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Research Article

Effect of morin hydrate on porcine oocytes in vitro maturation

Efeito da morina na maturação in vitro de ovócitos suínos

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ABSTRACT

This study aimed to evaluate Morin's effects on pig *cumulus*-oocyte complexes (COC), when added at different concentrations in the *in vitro* maturation (IVM) media. Porcine COC were matured *in vitro* in a base medium (control group), and with 5, 10, 50 and 100 µM of Morin. Viability, nuclear maturation rates and reactive oxygen species (ROS) levels were evaluated on oocytes; *cumulus* cell apoptosis was measured through flow cytometry. Progesterone levels from the IVM media at 22h and 44h were measured through chemiluminescent microparticle immunoassay. The addition of Morin during IVM did not affect oocyte viability. The exposure to 50 µM and 100 µM of Morin in the maturation media significantly decreased oocyte nuclear maturation. Progesterone levels in IVM media significantly increased in all experimental groups between 22 h and 44 h. The addition of 100 µM of Morin significantly decreased progesterone production at 22 and 44 h of incubation. ROS levels in the 5 µM and 10 µM groups were significantly lower than in the control. Morin significantly modifies the apoptosis profile and affects the viability of *cumulus* cells. Morin acts as an antioxidant and has both beneficial and detrimental effects on pig oocytes in a dose-dependent manner.

KEYWORDS: Morin. Porcine oocyte. In vitro maturation. Antioxidant.

RESUMO

Este estudo teve como objetivo avaliar os efeitos da morina em complexos cúmulo-oócito (COC) suínos, quando adicionada em diferentes concentrações no meio de maturação *in vitro* (MIV). Os COC suínos foram maturados *in vitro* em um meio base (grupo controle) e com 5, 10, 50 e 100 µM de morina. A viabilidade, as taxas de maturação nuclear e os níveis de espécies reativas de oxigênio (ROS) foram avaliados nos oócito; a apoptose das células do cúmulo foi medida por citometria de fluxo. Os níveis de progesterona do meio de MIV em 22h e 44h foram medidos por imunoensaio de micropartículas quimioluminescente. A adição de morina durante a MIV não afetou a viabilidade dos oócitos. A exposição a 50 µM e 100 µM de morina no meio de maturação diminuiu significativamente a maturação nuclear dos oócitos. Os níveis de progesterona no meio de MIV aumentaram significativamente em todos os grupos experimentais entre 22h e 44h. A adição de 100 µM de morina diminuiu significativamente a produção de progesterona em 22h e 44h de incubação. Os níveis de ROS nos grupos de 5 µM e 10 µM foram significativamente menores do que no controle. A morina modifica significativamente o perfil de apoptose e afeta a viabilidade das células do cúmulo. A morina atua como um antioxidante e possui efeitos tanto benéficos quanto prejudiciais nos oócitos suínos de maneira dose-dependente.

PALAVRAS-CHAVE: Morina. Oócito suíno. Maturação *in vitro*. Antioxidante.

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INTRODUCTION

The porcine species can be used as an animal model to study human diseases and a source for xenotransplants. The *in vitro* embryo production (IVP) shortens the generation interval, facilitates the transition of genetics and allows other more complex biotechnologies, such as transgenesis. However, IVP of porcine embryos is less efficient than other livestock species, such as cattle and goats (LUCHETTI et al. 2023). The IVP success depends on many variables associated with gamete quality and culture conditions. In particular, *in vitro* oocyte maturation under basic conditions is inefficient in producing developmentally competent oocytes.

This developmental incompetence can be overcome by supplementing the culture medium (PONIEDZIAŁEK-KEMPNY 2020). Abattoir-derived ovaries are an available and low-cost source of oocytes. However, they arrive in ischemic conditions to the laboratory (AGARWAL et al. 2022, IWATA et al. 2003), and reoxygenation of ischemic tissues stimulates the production of reactive oxygen species (ROS), which are involved in the induction of apoptosis (SENDTNER N et al. 2025).

Furthermore, *in vitro* matured oocytes are manipulated and exposed to higher oxygen concentrations than those matured *in vivo* (AGARWAL et al. 2022, COMBELLES et al. 2009, RAKHA et al. 2022). This also contributes to the formation of intracellular ROS during *in vitro* maturation (IVM). Consequently, to minimize damages produced by ROS, the addition of antioxidants in the maturation medium has been widely used (AGARWAL et al. 2022, KHAZAEI & AGHAZ 2017, RAKHA et al. 2022).

Flavonoids are polyphenolic compounds widely distributed in fruits and vegetables primarily known for their antioxidant activity (GOPAL 2013, PANCHE et al. 2017). Recent studies have used flavonoids during IVM, obtaining dose-dependent results. Overall, low concentrations of flavonoids (up to 25-50 µM, depends on the flavonoid) either exerted no effect or had a beneficial effect on maturation rates, oocyte ROS levels and embryo development (HUANG et al. 2018, KANG et al. 2016, OH et al. 2023, YUAN et al. 2025, ZHAO et al. 2020).

