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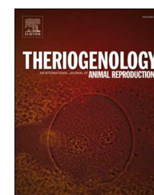

Original Research Article

Impact of reducing lipid content during *in vitro* embryo production: A systematic review and meta-analysis

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A R T I C L E I N F O

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A B S T R A C T

There is still no consensus regarding the role of lipid modulators during *in vitro* embryo production. Thus, we investigated how lipid reducers during the *in vitro* maturation of oocytes (IVM) or *in vitro* culture (IVC) of embryos impact their cryotolerance. A literature search was performed using three databases, recovering 43 articles for the systematic review, comprising 75 experiments (13 performed in IVM, 62 in IVC) and testing 13 substances. In 39 % of the experiments, an increase in oocyte and/or embryo survival after cryopreservation was reported, in contrast to 48 % exhibiting no effect, 5 % causing negative effects, and 8 % influencing in a dose-dependent manner. Of the 75 experiments extracted during IVM and IVC, 41 quantified the lipid content. Of those that reduced lipid content (n = 26), 50 % increased cryotolerance, 34 % had no effect, 8 % harmed oocyte/embryo survival, and 8 % had different results depending on the concentration used. Moreover, 28 out of the 43 studies were analyzed under a meta-analytical approach at the IVC stage in cattle. There was an improvement in the cryotolerance of bovine embryos when the lipid content was reduced. Forskolin, L-carnitine, and phenazine ethosulfate positively affected cryotolerance, while conjugated linoleic acid had no effect and impaired embryonic development. Moreover, fetal bovine serum has a positive impact on cryotolerance. SOF and CR1aa IVC media improved cryotolerance, while mSOF showed no effect. In conclusion, lipid modulators did not unanimously improve cryotolerance, especially when used in IVM, but presented positive effects on cryotolerance during IVC when reaching lipid reduction.

1. Introduction

In recent decades significant advances in improving techniques used in the cryopreservation of oocytes and embryos have been applied. These advances were triggered by the increased demand for superior quality *in vitro* production (IVP)-derived embryos [1]. Although cryopreservation is a well-established technology, gametes and embryos still face many challenges during this process. For instance, oocytes and embryos are highly sensitive to cryopreservation due to structural and physiological characteristics, compromising their viability [2]. In addition, IVP embryos show morphological, molecular, and metabolic differences compared to those produced *in vivo* [3]. This fact contributes to the low success of IVP embryos and limits their potential in the livestock industry [4].

Attempts to optimize the cryopreservation technique in embryos have been based on improving protocols and modulating the embryo composition, mainly the lipid content, to promote more cryo-resistance [5]. The impact of reducing lipid content to improve cryopreservation has been widely studied in several species, including ovine [2], bubaline [6], swine [7], and bovine [8,9]. Although lipids exhibit a variety of important cellular functions, including membrane composition, energy storage, and cell signaling [10], excessive lipid accumulation in IVP embryos is linked to reduced quality and increased susceptibility to the cryopreservation procedure [3]. This is because in the intracellular environment, they accumulate in lipid droplets, and when in excess, they influence the physical properties of the membrane, mainly by altering its fluidity. Changes in the level of unsaturation of membrane glycerophospholipids and the amount of cholesterol present in

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membranes can cause abnormalities in energy metabolism, gene expression, and epigenetic programming (Fig. 1; [5,11]).

The exact mechanisms by which IVP embryos accumulate more lipids are unclear. It appears that this abnormal accumulation may be related to the presence of cytokines and factors that influence de novo lipid synthesis [10] as well as the incorporation of lipids present in components of the culture medium, such as fetal bovine serum (FBS) [12]. These substances can promote changes in the composition of phospholipids in the membrane in proportion to the total content of saturated and unsaturated fatty acids, the number and position of double bonds within the phospholipid, and the level of cholesterol and protein content [13]. Some strategies have been used to reduce lipid content, including centrifugation and micromanipulation of oocytes and embryos [14,15], serum delipidation [2], and modulation of lipid content, for example, with linoleic acid [8,16], forskolin [17], and L-carnitine [18]. Some of these substances also have alternative functions, in addition to lipid modulation, which can interfere with embryonic competence and, consequently, cryosurvival (Fig. 2). Those functions will be detailed later [19,20]. However, information regarding how lipids affect the gamete during IVM, and embryonic development is conflicting [11]. On one hand, studies have shown that excess lipids in blastomeres contribute to low post-thaw embryo survival rates [21,22]. On the other hand, elevated lipid content may not be the primary factor contributing to the diminished cryotolerance of vitrified IVM oocytes. For example, utilizing serum from delipidated estrus sheep during IVM led to a reduction in oocyte lipid content. However, this alteration did not enhance cryotolerance in the vitrified oocytes [2].

A high amount of structural lipids and the consequent maintenance of cell membranes and the embryo's metabolic recovery after thawing are relevant for cryopreservation [11,23]. It is worth mentioning that *Bos taurus* naturally has greater lipid accumulation in IVF-derived embryos than *Bos indicus*, and the former still has greater cryotolerance [24]. Altogether, these data demonstrate that there is still no consensus on the real effect of lipid accumulation during IVM and embryonic development, especially regarding cryotolerance. Therefore, this study aimed to investigate systematically and meta-analytically the impact of reducing the lipid content during IVM and/or *in vitro* culture (IVC) on the cryotolerance of oocytes and/or IVP embryos.

2. Methodology

2.1. Sources of information and research strategy

The study was performed in accordance with the PRISMA statement [25]. The research question to guide this review was: Does reducing the lipid content during IVM and/or IVC improve the cryotolerance of the IVP oocyte or embryo? Three databases, MEDLINE - PubMed, Web of Science, and Scopus, were consulted on July 18, 2023. No language filters or publication date ranges were applied to the search. Multiple (duplicate) article counts were tracked and excluded. The search key adapted to the different databases is shown in Fig. 3. A manual search was performed on the articles included in this review to identify possible articles not found in the electronic search.

2.2. Selection of articles and exclusion criteria

The initial screening of the articles and evaluation of the title and abstract were carried out by two independent authors (EMV, RFB), and the articles were classified as "included," "excluded," or "maybe" in the Rayyan© software. The exclusion criteria included: "off-topic", when another subject was addressed; "wrong publication", for reviews or abstracts; "wrong population", when other cells than oocytes or mammalian embryos were used; "wrong study design", when different substances of interest were used; and "wrong result", when cryopreservation was not performed. Disagreements about the inclusion of the articles were discussed with two other authors (GRL, JMGSF). Then, the included articles were read in full, and one author (EMV) collected the data. The PICO structure was used as the basis for the eligibility criteria of the articles included in this study (Table 1).

2.3. Quality assessment

To the best of our knowledge, there is no specific checklist for assessing the risk of bias that has been validated for *in vitro* studies [26].

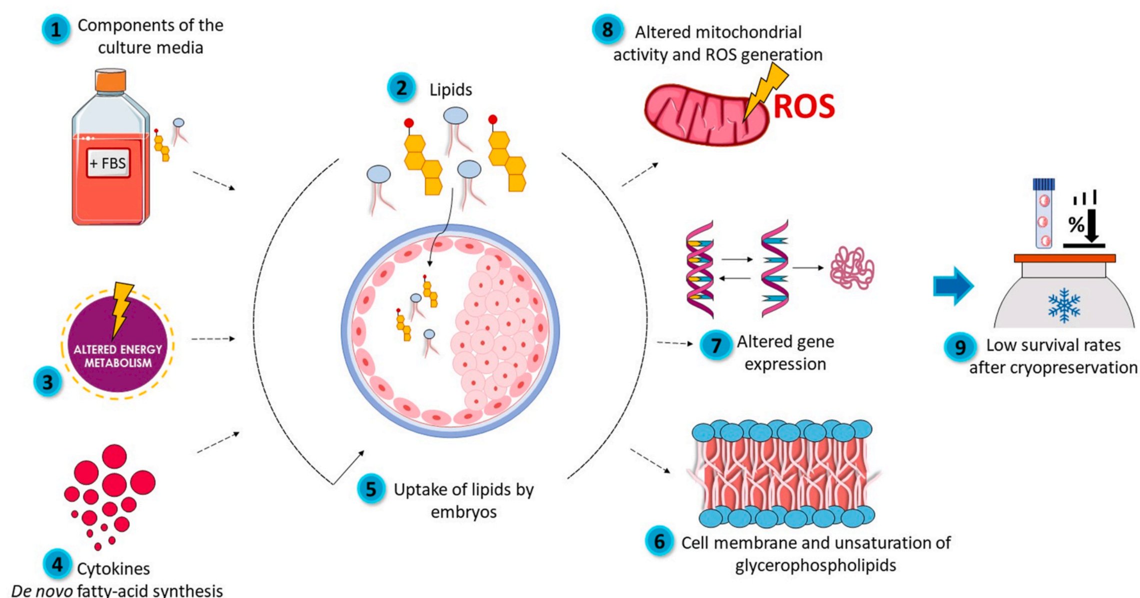

Fig. 1. Schematic representation of sources and lipid uptake by the embryo during *in vitro* culture. 1 – Culture media used during *in vitro* culture provide lipids from components such as FBS; 2 – Lipids; 3 – Alteration of energy metabolism due to the *in vitro* environment promotes the increase of intracellular lipids; 4 – Cytokines promote De novo fatty-acids synthesis; 5 – Uptake of lipids by the embryo; 6 – Excess lipids alter the levels of unsaturation of membrane glycerophospholipids; 7 – Excess lipids alter gene expression; 8 – Mitochondrial activity is altered by excess lipids, and there is an increase in reactive oxygen species (ROS); 9 – Damage caused to the embryo due to excess lipids reduces survival rates after cryopreservation.

