistical analysis [11,19]. Therefore, these articles could not be considered for future comparisons and conclusions. However, other articles also had "technical quality" or "adequacy to the review's question" criteria scored as "0", even so, it was possible for them to be explored and their data compared in this review: regarding the objective, the inhibitor was used to induce apoptosis [34], analyze autophagy [25], understand the role of FSH and amphiregulin (AREG) on the pathway [21], and the correlation between the pathway and protein kinase C [38], or CaMKII [37]; as for the addition of pre-IVM modulators, IBMX [22],

Table 4
List of articles and experiments that evaluated the effects of PI3K/AKT/PTEN inhibition during *in vitro* maturation on meiosis resumption and/or progression in bovine species. Except for the inhibitor concentration, different experimental conditions within the same article allowed their allocation into experiments.

Reference	Experiment	Inhibitor	COC/DO	Dose (M)	Exp.	Dur. (h)	IVM Media (protein source and hormones)	Effects on*	
								Resumption	Progression
[30]	1	LY	COC	2.5×10^{-5}	24 h	24	TCM-199; 10% FCS; 1.3 μg/mL LH; 0.6 μg/mL FSH	Negative	Positive
				5×10^{-5}				Negative	Positive
				7.5×10^{-5}				Negative	Positive
[31]	2			10^{-4}	22–25 h	22–25	MIVB	–	Negative
	3			10^{-4}			MIVB; 10 ng/mL FSH	–	Positive
[30]	4		DO	2.5×10^{-5}	24 h	24	TCM-199; 10% FCS; 1.3 μg/mL LH; 0.6 μg/mL FSH	Negative	Negative
				5×10^{-5}				Negative	Positive
				7.5×10^{-5}				Negative	Positive
[34]	5	SH6	COC	5×10^{-5}	22–24 h	22–24	TCM-199; 1 μg/mL E2; 10 μg/mL FSH; 10 ng/mL EGF; 5% FCS	–	Positive
[35]	6			10^{-5}	24 h	24	TCM-199; 3 mg/mL BSA	–	Negative
				2.5×10^{-5}				–	Negative
				5×10^{-5}				–	Positive
				7.5×10^{-5}				–	Positive
[30]	7	Wo		10^{-8}	24 h	24	TCM-199; 10% FCS; 1.3 μg/mL LH; 0.6 μg/mL FSH	Negative	Positive
				10^{-7}				Negative	Positive
				10^{-6}				Negative	Positive
[32]	8			10^{-7}	4 h	4	TCM-199; 10% FCS; 1.3 μg/mL LH; 0.6 μg/mL FSH	Negative	–
	9			10^{-7}	6 h	6		Negative	–
	10			10^{-7}	8 h	8		Negative	–
	11			10^{-7}	10 h	10		Negative	–
[33]	12			2×10^{-8}	22 h	22	TCM-199; 5 μg/mL LH; 0.5 μg/mL FSH	Negative	Positive
	13			2×10^{-8}	28 h	28		Negative	Negative
	14			2×10^{-8}	34 h	34		Negative	Negative
	15			2×10^{-8}	10 min	22		–	Positive
	16			2×10^{-8}	6 h	22		–	Positive
[30]	17		DO	10^{-7}	24 h	24	TCM-199; 10% FCS; 1.3 μg/mL LH; 0.6 μg/mL FSH	Negative	Positive
				10^{-6}	24 h	24		Negative	Positive

* A positive effect was characterized when the inhibitor supplementation led to the inhibition, while the absence of inhibition was considered as negative effect. Abbreviations: COC: *cumulus*-oocyte complex; DO: denuded oocytes; Exp: duration of inhibitor exposure; Dur: *in vitro* maturation duration; FSH: follicle-stimulating hormone; EGF: epidermal growth factor; E2: estradiol; BSA: bovine serum albumin; FCS: fetal calf serum; LH: luteal hormone.

Table 5
List of articles and experiments that evaluated the effects of PI3K/AKT/PTEN inhibition during *in vitro* maturation of complex *cumulus*-oocyte on *in vitro* culture (IVC) of bovine embryos, after *in vitro* fertilization. Except for the inhibitor concentration, different experimental conditions within the same article allowed their allocation into experiments. The effects were registered based on the statistical analysis of each article and its control group.