However, flavonoids in high concentrations had detrimental effects on IVM rates and blastocyst formation. Besides, it affects steroidogenesis, *cumulus* cell progesterone production, and granulosa cell proliferation during IVM (BASINI et al. 2005, KANG et al. 2016, SANTINI et al. 2009). Regarding the effects of flavonoids on IVM and embryo development in pigs, several compounds, including epigallocatechin-3-gallate (SPINACI et al. 2008), Quercetin and Taxifolin (KANG et al. 2016), Kaempferol (ZHAO et al. 2020), Isorhamnetin (OH et al. 2023) and Mangiferin (YUAN et al. 2025), have been shown to improve oocyte developmental competence and reduce ROS levels.

Morin hydrate (3,5,7,2',4' pentahydroxyflavone) is a flavonol that can be isolated from plants from the Moracae family and C*hlorophora tinctoria* (PANCHE et al. 2017, RAJPUT et al. 2021). Studies have shown a wide range of Morin's effects on different biological tissues (RAJPUT et al 2021). It has anti-inflammatory activity (WANG et al. 2016, UNSAL et al. 2024), protects from free radicals (SHYMA & MINI 2022, UNSAL et al. 2024), promotes tissue regeneration (ABDELMENEM et al. 2025, PANDI et al. 2025, QU et al. 2022) and has anti-cancerigenous activity (FAKHREDINI et al. 2024,

MOTTAGHI & ABBASZADEH 2021, ZHAO et al. 2023). Nonetheless, there is no evidence of Morin hydrate being used in *in vitro* reproductive biotechnologies.

Thus, the aim of this study was to evaluate Morin´s effects on pig *cumulus*-oocyte complexes (COC), when added at different concentrations in the IVM medium.

MATERIAL AND METHODS

Experimental Design

Experiment 1:

The objective of this experiment was to determine a working concentration of Morin in IVM medium. COC were collected from abattoir derived ovaries, in three replicates, washed and cultured in base media (control), and media containing 5, 10, 50 and 100 µM of Morin hydrate for 44 h. Oocyte viability and nuclear maturation were assessed to discard toxic doses of Morin. The concentrations that either decreased viability or nuclear maturation were considered toxic. Progesterone levels in culture media were also assessed.

Experiment 2:

The objective of this experiment was to determine Morin's antioxidant effect on pig oocyte and on apoptosis of *cumulus* cells. For this experiment, only the treatments that did not show toxic effect of experiment 1 were used (i.e. 5 and 10 µM of Morin). COCs were obtained from abattoir derived ovaries, in three replicates, washed and cultured for 44 h. Reactive oxygen species (ROS) levels on oocytes and *cumulus* cell apoptosis were assessed.

COC collection

Ovaries were collected from a local abattoir and transported to the laboratory in an adiabatic container at 30-37 °C within 3 h from the death of the animals. Ovaries were washed with 0,9% NaCl saline solution at 37 °C. COC were obtained by aspiration of 3–8mm antral follicles using an 18-gauge needle mounted on a 10 mL syringe.

In vitro maturation (IVM)

COC were washed thrice in PBS medium (14190136, Gibco) supplemented with 10% porcine follicular fluid (pFF) and 100 UI/mL of penicillin, 100 µg/mL of streptomycin. Only those oocytes with uniform ooplasm, surrounded with compact and whole *cumulus* layers, were selected to be matured. Groups of 50 COC were placed in a well with 500 µL of 199 medium (11150, Gibco®) supplemented with 10% of pFF, 0,3 mM of sodium pyruvate, 5 µM of 2-mercaptoethanol, 100 UI/mL of penicillin, 100 µg/mL of streptomycin, 1,5 UI/mL of human menopausal gonadotrophin (hMG, Lifecell, Buxton laboratory) and 1 mM of dibutyryl cyclic adenosine monophosphate (cAMP) modified from (BAGG et al. 2006). After 22 h, COC were changed to a fresh maturation medium without hMG and cAMP. Culture was performed in a humidified atmosphere with 5% CO_2 at 39°C for 44 h. After IVM, oocytes were denuded with 1 mg/mL of hyaluronidase in 0.1% polyvinyl alcohol in PBS and 3 min of vortexing (CRUZANS et al. 2026).

Morin treatment

Morin treatments were performed during the 44 h of IVM; COC in the control group were matured in IVM medium, as previously described. Morin hydrate (Sigma M4008) was dissolved in methanol and diluted in IVM medium to obtain a final concentration of 5, 10, 50 and 100 µM. The maximum amount of methanol added was 1 µL in 500 µL of IVM medium. The addition of 1 µL of methanol during IVM was previously tested, and no toxic effects for oocyte viability and maturation rates were observed (supplementary tables 1 and 2).

Assessment of oocyte viability

After IVM, denuded oocytes were stained with 0,16% Tripan Blue vital stain (Gibco 15250-061) for 5 min at 37 °C (GUPTA et al. 2002). Oocytes were washed in 0.1% PVA in PBS and placed under a stereoscopic microscope for observation. Those oocytes that presented a blue cytoplasm were considered non-viable (Figure 1). For each treatment, 5 µM (n=143), 10 µM (n=154), 50 µM (n=139), 100 µM (n=123) and control group (n= 281), the percentage of viable oocytes was determined.