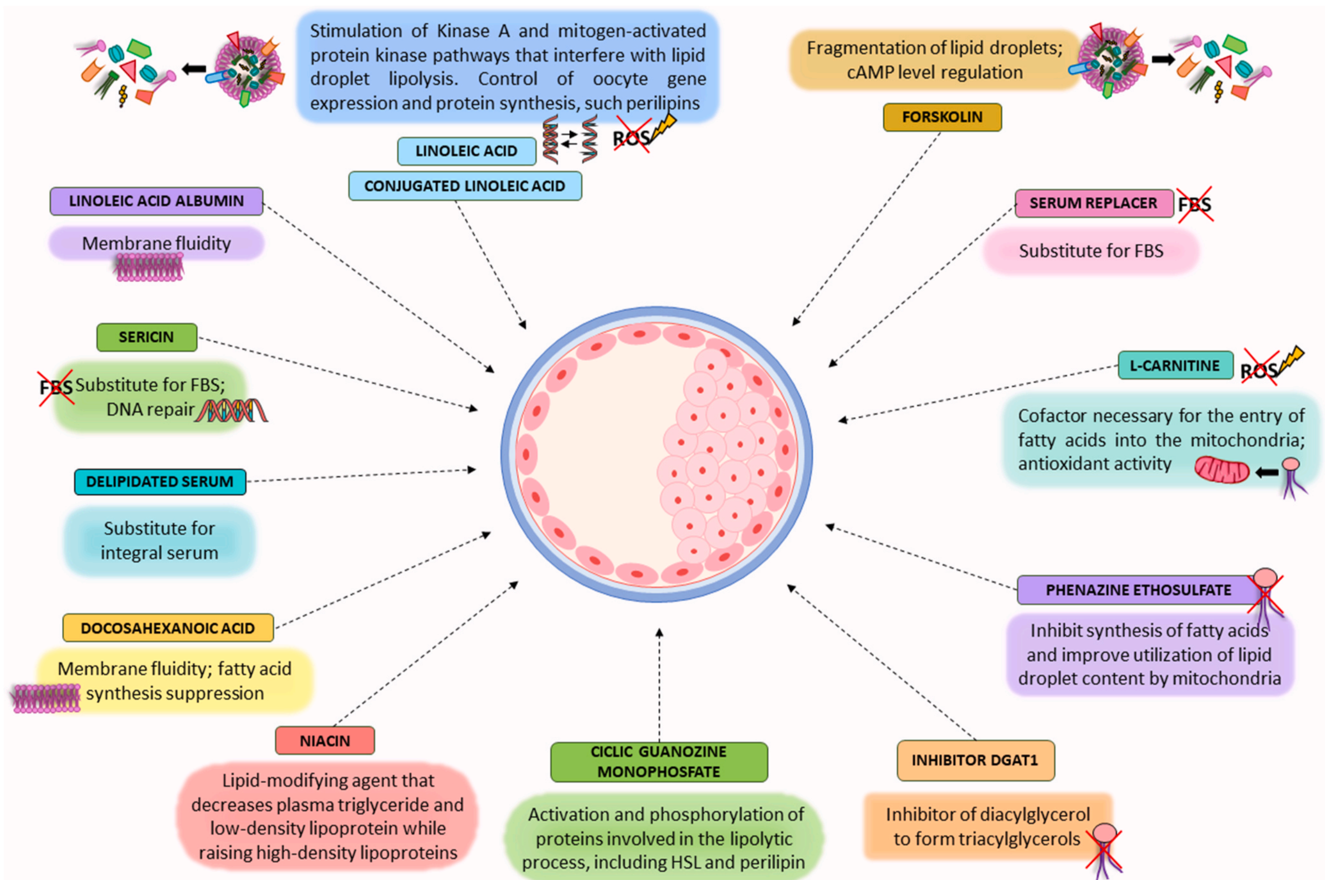

Fig. 2. Schematic representation of the action of lipid reducers investigated in this study. For each substance, the mechanism for reducing intracellular lipids is described, as well as its secondary functions to delipidation.

In this study, we adapted the SciRAP (http://www.scirap.org/) and Parsifal (https://parsif.al/) tools to assess the quality of the included articles. SciRAP includes predefined criteria that allow the evaluation of methodological quality and Parsifal allows the planning and conduction of systematic reviews, also making it possible to create questions directly related to the objective of the studies. The questions assessed in each article were the same as in SciRAP with slight modifications and can be accessed in Supplementary Table 1. The value of responses from each article was shown as a total score for each article. The outcomes for each evaluated criterion were the same as described in SciRAP and judged as fulfilled, partially fulfilled, and not fulfilled. Their respective weights were 1, 0.5, and 0. The maximum score that each article could reach was 16. Then, the points were converted into percentages, and each study article was classified as suggested [27] using the categories of Klimisch: "reliable without restrictions" (score >80 %), "reliable with restrictions" (score >60 %), or "unreliable" (score <60 %).

2.4. Data extraction

Information on species, intervention used (lipid modulator, delipidated serum, or synthetic serum), concentration, IVM, IVC conditions, medium, and survival rates were obligatorily extracted. Data from assessments of lipid content in oocytes and embryos were also extracted, if available. In unfertilized oocytes, the survival rate was defined by membrane integrity and/or hardening of the zona pellucida. Survival rates of IVP embryos were defined by the capacity for re-expansion and embryonic development after cryopreservation. When the article assessed the *in vitro* survival rate at more than one time point, it was classified as follows: when at least one time point (or all of them) showed an improvement, and the others remained similar, it was considered a "positive" result; similarly, if at least one time point (or all of them) showed a reduction, and the others remained similar, it was considered a "negative" result. Obviously, if there was no effect throughout all periods evaluated, it was considered "no effect".

2.5. Meta-analysis

The meta-analysis was conducted on experiments carried out during bovine IVC and extracted during the systematic review stage. This selection was made due to the significantly higher number of bovine-related studies available and presenting the minimum parameters necessary for the analyses. The limited number of studies on other species and in bovine oocytes made it impossible to incorporate them into the meta-analysis. Forty-three eligible experiments extracted from 28 articles were used for the meta-analysis, generating 56 and 62 independent comparisons for the two evaluated variables, 'cryotolerance' and 'blastocyst development', respectively. The other 12 experiments collected during IVC could not be evaluated at the metanalysis due to the lack of information regarding the exact number of embryos and oocytes evaluated during the experiments. Initially, we performed a separate meta-analysis of each variable affected by lipid reduction in IVP. Moreover, according to the information provided by the authors, a subgroup meta-analysis was used to evaluate the studies based on the following methodological characteristics, whenever possible: lipid content, intervention, cryopreservation method, presence of FBS, and culture medium. The analysis was performed using Rev Man 5.1 software (The Nordic Cochrane Centre, The Cochrane Collaboration 2011). The results for each article were expressed as risk ratios with 95 % confidence intervals (CI). The extent of variability across studies and heterogeneity of the summary effect estimates were evaluated using I^2

Database	Search key
Pubmed	(((oocyte OR coc OR cocs OR egg OR ovocyte) AND (delipidation OR "lipid modulator" OR "serum-free culture" OR "synthetic serum" OR "delipidated serum" OR l-carnitine OR forskolin OR "linoleic acid" OR cla OR "phenazine ethosulfate" OR pes OR "lipid metabolism" OR cgmp OR pufa OR "polyunsaturated fatty acids" OR lipids OR lipolysis OR acil-coa OR triacylglycerol OR "cytoplasmic lipids" OR "lipid content" OR "lipid modulation" OR "estrus sheep serum" OR "serum free medium" OR "lipid droplets" OR ld OR "eicosapentaenoic acid" OR "intracellular lipids")) AND ("fetal calf serum" OR fcs OR "fetal bovine serum" OR fbs)) AND (cryotolerance OR cryopreservation OR embryo OR "in vitro embryo production" OR "in vitro development" OR "in vitro maturation" OR "oocyte maturation" OR "in vitro fertilization" OR blastocyst OR Cleavage OR "in vitro development" OR "embryo development" OR "embryonic development" OR "in vitro development" OR ivf OR ivm OR ivc OR ivp OR ivd OR "embryo quality" OR "embryo viability" OR "embryo survival" OR "embryo survivability" OR "oocyte survival" OR "oocyte survivability" OR "embryo cryopreservation" OR "oocyte cryopreservation" OR oocyte cryotolerance OR cryosurvival OR freezing OR vitrification OR "slow freezing" OR "reproductive techniques")
Web of Science	(((oocyte OR coc OR cocs OR egg OR ovocyte) AND (delipidation OR "lipid modulator" OR "serum-free culture" OR "synthetic serum" OR "delipidated serum" OR l-carnitine OR forskolin OR "linoleic acid" OR cla OR "phenazine ethosulfate" OR pes OR "lipid metabolism" OR cgmp OR pufa OR "polyunsaturated fatty acids" OR lipids OR lipolysis OR acil-coa OR triacylglycerol OR "cytoplasmic lipids" OR "lipid content" OR "lipid modulation" OR "estrus sheep serum" OR "serum free medium" OR "lipid droplets" OR ld OR "eicosapentaenoic acid" OR "intracellular lipids")) AND ("fetal calf serum" OR fcs OR "fetal bovine serum" OR fbs)) AND (cryotolerance OR cryopreservation OR embryo OR "in vitro embryo production" OR "in vitro development" OR "in vitro maturation" OR "oocyte maturation" OR "in vitro fertilization" OR blastocyst OR Cleavage OR "in vitro development" OR "embryo development" OR "embryonic development" OR "in vitro development" OR ivf OR ivm OR ivc OR ivp OR ivd OR "embryo quality" OR "embryo viability" OR "embryo survival" OR "embryo survivability" OR "oocyte survival" OR "oocyte survivability" OR "embryo cryopreservation" OR "oocyte cryopreservation" OR oocyte cryotolerance OR cryosurvival OR freezing OR vitrification OR "slow freezing" OR "reproductive techniques")
Scopus	(ALL (oocyte OR coc OR cocs OR egg OR ovocyte) AND ALL (delipidation OR "lipid modulator" OR "serum-free culture" OR "synthetic serum" OR "delipidated serum" OR l-carnitine OR forskolin OR "linoleic acid" OR cla OR "phenazine ethosulfate" OR pes OR "lipid metabolism" OR cgmp OR pufa OR "polyunsaturated fatty acids" OR lipids OR lipolysis OR acil-coa OR triacylglycerol OR "cytoplasmic lipids" OR "lipid content" OR "lipid modulation" OR "estrus sheep serum" OR "serum free medium" OR "lipid droplets" OR ld OR "eicosapentaenoic acid" OR "intracellular lipids") AND ALL ("fetal calf serum" OR fcs OR "fetal bovine serum" OR fbs) AND ALL (cryotolerance OR cryopreservation OR embryo OR "in vitro embryo production" OR "in vitro development" OR "in vitro maturation" OR "oocyte maturation" OR "in vitro fertilization" OR blastocyst OR cleavage OR "in vitro development" OR "embryo development" OR "embryonic development" OR "in vitro development" OR ivf OR ivm OR ivc OR ivp OR ivd OR "embryo quality" OR "embryo viability" OR "embryo survival" OR "embryo survivability" OR "oocyte survival" OR "oocyte survivability" OR "embryo cryopreservation" OR "oocyte cryopreservation" OR oocyte AND cryotolerance OR cryosurvival OR freezing OR vitrification OR "slow freezing" OR "reproductive techniques")))