Reference	Experiment	Inhibitor	Dose (M)	Exp	Dur. (h)	IVM Media (protein source and hormones)	Effects on the rates:			
							Cleavage	Blastocyst	Blast/ embryo	Apoptosis
[31]	1	LY	10^{-4}	22–25 h	22–25	MIVB	Similar	Similar	–	–
	2		10^{-4}			MIVB; 10 ng/mL FSH	Lower	Lower	–	–
[34]	3	SH6	2.5×10^{-5}	22–24 h	22–24	TCM-199; 1 μg/mL E2; 10 μg/mL FSH; 10 ng/mL EGF; 5% FCS	Similar	Lower	–	–
			5×10^{-5}				Lower	Lower	Lower	Greater
			7.5×10^{-5}				Lower	Lower	–	–
[33]	4	Wo	2×10^{-8}	10 min	22	TCM-199; 5 μg/mL LH; 0.5 μg/mL FSH	Similar	Similar	–	–
	5		2×10^{-8}	6 h			Similar	Similar	–	–
	6		2×10^{-8}	22 h			Greater	Greater	–	–

Abbreviations: LY: LY294002; Wo: wortmannin; Exp: duration of inhibitor exposure; Dur: *in vitro* maturation duration; Blast: blastomere; Similar: similar to the control; Lower: lower than the control; Greater: greater than the control.

hypoxanthine (Hx) [36], and dibutyryl-cAMP [29] were used; and for experimental design category, Song et al. [27] used a sequential medium with/without modulators to assess the effect of pathway inhibition.

In swines, the selected papers show that the pathway regulates the expression of proliferation and survival genes [23,26], mitochondrial activity, and reactive oxygen species (ROS) production [25], in addition to being involved in cumulus cells (CCs) expansion [15,21,26,28]. Pathway modulation was shown to not alter viability [28] and, as reported by Song et al. [27] may be dependent on the oocyte morphological class. The retrieved control of meiosis resumption in this species is still not well defined, according to the data from the included studies, with slightly different results for each employed modulator, and its substrate (Fig. 1).

Different selected articles show that, with 3 MA and Wo supplementation to swine IVM medium, GVBD is pathway-dependent, acting directly through the oocyte [15,27,28]. However, when LY is added to the basic IVM medium, the pathway appears to have no central role in meiosis resumption, as shown by Shimada et al. [24]. Nevertheless, according to other selected references, inhibitor supplementation stimulates GVBD through a CCs-dependent activation [22] and regulates pAKT activity [27], as well as amphiregulin-induced IVM [21]. Regarding retrieved data on FSH-induced IVM, it appears to be dependent on activation of the PI3K/AKT/PTEN pathway [23,29], although there are divergent data reported by Prochazka et al. [21], on a study with similar technical quality, according to the performed critical appraisal. It is possible to hypothesize that the addition of fetal calf serum (FCS) to the IVM medium stimulates pathway activation or delays the GVBD in the first 24 h of IVM. Also, according to data available from the wider literature, Hx was reported as an additive to the IVM medium used to maintain high levels of intraoocyte cAMP [6]. However, when IVM was performed under Hx supplementation condition, as described in the selected study by Shimada et al. [23], LY promoted meiosis resumption. At the end of IVM in the presence of SH6, the rates were similar to the control although meiosis resumption was

Table 6
List of articles and experiments that evaluated the effects of PI3K/AKT/PTEN inhibition during *in vitro* maturation on meiosis resumption and/or progression in murine species. Except for the inhibitor concentration, different experimental conditions within the same article allowed its division into experiments.