Assessment of oocyte nuclear maturation

After IVM, denuded oocytes were fixed with 4% paraformaldehyde, stained with 10 µg/mL Hoechst 33342 (H1399; Invitrogen), washed in 0,1% PVA in PBS and mounted on slides for observation under an epifluorescent microscope (DM4000B LED LEICA), using excitation and emission wavelengths of 350 and 461 nm respectively. Oocytes with a metaphase II (MII) nuclear stage were considered mature (Figure 2). The percentage of matured oocytes for each treatment 5 µM (n=141), 10 µM (n=149), 50 µM (n=125), 100 µM (n=119) and control group (n= 265) was determined (CRUZANS et al. 2026).

Assessment of progesterone levels in *in vitro* maturation medium

The *in vitro* maturation medium was collected after 22 and 44 h. Progesterone levels were measured through chemiluminescent microparticle immunoassay (CMIA) (modified from ANCHORDOQUY et al. 2022). The medium of all treatments was evaluated, including the control group, in three replicates.

Assessment of ROS in oocytes

After IVM, denuded oocytes were incubated with 153 µM of 2',7'-dichlorodihydrofluorescein diacetate (DCHFDA; D6883) for 30 minutes at 37°C. Oocytes were then washed (0.1% PVA in PBS) and assembled for observation under an epifluorescent microscope (DM4000B LED LEICA), using excitation and emission wavelengths of 450–490 and 515–565 nm, respectively.

Fluorescence emission intensity was determined by the transmittance variable, measured by digital morphometry on images captured using Qwin Plus V3.1 digital analysis software (Leica). The transmittance arbitrary units corresponded to levels between 0 and 256 for each of the Red-Green-Blue (RGB) channels for the pixels of the analyzed matrix. The mean transmittance of each experimental group was recorded and compared (modified from LORENZO et al. 2019). The greater the intensity of green fluorescence, the greater the amount of ROS. Control (n=107), 5 µM (n=85), 10 µM (n=109).

Assessment of *cumulus* cell apoptosis

After IVM, COC were denuded, and oocytes were discarded. The supernatant was centrifuged at 200g for 10 min at RT. Fluorescence-activated flow cytometry analysis, as described by CRUZANS et al. (2026), was used to quantify *cumulus* cell apoptosis. Based on propidium iodide (PI) and/or annexin V-FITC staining, cells were assigned to one of the following categories: viable cells (negative to IP and anexin V-FITC), necrotic cells (positive to PI), cells in late apoptosis (positive to PI and annexin V-FITC) or cells in early apoptosis (positive to annexin V-FITC). Control (n= 15274), 5 µM (n= 15984) and 10 µM (n= 15506).

Statistical analysis

Data were analyzed with the software R v. 4.1.2. Oocyte nuclear maturation, oocyte viability and *cumulus* cell apoptosis were analyzed with a Chi-square test and expressed as a percentage over the total. Levels of ROS and Progesterone were analyzed with Kruskall-Wallis test followed by pairwise comparison with Dunn's test and presented as mean ± SEM. $P< 0.05$ was considered significant.

RESULTS

Effect of Morin on oocyte viability

The addition of Morin to the maturation medium did not affect oocyte viability at any concentration. The number of viable oocytes were 263 (93.6%), 135 (94%), 143 (92.8%), 128 (92%), 112 (91%) for the control, 5 µM, 10 µM, 50 µM and 100 µM Morin treatments, respectively (Figure 1).

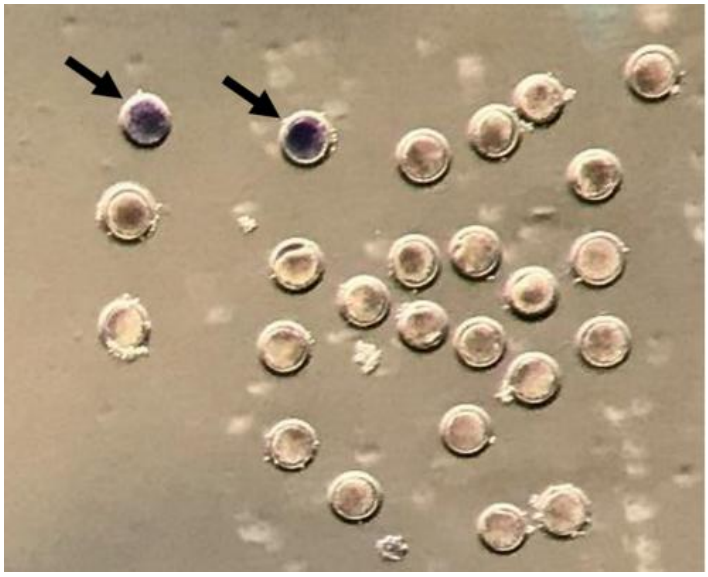


Figure 1. Representative image of post-IVM oocytes stained with 0.16 % Tripan Blue under stereoscopic microscope. Oocytes with a blue cytoplasm (arrows) were considered dead.