Fig. 3. Search keys applied to three different databases: Pubmed, Web of Science, and Scopus.

Table 1
Eligibility criteria were determined by the PICO structure (Population, Intervention, Comparator, and Outcome) for the selection of articles.

PICO structure	
Population (P)	Mammalian oocytes and embryos.
Intervention (I)	Exposure to delipidated serum, lipid modulators, or synthetic serum during IVM and/or IVC.
Comparator (C)	Exposure to FBS, female serum in estrus (integral), or control medium without modulator during IVM and/or IVC.
Outcome (O)	Effect on cryotolerance of oocytes and formed embryos.

test statistics. A random effect model was employed where I^2 statistics ≥50 %; otherwise, a fixed effect model was used.

3. Results

The searches yielded 717 articles. After removing the duplicates, 585 articles were evaluated, and 31 articles were selected according to the inclusion criteria after preliminary screening by reading the abstracts. In addition, 12 articles referenced in the selected articles were included in this systematic review, totaling 43 (Fig. 4). Also, 28 out of the 43 articles were analyzed under a meta-analytical approach because they conducted experiments in bovine IVC and presented enough comparisons.

3.1. Quality assessment

All evaluated articles reached scores above 80 %, considered reliable without restriction following the Klimisch criteria.

3.2. Characterization of the eligible studies

In the 43 eligible studies, the use of substances that reduce lipid content and its relationship with cryotolerance was more intensively investigated in bovine, although there are reports in other species as well (Fig. 5). In most of the articles included, the developmental stage at which the embryos underwent cryopreservation was blastocyst; in only two articles, morulae were used [28,29]. The oxygen level was consistent in all articles used in the IVM (high-) and IVC (low-tension) stages. In some cases, it was possible to extract experiments from both the IVM and IVC stages from a single article, even though in different experiments (no article assessed both steps simultaneously). Thus, out of the 43 articles included in this study, 35 evaluated lipid reducers in IVC and 12 in IVM. From these studies, 75 experiments were extracted and analyzed in this systematic review, with 17 % (n = 13) carried out in the IVM and 83 % (n = 62) during IVC. Differences in the experimental design include the type of control group and alterations in the duration of exposure to substances used as an intervention to reduce lipids. Thirteen substances were described as potential reducers of lipid content. L-carnitine was the most studied in all animal models, followed by

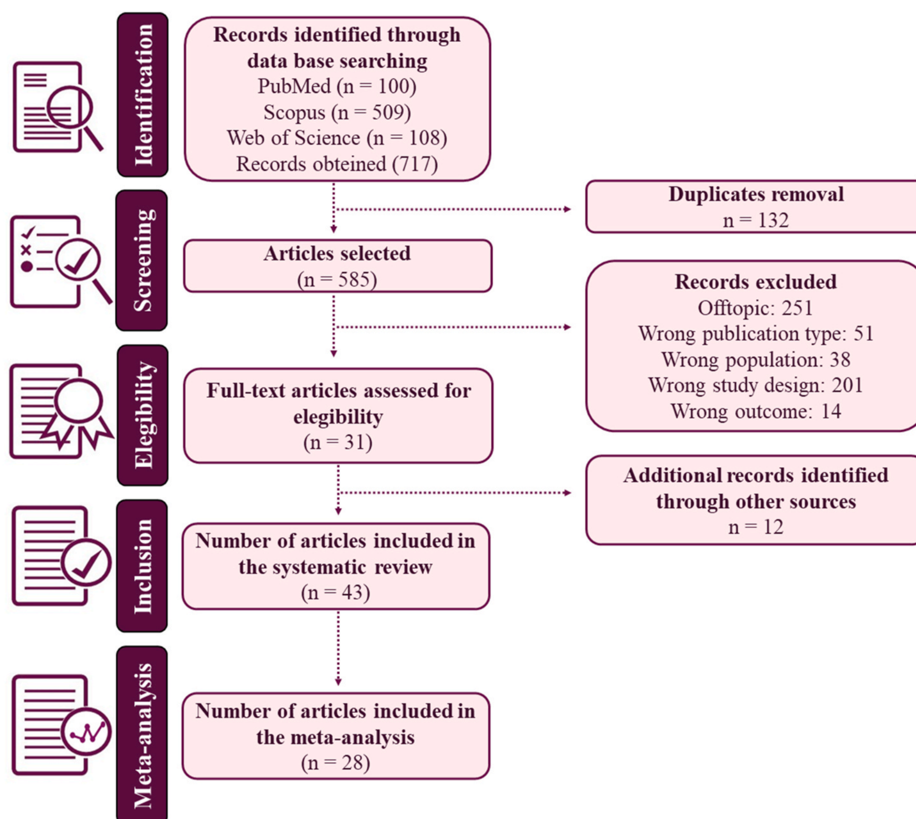

Fig. 4. Flowchart of identification, screening, eligibility, and articles included in the systematic review and meta-analysis.

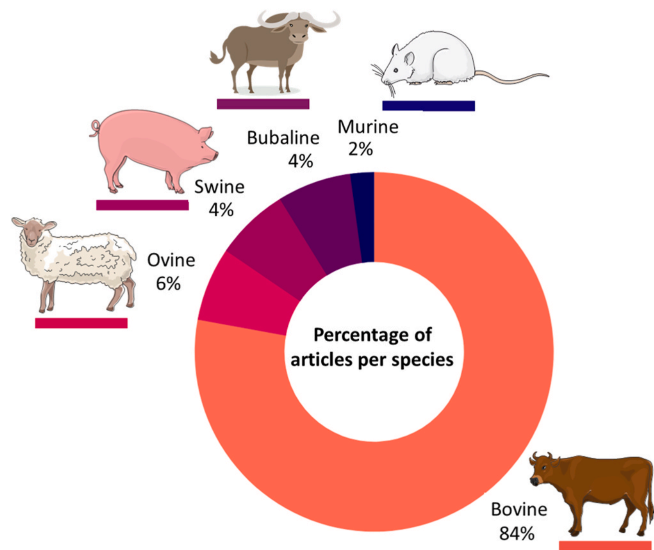

Fig. 5. Percentage of the eligible studies (n = 43) that used domestic species to evaluate the impact of reducing the lipid content during IVM and/or IVC on the cryotolerance of oocytes and/or IVP embryos. The articles were conducted in cattle (n = 35), ovine (n = 3), swine (n = 3), buffaloes (n = 3), and murine (n = 1).

conjugated linoleic acid and forskolin (Fig. 6D). Of the 75 experiments evaluated, 62 were performed in cattle, three in sheep, four in swine, three in buffaloes, and one in murine (Fig. 6A).

Overall, 48 % (n = 36) of the experiments evaluated here demonstrated no effect on post-cryopreservation survival of oocytes and embryos, whereas 39 % (n = 29) of the experiments observed an increment in this parameter. Additionally, 8 % (n = 6) of the experiments showed contrasting effects on post-cryopreservation survival of oocytes and embryos depending on the dose used to reduce lipid content, and 5 % (n = 4) obtained a reduction in embryo/oocyte survival after cryopreservation. Moreover, only 55 % (n = 41) out of the 75 experiments evaluated the reduction of lipid content in the IVM and IVC steps. Of those, 63 % (26/41) reduced lipid content. Specifically, out of those 26 experiments that reduced lipid content, 50 % (13/26) improved cryotolerance, while 34 % (9/26) had no effect, 8 % (2/26) decreased, and 8 % (2/26) had different results depending on the concentration used.