Reference	Experiment	Inhibitor	COC/DO	Dose (M)	Exp/Dur.	IVM Media (protein source and hormones)	Effects on*	
							Resumption	Progression
[36]	1	LY	COC	10^{-5}	18 h	MB752/1; 5% FCS	Negative	Negative
				10^{-4}			Negative	Negative
	2			10^{-5}		MB752/1; 5% FCS; 4 mM Hx; 100 IU/L FSH	Negative	Positive
				10^{-4}			Positive	Positive
	3		DO	10^{-5}	18 h	MB752/1; 5% FCS	Negative	Negative
				10^{-4}			Negative	Negative
	4			10^{-5}		MB752/1; 5% FCS; 4 mM Hx; 10 μM FFMAS	Negative	Positive
				10^{-4}			Negative	Positive
[37]	5			5×10^{-4}	4 h	M2	Positive	–
				10^{-3}			Positive	–
[11]	6			10^{-4}	55 min	Sequential: (1) M-199; 2.5 μM milrinone and (2) without milrinone	***	–
	7			10^{-4}	120 min		***	–
	8			10^{-4}	18 h		***	–
[19]	9			2×10^{-5}	16 h	KSOM	–	***
[20]	10		**	10^{-4}	55 min	M16	Positive	–
	11			10^{-4}	2 h		Positive	–
[38]	12	SH6	DO	10^{-6}	4,5 h	M2	Negative	–
				10^{-5}			Positive	–
				10^{-4}			Positive	–
[20]	13		**	5×10^{-5}	55 min	M16	Positive	–
	14			5×10^{-5}	2 h		Positive	–

* A positive effect was characterized when the inhibitor supplementation led to the inhibition, while the absence of inhibition was considered as negative effect.
** It was not possible to identify in the reference which structured was cultivated.
*** Articles did not exhibit a sufficient statistics description for categorization of the effects over resumption and progression. Abbreviations: LY: LY294002; COC: *cumulus*-oocyte complex; DO: denuded oocytes; IVM: *in vitro* maturation; Exp/Dur: Inhibitor exposure and *in vitro* maturation duration; FCS: fetal calf serum; FSH: follicle-stimulating hormone; Hx: hypoxanthine; FFMAS: follicular fluid meiosis-activating sterol.