Effect of Morin on oocyte nuclear maturation

The exposure to 50 µM and 100 µM of Morin in the maturation medium significantly decreased oocyte nuclear maturation, but there was no difference in maturation rates between 5 µM and 10 µM of Morin and the control group (Table 1, Figure 2).

Table 1. Effect of Morin on oocyte nuclear maturation.

Morin (µM)	N° of oocytes	N° of MII oocytes (%)
0 (control)	265	191 (72.07)[a]
5	141	102 (72.34)[a]
10	149	108 (72.48)[a]
50	125	74 (59.2)[b]
100	119	60 (50.42)[b]

Values are given for three biological replicates. Within columns, different superscript letters indicate significant differences. Chi-square test (P < 0.05).

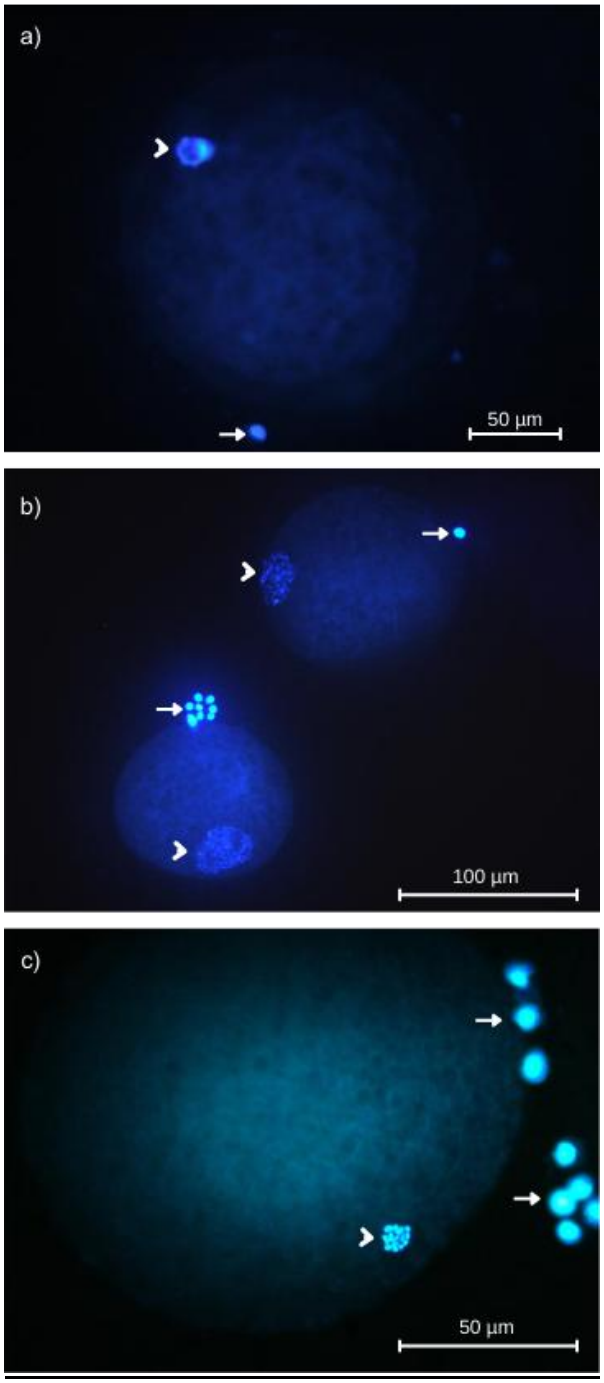


Figure 2. Representative images of post-IVM oocytes stained with Hoechst 33342. (a) Immature oocyte, nucleus in germinal vesicle stage (arrowhead) x400. (b) Immature oocytes with nucleus in prophase I (arrowhead) x200. (c) Mature oocyte with its nucleus in metaphase II (arrowhead) x400. White arrows indicate cumulus cells.

Effect of Morin on progesterone production

Progesterone levels in the IVM media showed no significant differences between control, 5 µM, 10 µM, and 50 µM Morin- treated groups at 22 h nor at 44 h. The addition of 100 µM of Morin significantly decreased progesterone levels at both 22 h and 44 h in comparison with the other groups. Regarding progesterone production, the hormone levels found in the IVM media significantly increased in all groups between 22 h and 44 h (Table 2 and Figure 3).

Table 2. Effect of Morin on progesterone production.

Morin (µM)	Progesterone level (nG / mL) ± SEM	
	22 h	44 h
0 (control)	61.48 ± 10.18 [a]	128.18 ± 33.77 [c]
5	63.74 ± 7.62 [a]	215.94 ± 42.5 [c]
10	60.37 ± 7.23 [a]	194.92 ± 28.66 [c]
50	40.77 ± 3.9 [a]	90.53 ± 10.45 [c]
100	28.75 ± 0.91 [b]	64.43 ± 6.9 [d]

Values are given for three biological replicates. Mean ± S.E.M. Within columns and rows, different superscript letters indicate significant differences. Kruskall-Wallis test followed by pairwise comparison with Dunn's test ($P < 0.05$).