3.3. Bovine

3.3.1. In vitro maturation

Eight articles and nine experiments were extracted from cattle in the IVM stage (Table 2), using niacin, L-carnitine, forskolin, and conjugated linoleic acid. All experiments used interventions in the IVM stage that lasted 20–24 h. Five experiments evaluated the lipid content of embryos produced after using the intervention in the IVM stage. Of those, three reduced lipids, but without improvement in cryotolerance. Survival rates of IVP embryos after IVF from oocytes matured in the presence of 400 mM niacin were improved. Only the lowest molarity enhanced survival rates in the five experiments that used L-carnitine between 3.03 and 3.8 mM [20]. Interestingly other two experiments using 3.03 mM of L-carnitine did not affect survival [30,31]. All other experiments did not affect survival, although one [32] demonstrated decreased lipid content. Two experiments evaluated the effect of different conjugated linoleic acid isomers (9, 11, and 10,12) at a concentration of 15 μM [33], which, respectively, unchanged, and decreased cryotolerance. One experiment [34] evaluated the combination of L-carnitine (2.5 mM), forskolin (15 μM), and conjugated linoleic acid (150 μM) during IVM. Combining the three modulators reduced the lipid content but did not improve cryotolerance. Regarding the culture media, the medium used in all

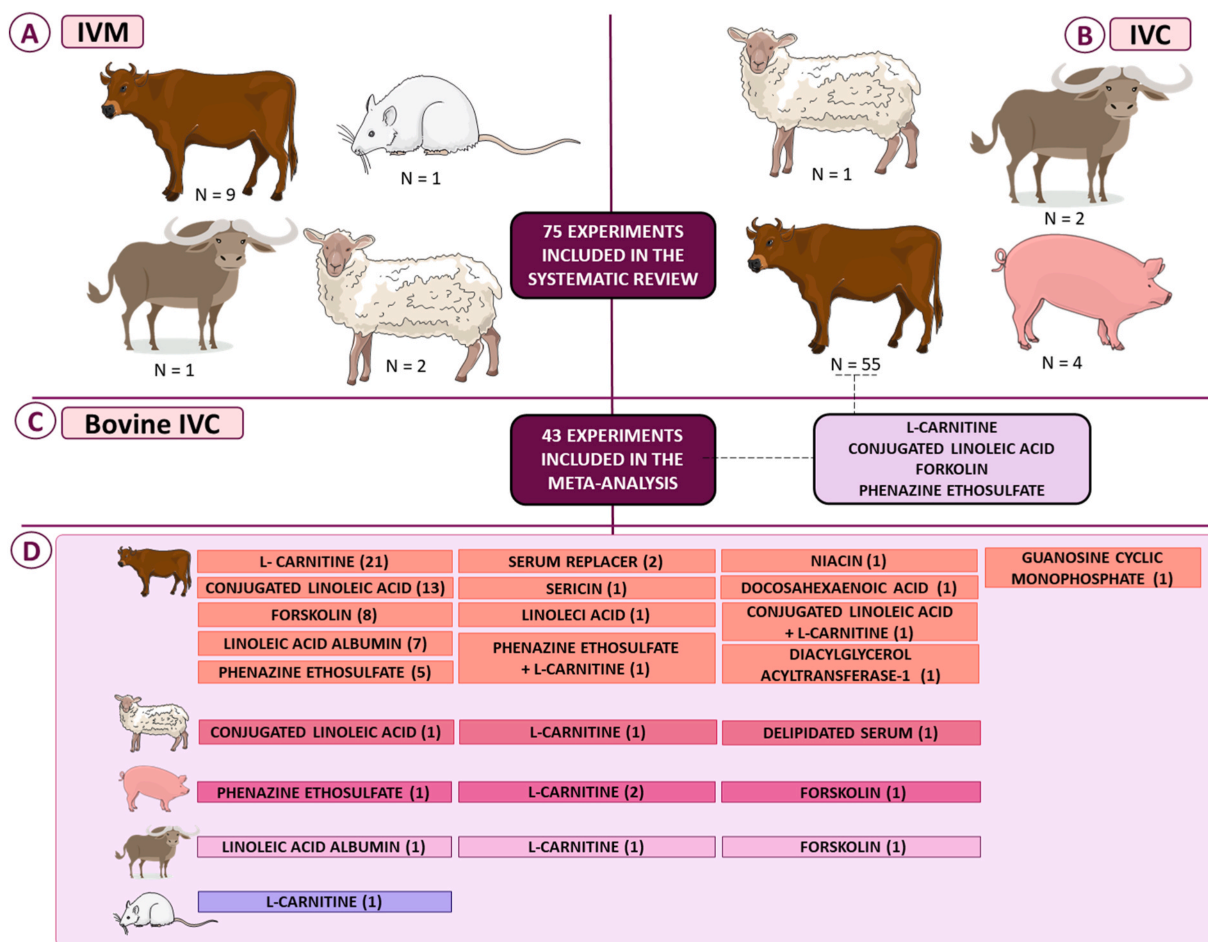

Fig. 6. Schematic representation of the number of experiments extracted from studies using domestic species. **A** – Experiments included in the systematic review evaluating reduce of lipid content and number of experiments extracted during the *in vitro* culture (IVC) step in cattle (57), sheep (1), swine (4), and buffaloes (2). **B** – Interventions used to reduce lipid content during *in vitro* maturation (IVM) of cattle (9), buffaloes (1), sheep (2), and murine (2). **C** – Number of experiments included in the meta-analysis. **D** – Substances used for reducing the lipid content in the different species.

experiments was TCM-199 supplemented with different components, and only one experiment used FBS during IVM [35].

3.3.2. *In vitro culture*

In total, 55 experiments were extracted from 35 articles in cattle in the IVC stage, estimating the effects of 13 substances. Two experiments evaluated the association between L-carnitine and phenazine ethosulfate and between conjugated linoleic acid and L-carnitine (Table 3). In total, 31 articles evaluated the lipid content after intervention with the lipid modulators. Of the 55 experiments that evaluated the cryosurvival of IVP embryos exposed to those interventions, 42 % (n = 23) improved, 47 % (n = 26) had no effect, 2 % (n = 1) decreased, and 9 % (n = 5) improved or not depending on the concentrations of the substances.

Linoleic acid was used in two experiments. The concentration of 100 μM reduced lipids, and improved embryonic survival [16]. The second experiment showed different results depending on the linoleic acid concentration, but 100 μM also improved survivability [36]. Docosahexaenoic acid did not show any role in embryo survival at any of the concentrations tested. Five experiments tested 0.3 μM phenazine ethosulfate. In three of them, there was an improvement in survival 22,37 and lipid reduction in two [22]. The other experiments evaluated did not affect survival [38].

The use of 100 μM conjugated linoleic acid was investigated in 10 experiments under different culture conditions. An improvement in survival was observed in three of these experiments [8,39,40], accompanied by a reduction in lipid content in two [8,39]. The others did not change embryonic survival [33,36,41].

Albumin linoleic acid was evaluated in six experiments throughout the culture or at different exposure times at concentrations from 0.03 % to 0.10 %. All experiments showed a survival improvement, except for using 0.3 % and 0.10 %, which did not improve or unchanged survival, respectively [28,42].

A single experiment investigated the use of diacylglycerol acyltransferase 1 in supplementing the culture medium at concentrations of 10 and 50 μM [3]. The two concentrations tested were able to reduce the lipid content, but only 10 μM improved embryonic survival. The treatment with 50 μM did not affect survivability.

Fifteen experiments were extracted using L-carnitine at concentrations ranging from 0.1 to 6.072 mM. Four experiments showed no effect on survival when LC was used at concentrations of 0.75 mM [35] from day one to four or from day four to seven, as well as 0.5 mM [9]. Two experiments showed different results according to the concentration of L-carnitine used [43,44]. In one article [43], of the three concentrations tested, two improved survival (1.518 and 3.030 mM) and one (6.072 mM) showed no effect. In this experiment, only 3.030 mM was evaluated when reducing lipids and was shown to be effective. In the study by Verma et al. [45], all the concentrations reduced the lipid content, but 0.1 and 2 mM had no effect, just 1.5 mM improved survival. Another experiment evaluating 3.03 mM of L-carnitine [44] reduced the lipid content but also decreased cryosurvival. All other experiments using L-carnitine had positive results regarding survival rates [4,9,35,46].

Sericin at 0.1, 0.5, and 0.15 % reduced lipid content, but did not

Table 2
Compilation of the experiments evaluated in bovine, ovine and murine evaluating the effects of the reduction of lipid content on the cryotolerance of oocyte matured *in vitro*. Data are presented according to intervention, substance concentration, exposure period, IVM medium, assessment of lipid reduction, cryopreservation method and effect on oocyte survival.