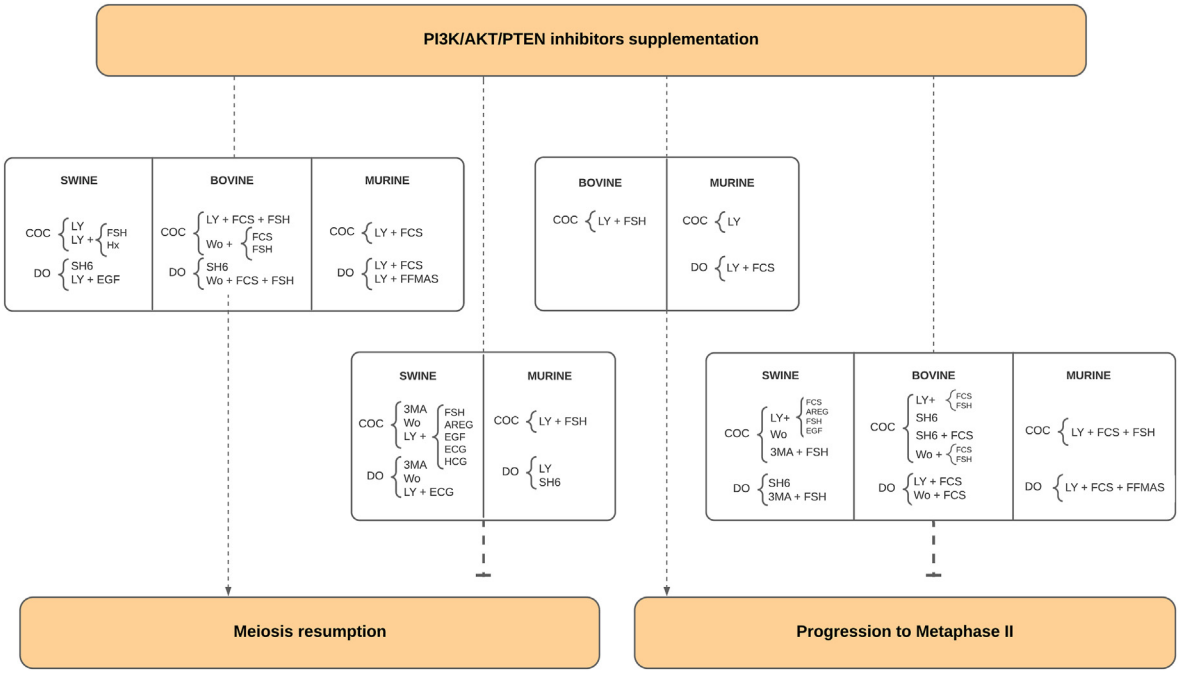

Fig. 3. Summary of evidence from included articles of the systematic review regarding the modulation of the Phosphoinositide 3-kinase/protein kinase B/phosphatase and tensin homologue (PI3K/AKT/PTEN) pathway during *in vitro* maturation of mammalian oocytes. Supplementation of direct inhibitors of PI3K and AKT, along with or without the addition of hormones and/or protein source, in the *in vitro* maturation medium of swines, bovines and murines and its effects on resumption and progression of meiosis. Abbreviations: COC: *cumulus*-oocyte complex; DO: denuded oocytes; FSH: follicle-stimulating hormone; Wo: wortmannin; LY: LY294002; 3 MA: 3-metiladenina EGF: epidermal growth factor; FCS: fetal calf serum; eCG: equine chorionic gonadotropin; hCG: human chorionic gonadotropin; Hx: hypoxanthine; AREG: amphiregulin; FFMAS: follicular fluid meiosis-activating sterol.

accelerated. This could be due to a parallel inhibition of protein kinase A, compensating for AKT inhibition, as proposed in the selected study by Kalous et al. [29]. Nevertheless, control of meiosis progression in swines is highly regulated by PI3K/AKT/PTEN activity since all tested modulators were able to reduce MII rates. Furthermore, Kalous et al. [29] demonstrated the importance of AKT for meiotic spindle formation, since when inhibited the oocytes presented an aberrant spindle, blocking the MI/MII transition.

In bovine oocytes, the identified evidence indicates that the PI3K/AKT/PTEN pathway acts on proliferation and survival, maturation, activity, and distribution of mitochondria, and ROS production [34,35], in addition to regulating steroidogenesis, apoptosis, and expression of LH and FSH receptors in CCs [31]. Expansion of CCs seems to be linked to the activity of AKT and not PI3K, as the addition of LY and Wo did not alter the expansion pattern [30]. However, when SH6 was added, both expansion and related genes were inhibited, as reported by Sheikh et al. [34]. Furthermore, according to the selected studies, pathway modulation affects neither oocyte viability [33], nor GVBD kinetics [32], revealing that meiosis resumption seems to be PI3K/AKT/PTEN independent. The inhibition of other pathway-related enzymes was also explored in some selected references, indicating the modulation does not modify mitogen-activated protein kinase (MAPK) activity [32,35]. However, when Wo was added, cyclin-dependent protein kinase (cdc2) was kept active, inhibiting the MI/MII transition, which is dependent on the reduction of this protein's activity [32]. The same does not occur with the pathway inhibition by SH6, which does not alter MPF, as reported in the study by Tomek & Smijakovi [35]. According to the retrieved data, progression to MII depends on the pathway activation, even when the exposure time to the inhibitor is short [33]. This regulation seems to take place directly through the oocyte, since the culture of both COCs and DOs exhibits consonant data. The SH6 and Wo inhibition proved to be reversible and, when the IVM duration was extended, the MII rates were similar to the control [33,35].