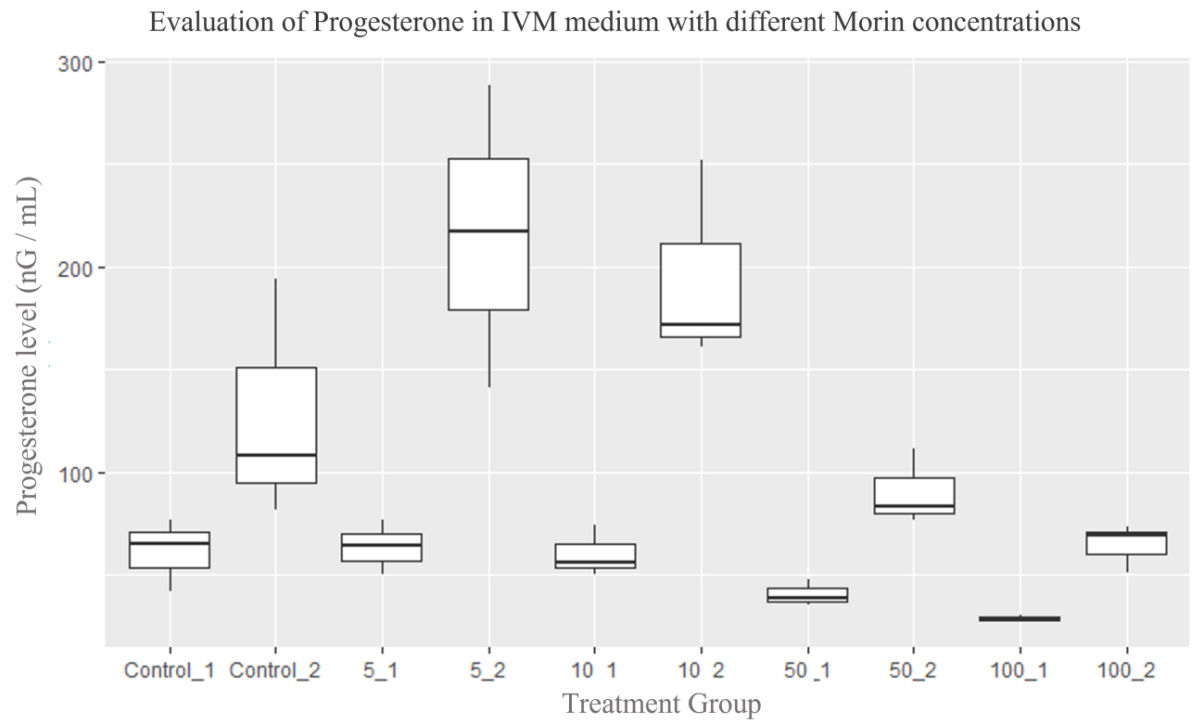


Figure 3. Box plot with progesterone levels found in the IVM mediums after 22 h (_1) and 44 h (_2) of COC incubation with different concentrations of Morin hydrate in three replicates. Graphic obtained with R studio. Within each box, horizontal black line represents the median values; boxes extend from the 25th to 75th percentile of each; edges of whiskers denote minimum and maximum values.

Effect of Morin on mature oocyte ROS levels

ROS levels in the 5 µM and 10 µM treated groups were significantly lower than in the control group. No significant differences were observed between the different concentrations of Morin. (Figures 4a and 4b)