Reference	Intervention	Concentration	Exposure	IVM medium	Cryo method	Lipid reduction	Survival
Bovine							
Kafi et al., 2019	Niacin	400 μM	24 h	TCM-199	VIT	–	Positive
Zolini et al., 2019	LC	3.03 mM	20–22 h	TCM-199	SF	–	No effect
Carrillo-González et al., 2020	LC	3.8 mM	24 h	TCM-199	VIT	Yes	No effect
Phongnimitr et al., 2013	LC	3.03 mM	23 h	TCM-199 + 10 % FBS	VIT	–	No effect
Chankitisakul et al., 2013	LC	3.03 mM	21	TCM-199 + 5 % FBS	VIT	–	Positive
Sprícigo et al., 2017	LC	3.03 mM	24 h	TCM-199 + 10 % FBS	VIT	–	No effect
Absalón-Medina et al., 2014	CLA (9,11)	15 μM	23 h	TCM-199 + 10 % FBS	VIT	Not	No effect
	CLA (10,12)	15 μM	23 h	TCM-199 + 10 % FBS		Not	Negative
Oliveira et al., 2022	FK + LC + CLA	15 μM/2.5 mM/150 μM	24 h	TCM-199 + 10 % FBS	VIT	Yes	Negative
Ovine							
Davachi et al., 2018	LC	3.03 mM	24 h	TCM 199	VIT	–	No effect
Barrera et al., 2018	Delipidated serum	10 %	22–24 h	TCM-199	VIT	Yes	No effect
Murine							
Moawad et al., 2013	LC	3.03 mM	16 h	MEM- alpha +5 % FBS	VIT	–	No effect
Bubaline							
El-Sokary et al., 2021	LC	0.25 mM	24 h + IVC	TCM 199 + 10 % FBS	VIT	–	No effect
		0.5 mM	24 h + IVC	TCM 199 + 10 % FBS		–	Positive
		1 mM	24 h + IVC	TCM 199 + 10 % FBS		–	No effect

Abbreviations:=Not determined, Cryo=Cryopreservation, LC=L-carnitine, CLA = Congugated Linoleic Acid, FK = Forskolin, VIT = Vitrification, SF = Slow freezing, FBS = Fetal Bovine Serum, IVC=*In vitro* Culture, IVM = *In Vitro* Maturation.

affect survival. For forskolin, six experiments evaluated the effects of concentrations ranging from 2.5 to 10 μM, and an improvement in survival associated with lipid reduction was observed with the use of 5 and 10 μM in two experiments [47,48]. The remaining experiments showed no effects on survival rates [17,49,50].

Two experiments evaluated the combination of 0.5 mM L-carnitine and 0.3 μM phenazine ethosulfate [38] or between 0.3 mM L-carnitine and 100 μM conjugated linoleic acid [44]. The combination of L-carnitine and phenazine ethosulfate reduced intracellular lipids, but no experiment showed effects on survival. A combination of L-carnitine (5 mM), forskolin (30 μM), and conjugated linoleic acid (300 μM) was tested in two other experiments [34], but no effects on survival rates were observed either.

Sildenafil at a concentration of 10^{-5} was used to modulate cyclic guanosine monophosphate levels and, consequently, lipid content [51] but did not observe effects on embryonic survival. Furthermore, two experiments evaluated the use of 10 % serum replacer under two different experimental conditions: throughout the culture and from the third to the seventh day of culture [52]. Both experiments presented an improvement in survival rates.

3.3.3. *Meta-analysis – in vitro culture*

Regarding the first variable, the overall risk ratio showed that the use of potential lipid content reducers increased the cryotolerance of bovine embryos (Fig. 7). Furthermore, when evaluating only studies in which a reduction in lipid content was confirmed, a significant improvement in the cryotolerance of bovine embryos was observed, while studies in which there was no reduction in lipid content showed no effects on cryotolerance (Fig. 7). Forskolin, L-carnitine, and phenazine ethosulfate promoted an improvement in cryotolerance, proving that both slow freezing and vitrification were effective for the cryopreservation of bovine embryos (Fig. 7). Additionally, the presence of FBS, as well as the use of SOF and CR1aa, increased the cryotolerance of bovine embryos. Interestingly, the mSOF culture medium did not affect the cryotolerance of bovine embryos (Fig. 7).

The overall risk ratio indicated that the use of potential lipid content reducers does not influence the blastocyst development of bovine embryos (Fig. 8). However, in the subgroup analysis of tested substances, conjugated linoleic acid decreased the blastocyst development rates (Fig. 8). The other substances (L-carnitine, Forskolin, and phenazine ethosulfate) did not affect the blastocyst development in bovine embryos. The heterogeneity I^2 was mostly more than 50 % up to more than 75 % for both variables, cryopreservation and embryonic development, indicating substantial and considerable heterogeneity.

3.4. *Ovine*

3.4.1. *In vitro maturation*

Two articles and two experiments were extracted from sheep in the IVM stage (Table 2). The substances used as intervention were L-carnitine [53], and delipidated estrus sheep serum [2]. In both experiments, the IVM stage lasted 22–24 h. Survival rates were evaluated after vitrification of oocytes matured in the presence of 0.6 mg/mL L-carnitine, and in embryos produced from oocytes matured in the presence of 10 % delipidated estrus sheep serum. The use of 0.6 mg/mL L-carnitine had no effect on oocyte survival, and the use of 10 % delipidated estrous sheep serum, showed no effect on oocyte survival, even reducing the lipid content.

3.4.2. *In vitro culture*

One article and one experiment were extracted from sheep in the IVC stage (Table 4). Embryos were produced *in vitro* in SOF medium supplemented with 25 mM or 50 mM conjugated linoleic acid [54], but survival rates after cryopreservation were not affected.

3.5. *Swine*

3.5.1. *In vitro culture*

In swine, three articles were selected, and four experiments were extracted in the IVC stage (Table 4). One experiment evaluated the effect of using 0.05 μM phenazine ethosulfate throughout the culture [55] and was able to reduce embryonic lipid content, it did not affect survival rates. Two experiments evaluated the effects of 3 mM L-carnitine during the entire IVC or the initial 72 h [7]. The survival rates were positive in

Table 3
Compilation of the experiments in cattle evaluating the effects of the reduction of lipid content on the cryotolerance of bovine embryos produced *in vitro*. Data are presented according to intervention, substance concentration, exposure period, IVC medium, assessment of lipid reduction, cryopreservation method, and effect on embryonic survival.