According to the selected studies on the PI3K/AKT/PTEN pathway in murines, this pathway appears to have high control over meiosis resumption in IVM with basic medium [20,37,38] and during FSH-induced IVM [36]. It is important to report that two further selected articles [11,19] also seem to describe the inhibition of meiosis resumption and progression rates, corroborating the aforementioned data, even though they reached low critical appraisal scores due to the lack of statistical analysis to support their findings. Alongside, follicular fluid meiosis-activating sterol-induced IVM in DOs demonstrated that the pathway is dependent on CCs during meiosis resumption, but not for meiosis progression, which acts directly on the oocyte. Still, spontaneous IVM supplemented by FCS was reported by the studies as promoting meiosis resumption and progression to MII [36], since serum typically has molecules such as EGF and insulin [30], which are efficient PI3K/AKT/PTEN pathway promoters [39]. The expansion of CCs appears to be pathway-regulated during FSH-induced IVM according to the results from Hoshino et al. [36]. Furthermore, SH6 supplementation reduced pAKT levels and led to the aberrant oocyte distribution pattern of this kinase, cdc2, and alpha-tubulin [36,38].

A major obstacle to IVP efficiency is still the low quality of the IVM step, since the removal of COCs from the follicular environment promotes the reduction of cyclic nucleotides, resulting in premature meiosis resumption and, thus, desynchronization of nuclear and cytoplasmic maturation. It was previously reported in the scientific literature that some modulators such as cilostamide, IBMX, and forskolin have already been studied aiming to maintain high cAMP levels, resulting in increased blastocyst yield [6]. Likewise, according to data identified in this review, the PI3K/AKT/PTEN pathway has major potential to improve IVP efficiency [33]. Among the selected studies, those focused only on the reversibility of the pathway inhibition showed that the addition of the modulator at the beginning of maturation followed by IVM in a medium without

the inhibitor produced MII rates similar to the control group [15,28,35], except for the study by Song et al. [27]. This difference seems to be dependent on the Wo concentration (10^{-5} to 5×10^{-6} M) used, which probably leads to irreversible inhibition of all classes of PI3K, which was not observed by the selected study of Pereira et al. [33], where PI3K activity was reversibly reduced by 30% by adding 2×10^{-8} M of Wo.

The role of the PI3K/AKT/PTEN pathway during IVM becomes more explicit when the impacts of its inhibition are visualized on blastocyst production. Most of the selected articles demonstrate lower efficiency of cleavage rates and blastocyst yield when the pathway was inhibited. However, two articles reported similar or even higher rates than the control groups by temporarily blocking meiosis resumption [28] and reducing PI3K activity [33]. These strategies proposed by selected articles [15,33] highlight the potential of modulating this pathway for synchronizing nuclear and cytoplasmic maturation, reducing the detrimental effects of COC removal from the follicular environment, being a possible strategy to improve the overall IVP efficiency.

Nevertheless, an important limitation of this systematic review is the data on the oocyte source. Apart from studies in mice, which are generally performed under a controlled environment, the oocytes from bovines and swines come from the abattoir, where little information about the animal is available. Furthermore, as the review was based solely on the statistical analysis of each article, it was not possible to infer other information, such as GVBD inhibition when only MII data were presented or the opposite. However, despite these limitations, the information extracted from the studies allowed a better general understanding of the PI3K/AKT/PTEN pathway among mammalian species.

5. Conclusions

The available data in the literature points out that PI3K/AKT/PTEN pathway plays a crucial role in proliferation, survival, meiotic spindle formation, cytoplasmic protein distribution, regulation of mitochondrial activity, ROS production, and CCs expansion. However, there is evidence that it does not regulate the resumption and progression of meiosis in the same way in different groups of mammals. In swines, progression to MII is reported as highly regulated by this pathway, however, GVBD has different control depending on the inhibitor. In bovines, the resumption is independent and progression dependent on PI3K/AKT/PTEN activity. In murines, the pathway controls resumption if there is no supplementation of pathway activators, whereas progression is reported as pathway-independent when not induced by gonadotropins. Furthermore, the mapped data indicate that reducing pathway activity or temporarily inhibiting meiosis during IVM can be used as a strategy for increasing the efficiency of blastocyst production.

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CRediT authorship contribution statement

Leticia Pereira Alcaráz: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Lucia Prellwitz:** Validation, Investigation, Writing – original draft. **Gutemberg Alves:** Methodology, Writing – review & editing. **Joanna Maria Gonçalves Souza-Fabjan:** Conceptualization, Writing – review & editing. **Angelo José Burla Dias:** Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.theriogenology.2022.06.009.

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