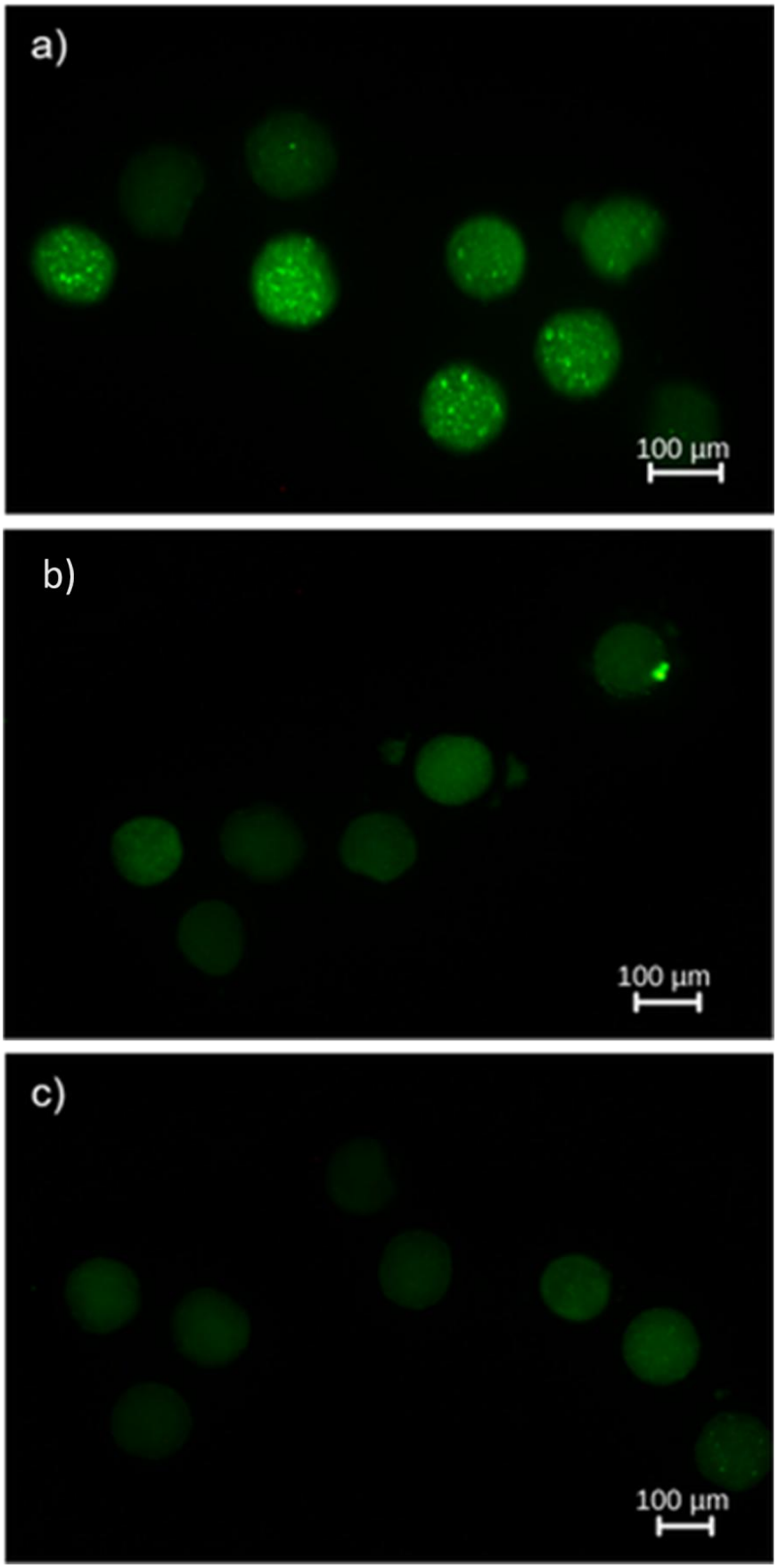


Figure 4a. Post-IVM oocytes stained with DCHFDA under fluorescent microscope x100. The intensity of the green fluorescence is directly proportional to ROS levels. Each letter represents a different experimental group: (a) control; (b) 5 µM of Morin; (c) 10 µM of Morin.

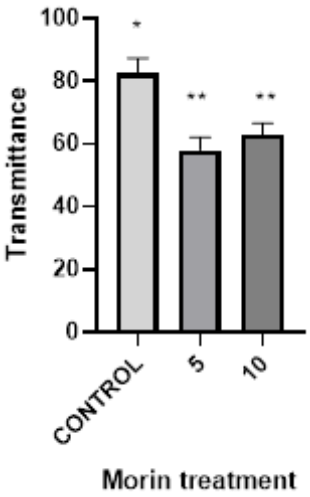


Figure 4b. Bar graph with standard error showing ROS levels on oocytes post IVM cultured in base media or base media with 5 or 10 µM of Morin. Different number of asterisks show significant differences with a p-value <0,05.

Effect of Morin on *cumulus* cell apoptosis

Morin significantly modifies the apoptosis profile and affects the viability of *cumulus* cells during IVM. Morin increased viability in all treatments. 5 µM increased necrosis, whereas 10 µM did not affect it. Treatment with 5 µM and10 µM decreased early apoptosis. Treatment with 5 µM and 10 µM enhanced late apoptosis, (Table 3, Figure 5).

Table 3. Effect of Morin on *cumulus* cell apoptosis.

Morin (µM)	N° of cells	Viable		Necrosis		Late Apoptosis		Early Apoptosis	
		n	(%)	n	(%)	n	(%)	n	(%)
0 (control)	15274	3786	24.79[a]	90	0.59[a]	2938	19.24[a]	8460	55.39[a]
5	15984	4547	28.45[b]	137	0.86[b]	3842	24.04[b]	7458	46.66[b]
10	15506	3999	25.79[c]	87	0.56[a]	3454	22.28[c]	7966	51.37[c]

Values are given for three biological replicates. Within columns, different superscript letters indicate significant differences. Chi-square test ($P < 0.05$)