Reference	Intervention	Concentration	Exposure	IVC medium	Lipid reduction	Cryo method	Survival
Accorsi et al., 2016	LA	100 μM	IVC	SOF + BSA	Yes	VIT	Positive
Al Darwich et al., 2010	LA (C18:3)	1 μM	IVC	mSOF +5 % FBS	–	VIT	No effect
	LA (C18:3)	10 μM	IVC	mSOF +5 % FBS	–		No effect
	LA (C18:3)	100 μM	IVC	mSOF +5 % FBS	–		Positive
Al Darwich et al., 2010	DHA (22:6)	1 μM	IVC	mSOF +5 % FBS	–	VIT	No effect
	DHA (22:6)	10 μM	IVC	mSOF +5 % FBS	–		No effect
	DHA (22:6)	100 μM	IVC	mSOF +5 % FBS	–		No effect
Batista et al., 2014	CLA (10,12)	100 μM	IVC	CR1aa +10 % FBS/BSA	Yes	SF	Positive
Carvalho et al., 2019	CLA (10,12)	100 μM	IVC first72 h	SOF +5 % FBS	Not		No effect
	CLA (10,12)	100 μM	IVC	SOF +5 % FBS	Not		No effect
	CLA (10,12)	100 μM	IVC last72 h	SOF +5 % FBS	Not		No effect
Al Darwich et al., 2010	CLA (10,12)	100 μM	IVC	mSOF +5 % FBS	–	VIT	No effect
Dias et al., 2019	CLA (10,12)	100 μM	IVC	SOF +5 % FBS	Not	SF	No effect
Absalón-Medina et al., 2014	CLA (9,11)	100 μM	D 6.5 – IVC	SOF + BSA/5 % FBS	–	VIT	No effect
	CLA (10,12)	100 μM	D 6.5 – IVC	SOF + BSA/5 % FBS	–		No effect
Pereira et al., 2007	CLA (10,12)	100 μM	IVC	M199 + 10 % FBS	–	VIT	Positive
Pereira et al., 2008	CLA	100 μM	IVC	TCM199 + 10 % FCS	Yes	VIT	Positive
Tominaga et al., 2000	LAA	0.25 mg	D0 – D4	CR1aa + BSASOF + FBS	–	SF	Positive
Hochi et al., 1999	LAA	0.03 %	IVC	mSOF + BSA	–	SF	Positive
	LAA	0.1 %	IVC	mSOF + BSA	–		Positive
	LAA	0.3 %	IVC	mSOF + BSA	–		Negative
	LAA	0.1 %	20–90 h hpi	mSOF + BSA	–		Positive
	LAA	0.1 %	138 h hpi	mSOF + BSA	–		Positive
	LAA	0.1 %	20–138 h hpi	mSOF + BSA	–		Positive
Laowtammathron et al., 2005	LAA	0.10 %	IVC	mSOFaa + BSA	–	VIT	No effect
Barceló-Fimbres et al., 2007	PES	0.3 μM	D 2.5 - D7	CDA	–		No effect
Ghanem et al., 2014	PES	0.3 μM	IVC	CR1aa + BSA	Yes	VIT	No effect
Saber et al., 2021	PES	0.3 μM	IVC	TCM-199 + 10 % serum	–	VIT	Positive
Sudano et al., 2011	PES	0.3 mM	PES 60 h	SOF + FBS	Yes	VIT	Positive
	PES	0.3 mM	PES 96 h	SOF + FBS	Yes		Positive
Canõn-Beltran et al., 2020	DGAT1	10 μM	IVC	SOF +5 % FBS	Yes	VIT	Positive
	DGAT1	50 μM	IVC	SOF +5 % FBS	Yes		No effect
Carrillo-Gonzáles et al., 2020	LC	1.5 mM	D3 – D8	SOF + BSA-FAF + FBS	Yes	VIT	No effect
Ghanem et al., 2014	LC	0.5 mM	IVC	CR1aa/BSA	Yes	VIT	No effect
Held-Hoelker et al., 2017	LC	2.5 mM + BSA	IVC	SOF	Yes	SF	Positive
	LC	2.5 mM + BSA FAF	IVC	SOF	Not		Positive
Dias et al., 2019	LC	3.03 mM	IVC	SOF +5 % FBS	Yes	SF	Negative
Takahashi et al., 2012	LC	1.518 mM	IVC	CR1aa +5 % FBS	–	SF	Positive
	LC	3.030 mM	IVC	CR1aa +5 % FBS	Yes		Positive
	LC	6.072 mM	IVC	CR1aa +5 % FBS	–		No effect
Zolini et al., 2019	LC	0.75 mM	IVC	SOF-BE2 +5 % FBS	–	SF	Positive
	LC	1.5 mM	IVC	SOF-BE2 +5 % FBS	–		Positive
	LC	3.03 mM	IVC	SOF-BE2 +5 % FBS	–		Positive
	LC	0.75 mM	D1 – D4	SOF-BE2	–		No effect
	LC	0.75 mM	D4 – D7	SOF-BE2	–		No effect
Ghanem et al., 2022	LC	1.5 mM + 2.5 % FBS	LC all IVCFBS – D3	CR1aa + BSA	Yes	VIT	Positive
	LC	1.5 mM + 5 % FBS	LC all IVCFBS – D3	CR1aa + BSA	Yes		Positive
	LC	1.5 mM + 7.5 % FBS	LC all IVCFBS – D3	CR1aa + BSA	Not		Positive
	LC	1.5 mM + 10 % FBS	LC all IVCFBS – D3	CR1aa + BSA	Not		Positive
Verma et al., 2018	LC	0.1 mM	After IVF48 h	SOF +10 % FBS	Yes	VIT	No effect
	LC	1.5 mM	After IVF48 h	SOF +10 % FBS	Yes		Positive
	LC	2 mM	After IVF48 h	SOF +10 % FBS	Yes		No effect
Owen et al., 2022	LC	0.5 mM	IVC	SOF	Yes	SF	Positive
Hosoe et al., 2017	Sericin	0.5 %	IVC first5 Days	SOF	Yes	SF	No effect
	Sericin	0.1 %	IVC first5 Days	SOF	Yes		No effect
	Sericin	0.15 %	IVC first5 Days	SOF	Yes		No effect
Meneghel et al., 2017	Forskolin	5.0 μM	D6	SOF + BSA+ 2.5 % FBS	Yes	VIT	Positive
Panyaboriban et al., 2018	Forskolin	10 μM	Pre treatment 24 h	SOF +10 % FBS	Yes	SF	Positive
Paschoal et al., 2017	Forskolin	2.5 μM	Until D6	SOF +2.5 % FBS	Yes	VIT	No effect
	Forskolin	5 μM	Until D6	SOF +2.5 % FBS	Not		No effect
	Forskolin	10 μM	Until D6	SOF +2.5 % FBS	Not		No effect
Paschoal et al., 2012	Forskolin	2.5 μM + 0 % FCS	D6	SOF + BSA	–	VIT	No effect
	Forskolin	2.5 μM + 2.5 % FBS	D6	SOF + BSA	–		No effect
Sanches et al., 2013	Forskolin	10 μM	D5	SOF +2.5 % FBS	–	VIT	No effect
Owen et al., 2022	Forskolin	10 μM	D5	SOF-based	Yes	SF	No effect
Ghanem et al., 2014	PES + LC	0.3 μM + 0.5 mM	IVC	CR1aa/BSA	Yes	VIT	No effect
Dias et al., 2019	LC + CLA	0.03 mM + 100 μM	IVC	SOF +5 % FBS	Not	SF	No effect
Schwarz et al., 2018	SDF > PDE5> cGMP	10^{-5} SDF	IVC	mSOF + BSA/2 % FBS	Not	VIT	No effect
Moore et al., 2007	SR	10 %	KSOM – D3FBS – D7	KSOM + BSA	–	VIT	Positive
	SR	10 %	KSOM until D7	KSOM + BSA	–		Positive

(*continued on next page*)

Table 3 (*continued*)

Reference	Intervention	Concentration	Exposure	IVC medium	Lipid reduction	Cryo method	Survival
Oliveira et al., 2022	FK + LC + CLA	30 μM/5 mM/300 μM	D4	SOF +1.5 % FBS	Not	VIT	No effect
	FK + LC + CLA	30 μM/5 mM/300 μM	D6	SOF +1.5 % FBS	Not		No effect

Abbreviations:=Not determined, Cryo=Cryopreservation, LA=Linoleic acid, DHA=Docosahexaenoic Acid, CLA = Conjugated Linoleic Acid, LAA = Linoleic Acid-Albumin, PES = Phenazine Ethosulfate, DGAT1 = Diacylglycerol Acyltransferase-1, LC = L-carnitine, SDF = Sildenafil, PDE5 = Phosphodiesterase 5 inhibition, cGMP = Cyclic Guanosine Monophosphate, SR = Serum Replacer, FBS = Fetal Bovine Serum, BSA = Bovine Seric Albumin, IVC=*In vitro* Culture, VIT = Vitrification, SF = Slow freezing.

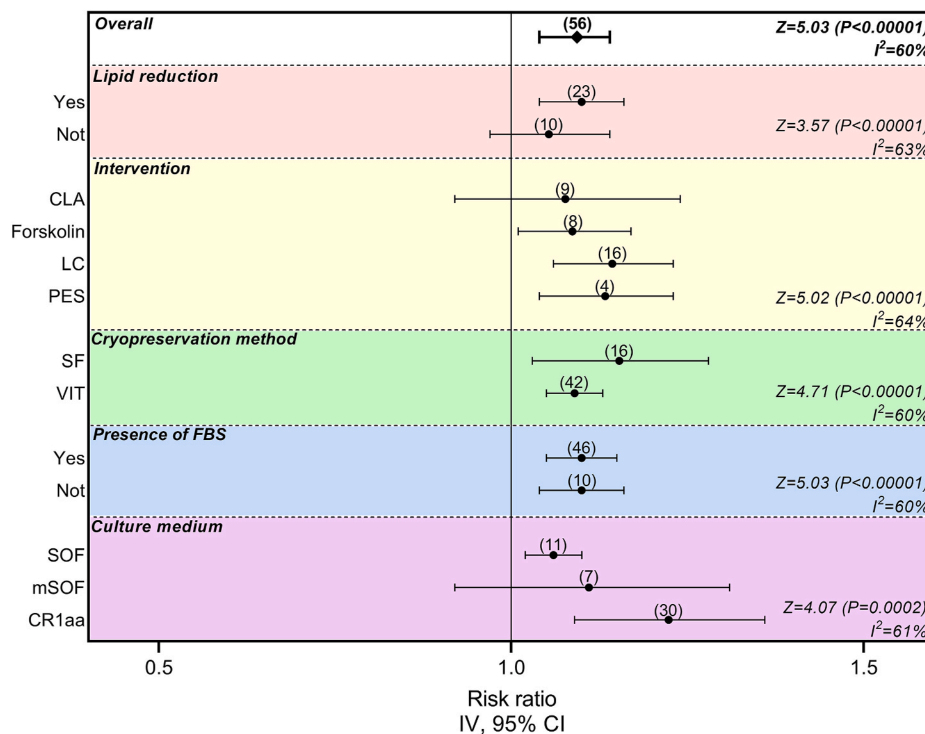

Fig. 7. Effects of the lipid modulators on the cryotolerance of bovine embryos produced *in vitro*. The risk ratio is reported with its 95 % confidence interval, and effects are significant if confidence intervals do not overlap with one. (n) = number of independent comparisons. Subgroup analysis was performed on lipid content, intervention, cryopreservation method, presence of FBS, and culture medium. SOF = synthetic oviductal fluid, mSOF = modified synthetic oviductal fluid.

both experiments, and the culture periods did not interfere with survival results at the tested L-carnitine concentrations. One experiment assessed the impact of forskolin from day five of IVC and revealed a decrease in lipid content along with an improvement in survival rates. The culture medium used was PZM-3 and NCSU-23 supplemented with BSA or FBS.

3.6. Bubaline

3.6.1. In vitro maturation

One experiment evaluated the effects of different L-carnitine concentrations during the IVM stage in buffaloes [6]. The lipid content was not evaluated, and the 0.5 mM concentration improved the oocyte survival rates (Table 2).

3.6.2. In vitro culture

Two articles and two experiments were extracted from buffaloes in the IVC stage (Table 4). One of the experiments evaluated the effect of 10 μM forskolin in the pretreatment of embryos cultured in SOF supplemented with 10 % FBS [48]. The second experiment evaluated the effect of 0.10 % linoleic acid-albumin during the entire culture period in co-culture with bovine oviductal epithelial cells (BOECs) in mSOF medium [42]. Only the experiment using 10 μM forskolin reduced embryonic lipid content, however, it did not show any effect on post-rewarm embryo survival rates. The use of 0.10 % forskolin also had no effect on embryonic survival rates.

3.7. Murine

3.7.1. In vitro maturation

Only one article (and experiment) was extracted from murine [56]. The IVM time was 16 h, and the concentration of 3.03 mM L-carnitine had no effect on oocyte survival (Table 2). Of note, cellular lipid content was not evaluated.