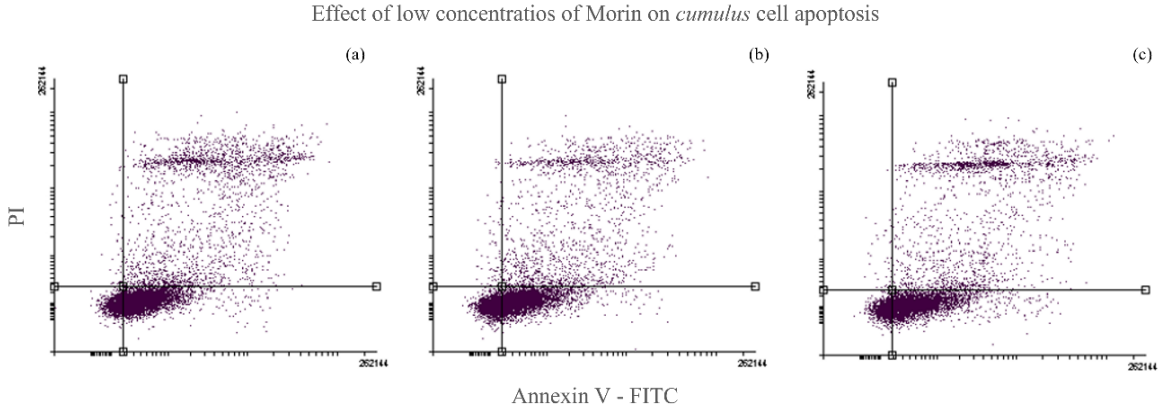


Figure 5. Distribution of cumulus cells, determined through flow cytometry, in the (a) control, (b) 5 µM of Morin and (c) 10 µM of Morin treated groups. Lower left quadrant: viable cells (negative to PI and annexin V); upper left quadrant: necrotic cells (positive to PI); lower right quadrant: cells exhibiting early apoptosis (positive to annexin V); and upper right quadrant: cells exhibiting late apoptosis (positive to annexin V and PI). Graphic obtained with Flowing Software.

DISCUSSION

Different studies have added antioxidants to the maturation media to enhance *in vitro* maturation rates and posterior *in vitro* production of embryos (PONIEDZIAŁEK-KEMPNY 2020). In this study, different concentrations of Morin hydrate were used in the IVM media of porcine COC. These concentrations were established by considering other flavonoids used in pig (KANG et al. 2013 and 2016, ORLOVSCHI et al. 2014, SPINACI et al. 2008) and bovine (HUANG et al. 2018) oocytes.

Previous studies show that the effects of flavonoids on oocyte quality are dose-dependent (HUANG et al*.* 2018, KANG et al. 2013 and 2016, OH et al. 2023, YUAN et al. 2025, ZHAO et al. 2020). Thus, we expected that the higher concentrations of Morin, i.e., 50 µM and 100 µM, would be toxic for oocytes, and this would be expressed as a decrease in oocyte viability and maturation rates. Indeed, our results show that these concentrations significantly decrease oocyte maturation rates.

Nonetheless, neither concentration influenced oocyte viability. The concentrations of Morin that produce apoptosis in tumoral cell cultures are higher than 50 µM (RAJPUT et al. 2021, ZHANG et al. 2017). Even though in our study both concentrations of 50 µM and 100 µM of Morin did not affect oocyte viability, they were considered toxic due to the detrimental effect on IVM. When oocytes leave their follicular environment, they spontaneously resume meiosis during in vitro culture. This resumption is inhibited at high concentrations of Morin.

Morin, like other flavonoids, has antioxidant activity (GOPAL 2013, PANCHE et al. 2017, RAJPUT et al. 2021, SHYMA & MINI 2022, UNSAL et al. 2024). However, higher concentrations of flavonoids have been shown to have pro-oxidative effects in a dose-dependent manner (SLICA et al. 2022, ZHANG et al. 2017). Thus, to further analyze Morin's effect on oocytes, we evaluated oocyte ROS levels after being matured with different concentrations of Morin.

Our results show that Morin up to 10 µM significantly decreases oocyte ROS levels. We expected dose-effect activity, but there are no differences between treatments. The reducing agents present in the maturation media used in our experiments, such as 2-mercaptoethanol, could have an additive effect, increasing the overall antioxidant activity and masking Morin's dose effects through the concentration range lower than 50 µM. Also, JOMOVÁ et al*.* (2019) states that flavonoids will act as antioxidants or prooxidants depending on their dose, stability of the metal-flavonoid complex and number of hydroxyl groups.

In accordance, Morin has been proven to have differential cytotoxicity on human hepatocellular carcinoma cells than on umbilical vein endothelial cells, being greater on the former (KOK et al. 2000). This allows us to presume that Morin may operate differently according to cell metabolism (i.e. tumoral or differentiated). In our study, Morin at lower concentrations acted as an antioxidant. Further investigation should be done to assess the oxidative effect of Morin at higher concentrations on porcine oocytes during maturation.

Cumulus cell steroidogenesis plays a key role during IVM; progesterone produced by *cumulus* cells induces germinal vesicle breakdown, increases sperm head decondensation and MPN formation and its inhibition reduces maturation rates (SHIMADA & TERADA 2002, SIQUEIRA et al*.* 2012). Some flavonoids from the

flavonol family, such as Quercetin and Morin hydrate, function as phytoestrogens (MOUTSATSOU 2007, PANCHE 2017). KANG et al. (2016) and SANTINI et al. (2009) studied Quercetin's effect on granulosa cell steroidogenesis.