4. Discussion

This review investigated the impact of lipid modulation during IVM and IVC on the cryotolerance of oocytes and IVP embryos. The systematic evaluation of data showed that the use of lipid reducers in 38 % of the experiments contributed to the survival of oocytes and embryos during IVM and IVC of domestic animals, and in cattle, species in most experiments were carried out, there were 40 % of improvement in cryotolerance. The meta-analysis confirmed a positive response in embryo cryotolerance according to the interventions used during IVC of bovine embryos. This finding indicates that the use of L-carnitine, forskolin, and phenazine ethosulfate as lipid reducers contributes to an improvement of cryotolerance [4,18,29,57,58]. In general, reducing lipid content has been a strategy to improve the cryotolerance of IVP embryos. This

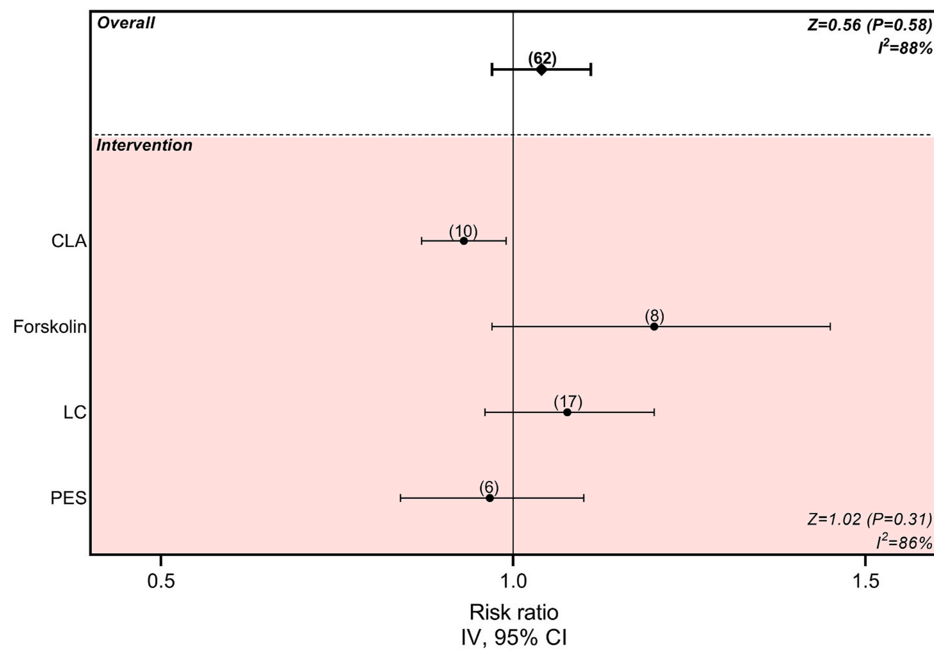

Fig. 8. Effects of the lipid modulators on the blastocyst development of bovine embryos produced *in vitro*. The risk ratio is reported with its 95 % confidence interval, and effects are significant if confidence intervals do not overlap with one. (n) = number of independent comparisons. Subgroup analysis was performed to intervention.

Table 4
Compilation of the experiments evaluated in ovine, swine, and buffaloes evaluating the effects of reducing lipid content on the cryotolerance of embryos produced *in vitro*. Data are presented according to intervention, substance concentration, exposure period, IVC medium, assessment of lipid reduction, cryopreservation method, and effect on embryonic survival.

Reference	Intervention	Concentration	Exposure	IVC medium	Lipid reduction	Cryo method	Survival
Ovine							
Romão et al., 2015	CLA	25 μM	IVC	SOF + BSA10 % FBS	–	VIT	No effect
		50 μM	IVC		–		No effect
Swine							
Gajda et al., 2011	PES	0.05 μM	IVC	NCSU-23 +BSA	Yes	VIT	No effect
Lowe et al., 2017	LC	3 mM	IVC	PZM-3	–	VIT	Positive
	LC	3 mM	IVC first 72 h	PZM-3	–		Positive
Men et al., 2006	Forskolin	10 μM	D5 IVC	NCSU + BSAD4 + 10 % FBS	Yes	VIT	Positive
Bubaline							
Panyaboriban et al., 2018	Forskolin	10 μM	Pre treatment	SOF + BSAFBS	Yes	SF	No effect
Laowtammathron et al., 2005	LAA	0.10 %	IVC	mSOF + BSA	–	VIT	No effect

Abbreviations:=Not determined, Cryo=Cryopreservation, CLA = Conjugated Linoleic Acid, PES = Phenazine Ethosulfate, LC=L-carnitine, LAA=Linoleic Acid-Albumin, VIT = Vitrification, SF = Slow freezing, FBS = Fetal Bovine Serum, IVC=*In vitro* Culture, BSA = Bovine Seric Albumin.

methodology was tested first in pigs [57] and follow-up in other species, such as bovine [23,39,40,47] and ovine [2]. Thus, a considerable number of substances have been used for this purpose during IVM of oocytes and IVC of embryos. L-carnitine was used for this purpose during IVM of bovine [31,33,35], ovine [53], and murine oocytes [56], as well as during IVC of bovine embryos [9,43,44]. Likewise, forskolin [17,47], conjugated linoleic acid [8,41], and phenazine ethosulfate [23,59,60] have been widely used during IVC of bovine embryos. An excessive accumulation of lipids has been associated with lower cryotolerance rates by interfering with the physical properties of the membrane, causing changes in its fluidity because of changes in the levels of glycerophospholipid unsaturation and the amount of cholesterol present in the membranes [5].

Our systematic data revealed that although lipid reducers do not present a unanimous improvement in embryonic cryotolerance, the number of experiments that presented negative results for this parameter was low (n = 7). In addition, other studies reported positive responses in embryos after adding substances capable of reducing lipid content, depending on the concentration of lipid reducers or the culture conditions to which they were exposed. As considered previously [41], differences in exposure time to the delipidant may influence the results, with the need, in some cases, for a longer embryo culture. Furthermore, the presence or absence of FBS or BSA used under culture conditions can influence the results together with the concentration of lipid reducers and the composition of the culture media [44]. Other points to consider can be the origin of the oocytes and the animal species, which can influence both lipid removal and embryonic development [7]. All these factors corroborate our findings in the systematic review, in which diverse studies evaluating the same substances at the same concentrations presented divergent results regarding embryonic survival. In some cases, we were able to reach a consensus regarding this divergence using a meta-analytic approach.

Experiments using L-carnitine, diacylglycerol acyltransferase 1, linoleic acid-albumin, and forskolin during bovine IVP showed divergent results according to the investigated concentrations, varying between the reduction or not of the lipid content and positive, negative and

no effect on embryo survival. None of the articles evaluated in this systematic review investigated whether there is a dose-response of lipid reduction by modulators, taking into account the energetic needs of the developing embryo. For example, phospholipids and cholesterol are prerequisites for the formation of cell membranes that are essential during rapid cell divisions after fertilization, while triglycerides function as an energy reserve for the maintenance of cellular activities [19]. Thus, lipid modulators could lead to an unbalanced reduction in intracellular lipid content. This would explain why some concentrations were able to reduce lipid content and, even so, have negative effects or no effect on survival [15,44]. Nevertheless, it is well established that L-carnitine promotes beneficial effects for embryonic development in cattle [30,60], as it is an essential cofactor necessary for the entry of fatty acids into the mitochondria and energy generation, in addition to functioning as an antioxidant, neutralizing free radicals derived from oxygen [16]. Its effects of improving cryotolerance were confirmed in our meta-analysis and, according to our systematic data, 1.5 mM appears to be a safe dose for improving cryotolerance of bovine embryos.

The combination of L-carnitine and phenazine ethosulfate or between L-carnitine and conjugated linoleic acid showed no effect on bovine embryo cryotolerance, even with a reduction in the lipid content when L-carnitine was associated with phenazine ethosulfate. L-carnitine and phenazine ethosulfate do not only target cytoplasmic lipids but can also affect membrane lipids. In the study of Barceló-Fimbres et al. [59], the expression of sphingosine-1-phosphate phosphatase 1, a transmembrane protein, was increased in embryos cultured with L-carnitine or phenazine ethosulfate when compared with groups where the two substances were combined. The expression of the SGPP1 gene is related to better post-cryopreservation hatching capacity, which could be a potential candidate gene for embryonic cryotolerance [9].

Previous studies showed that in bovine IVP embryos, forskolin had controversial effects, which could reduce [17] or not [60] intracellular lipid levels. The experiments evaluated in this systematic review also showed divergent results when evaluated individually depending on the concentration of forskolin used. When a meta-analysis was carried out on these experiments, forskolin showed a beneficial effect on cryotolerance and but did not contribute to improving the development of IVP bovine embryos. Forskolin is a regulator of cAMP levels and as the effects of cAMP are not specific to cellular metabolism, the use of different concentrations of forskolin can lead to hitherto unknown effects in oocytes and embryos; therefore, it is necessary to establish a concentration capable of stimulating lipolysis with no interference in cAMP levels [47,50].

In bovine embryos, the use of 100 μM of linoleic acid appears to have a beneficial effect on reducing lipid content and improving cryotolerance, which is not observed at lower concentrations (1 μM and 10 μM) [15,36]. Linoleic acid acts in a similar way to conjugated linoleic acid, reducing the content of cytoplasmic lipid droplets [40], changing the composition of membrane lipids, and increasing their fluidity [33]. Furthermore, linoleic acid is a modulator of arachidonic acid, which benefits the oocyte by reducing damage caused by free radicals [61]. Low concentrations of linoleic acid may not be sufficient to positively interfere with the modulation of arachidonic acid [33].

Conjugated linoleic acid showed different results even when tested at the same concentration (100 μM) [33,39,40,41,44]. The meta-analysis showed that the use of conjugated linoleic acid often harmed embryonic development and did not affect survival rates. In previous studies, conjugated linoleic acid had no effect on embryonic cryosurvival [19,62]. It is known that different culture conditions can interfere with the beneficial results of the molecule; for example, the use of conjugated linoleic acid throughout the culture appears to be more beneficial for the bovine embryo than in the initial or final moments of the culture [41]. Nonetheless, it appears that the effect of conjugated linoleic acid in the literature has been controversial. Despite having similar functionalities, the discrepancy in results between linoleic acid and conjugated linoleic acid may be related to the different configurations of these isomers. Different configurations allow some isomers to function in membrane composition, while others in energy metabolism [61]. The regulatory mechanisms that may be involved in the effects of LA supplementation in the culture medium need to be better elucidated 16, as well as those of CLA, as they are not yet fully understood [63].

The current meta-analysis showed that phenazine ethosulfate promotes an improvement in the cryotolerance of IVP bovine embryos. Phenazine ethosulfate is an inhibitor of fatty acid synthesis through the oxidation of NADH to NADP, which subsequently promotes the balance of energy metabolism and pentose phosphate pathway reactions [22,38]. Thus, the use of this substance has been associated with an improvement in embryonic quality and viability [61].

The diacylglycerol acyltransferase 1 inhibitor was used for the first time in the culture of bovine IVP embryos and has been demonstrated to reduce lipids in other cell types [3]. Diacylglycerol acyltransferase 1 is a catalytic enzyme that links fatty acyl-CoA molecules and diacylglycerol to form triacylglycerols (TAG). Therefore, its inhibition reduces triacylglycerol levels [64,65]. In bovine embryos, the use of 10 and 50 μM of the diacylglycerol acyltransferase 1 inhibitor prevented the conversion of diacylglycerol into triacylglycerol, reducing the lipid content, but blastocyst survival was better in the group treated with 10 μM of the inhibitor, suggesting an effect dose-dependent [3].

Sericin and serum replacers were investigated in the evaluated experiments with the aim of replacing undefined components of the culture media, such as FBS and thus reducing the lipid content. Sericin reduced lipid content in bovine embryos but did not affect survival. Although in the evaluated article, sericin did not improve survival rates, the results for development, freezing resistance, pregnancy, and birth rates were similar in embryos subjected to a medium containing 0.05 % sericin or 5 % FBS, suggesting that sericin can be used as a substitute for FBS to reduce the variability of harmful components in the medium, including the excess of supplied lipids [66]. It has been reported that supplementation with sericin in culture media containing FBS improves the formation of blastocysts in bovine embryos [67,68] and has already been used in freezing media for other cell types 69. Embryo survival rates obtained with serum replacer were similar to those of previous studies [70], showing that it is also an option as a substitute for FBS in media. Several studies have investigated the impact of using FBS during embryonic culture and its effects on cryotolerance since it is one of the sources of lipids in the culture media [12,22,70]. Interestingly, the meta-analysis showed that the presence of FBS improved embryonic cryotolerance rates.

Our findings point to the need to further investigate how delipidating substances can affect the content and composition of cellular lipids. Modulation of cGMP levels, for example, reduced embryonic lipid content but had no effect on post-cryopreservation survival [51]. It is important to understand the extent to which this reduction can have a beneficial effect on the oocyte or embryo. Furthermore, none of the articles included in this study evaluated the profile of cell membranes, highlighting the need for future investigations, as the composition of membrane lipids is directly associated with the success of cryopreservation [22,71]. Methods such as matrix-assisted laser desorption and ionization mass spectrometry (MALDI-MS) make it possible to detect changes in the structural composition of membrane's lipids and improve the understanding of lipid composition, allowing the tailoring of specific lipid modulators to increase cryotolerance [31,72].

Interestingly, the meta-analysis showed that the two cryopreservation methods had a similar effect on embryonic cryotolerance. Meta-analytical approaches performed in humans [73] and bovine [74], however, pointed out that slow freezing negatively affects embryonic survival when compared to vitrification. Thus, these findings imply that the compounds utilized as lipid-reducing agents in the examined studies possibly had a positive effect on cryotolerance. These effects were observed irrespective of the cryopreservation method employed for the embryos.

We investigated whether the culture media had any effect on the

cryotolerance of IVP embryos. SOF and mSOF had no effect, but CR1aa had a positive impact on survival. CR1aa already demonstrated superior effects for embryonic development in goats [75] and in buffaloes showed a similar effect to SOF [76]. It is known that culture media have different compositions, such as the energy sources used, and that they can even modify embryonic development and interfere in later stages [31,76]. For example, the mammalian embryo's energy needs change throughout development. At the 8–16 cell stage, they tend to primarily use pyruvate as an energy source, but after compaction, glucose consumption increases significantly [41]. In the *in vitro* environment, glucose metabolism occurs abnormally, causing an increase in glucose consumption and occasionally an increase in lipid content due to the greater presence of fatty acid precursors [77]. Embryos may have the ability to develop into blastocysts under certain conditions but may exhibit reduced cell counts, disrupted cell signaling, or nuclear functions that are incompatible with later-stage development [78]. These factors can impact parameters such as embryonic cryotolerance and perhaps the use of simpler formulations such as CR1aa, without the presence of additional energy sources, could be one of the contributing factors to the results observed in this study.

As already mentioned before, numerous factors could have contributed to the significant heterogeneity observed in the analyzed variables on metanalysis, such as the use of different substances with secondary functions and different concentrations [32,78,79], the cryopreservation method, laboratory conditions, and embryo characteristics [5].

In addition to bovine species, this review included sheep, murine, porcine, and buffalo. The results showed that of the substances tested in sheep, pigs, and buffaloes during IVC, there was only an improvement in embryonic survival in swine with the use of L-carnitine. However, the lipid content was not measured, which is why it is not possible to state whether L-carnitine improved survival by reducing the lipid content or by its antioxidant effect, providing protection against reactive oxygen species formed during the vitrification and warming process [7]. In the other experiments, there was no effect on the use of any of the evaluated substances. It is important to point out that, in addition to the factors already mentioned in cattle in this review, the specificities of the evaluated species, as well as the use of different culture systems, can influence the achieved results [54]. Furthermore, due to these specificities, lipid reduction may not be sufficient for a significant increase in embryo survival after vitrification [55].

Of the species in which the extracted experiments were performed in the IVM step, only niacin and L-carnitine were able to reduce lipids and improve cryotolerance. Niacin is an antioxidant, acts on DNA repair [80], and is also a lipid-modifying agent that acts by decreasing plasma triglyceride levels and low-density lipoproteins while increasing high-density lipoproteins [81]. The use of delipidated sheep serum in estrus during IVM of ovine oocytes reduced the lipid content but did not affect cryotolerance. It is known that lipid content is just one of multiple factors that lead to reduced cryosurvival rates [2]. In the *in vitro* environment, the embryo is able to change its energy source according to the availability of nutrients present in the medium [22]. This ability to seek survival and adapt to adverse conditions in an environment that does not meet their physiological needs such as the *in vivo* environment, known as embryonic plasticity, can cause serious damage to embryonic development competence [82]. Damage caused by the cryopreservation process is one of the factors intrinsically linked to low cryosurvival rates. Although much knowledge about IVP systems has been produced over the past decades, improvements in the efficiency of the IVP biotechnological process are still necessary to increase the production of blastocysts of exceptional quality [83].

5. Limitations

This review evidenced the different manners in which studies describe their data, mainly in relation to the survival rate of oocytes and embryos after cryopreservation. This difference impacted the viability of performing the meta-analysis in all eligible studies included in the systematic review. It was difficult to assess the real effect of lipid modulators in reducing intracellular lipids since a large number of studies did not assess the oocyte and embryo lipid content before and after IVM and/or IVC. Such factors prevent us from making definitive conclusions about how the reduction in lipid content impacts the cryotolerance of mammalian oocytes and IVP embryos. However, systematic review and meta-analysis continue to be strategies with a high level of confidence for evaluating studies in a blind and reasoned way and allow a broad understanding of the included studies in this type of approach [84].

6. Conclusions

Reducing lipid content by using forskolin, L-carnitine, and phenazine ethosulfate improves cryotolerance of IVP bovine embryos, as evidenced in the meta-analysis. The systematic review suggests lipid modulators did not unanimously improve cryotolerance, especially when used in the IVM stage. Future studies of an experimental nature in domestic species other than cattle are needed to provide an overview, including a meta-analytical approach, of how the reduction in lipid content affects the oocyte during IVM and embryos during IVC.

CRediT authorship contribution statement

Erlandia M. Vasconcelos: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Rachel F. Braga:** Data curation, Investigation, Writing – review & editing. **Gabriela R. Leal:** Data curation, Investigation, Writing – review & editing. **Renner P.R. Carvalho:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Mariana Machado-Neves:** Writing – review & editing, Investigation, Data curation. **Mateus J. Sudano:** Writing – review & editing, Investigation, Data curation. **Joanna M.G. Souza-Fabjan:** Supervision, Resources, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors have declared that no conflict of interest exists.

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Appendix A. Supplementary data

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