At a concentration of 1 µM, Quercetin added to the IVM media does not affect *cumulus* cell steroidogenesis (KANG et al. 2016). But 5 µM of Quercetin increases estrogen production, whilst 50 µM decreases it. Besides, Quercetin inhibits granulosa cell progesterone production in a dose-dependent way (SANTINI et al. 2009). Thus, we decided to assess the effects of different concentrations of Morin on *cumulus* cells steroidogenesis.

In our system *cumulus* cells produce progesterone during IVM in all the studied groups; this is reflected in the different progesterone levels between 22 h and 44 h. This production shows a tendency to be higher in the control, 5 and 10 µM compared to 50 and 100 µM groups. The addition of 100 µM significantly decreases progesterone levels both at 22 h and 44 h of incubation. Considering the progesterone behavior in our model, and the fact that it could be a reflection of *cumulus* steroidogenesis, it would provide an explanation for the harmful effect observed on IVM at the higher concentrations of Morin hydrate.

Morin has both pro and anti-apoptotic effects in a dose-dependent way, also depending on the cell type (KOK et al. 2000, PANDI et al. 2025, WANG et al. 2007, ZHAO et al. 2023). Thus, we assessed the effect of the different Morin doses on *cumulus* cell apoptosis and viability. *Cumulus* cells have a different behaviour from that of oocytes, and the former suffer programmed cell death during the maturation process.

In vivo, the LH surge coincides with *cumulus* cell expansion; *in vitro*, *cumulus* cell expansion can be induced by the addition of hormones. When cell expansion occurs, there is a decrease in gap junctions, and *cumulus* cells lose all physical contact with their associated oocyte, resulting in cell death through apoptosis (TEPLITZ et al. 2020). As we had expected, and in concordance with other authors which have evaluated apoptosis in *cumulus* by annexin V-PI flow cytometry, most cells were in different states of apoptosis, and with low viability percentages in the control groups (CRUZANS et al. 2026, LORENZO et al. 2019, LUCHETTI et al. 2023, TEPLITZ et al. 2020).

In the present work, Morin increases the viability of the *cumulus* cells in all the concentrations used, however, the results for necrosis and apoptosis varied among experimental groups. The tested concentrations of Morin (i.e 5 and 10 µM) showed a similarpattern of effects on *cumulus* cells: increasing cell viability, decreasing early apoptosis and enhancing late apoptosis. Although the increases in cell viability and late apoptosis (less than 5%) were statistically significant, they may not be biologically relevant.

In fact, the most notorious effect was observed on early apoptosis. Oxidative stress is a know trigger of apoptotic initiation (SENDTNER N et al. 2025), and the reduction in early apoptosis observed at these concentrations may be related to the decrease in ROS levels. *Cumulus* cells are physiologically destined to undergo apoptosis during IVM, therefore the concurrent decrease in early apoptosis and increase in late apoptosis may simply reflect differences in apoptotic progression at

the single time point evaluated (44h of IVM), rather than an increased apoptotic induction. In this context, Morin may reduce ROS-mediated entry into apoptosis, while allowing cells already committed to apoptosis to progress. These results indicate that *cumulus* cell viability and apoptosis are not reliable predictors of Morin's effect on oocytes. More experiments should be performed to understand the mechanisms underlying Morin's effect on cells already undergoing apoptosis, such as *cumulus* cells.

The information regarding the molecular mechanisms involved in Morin's actions is revised in RAJPUT et al. (2021). In particular, Morin's anti-cancerigenous and anti-inflammatory activity is related to the regulation of the the tumoral necrotic factor (TNF) pathway. Morin suppresses both TNFα and TNF- induced activation of NF-κB and NF-κB-regulated gene expression, leading to enhancement of apoptosis (RAJPUT et al. 2021).

TNFα is present in granulosa, theca and luteal cells of various mammalian species (SILVA et al. 2020). It can stimulate cell proliferation or cell death depending on the TNF-receptor (TNFR) it binds to (NAKAYAMA et al. 2003). In pigs, TNFα contributes to follicle atresia in subordinate follicles and cell proliferation and growth in dominant follicles (NAKAYAMA et al. 2003). Furthermore, TNFα has been shown to stimulate FSH-induced steroidogenesis in granulosa cells (SILVA et al. 2020). It is possible that high concentrations of Morin in the IVM medium might suppress TNF expression and TNF-induced pathways in *cumulus* cells.

The present study has some limitations that should be considered when interpreting the results. The efect on COC was evaluated using nuclear maturation, ROS levels, progesterone production, and cumulus cell apoptosis; however, these parameters do not fully predict the ability of oocytes to undergo fertilization and sustain embryo development. Therefore, the absence of *in vitro* fertilization or embryo culture prevents direct conclusions regarding the effects of Morin on oocyte developmental competence.

CONCLUSION

To our knowledge, this is the first report that studies the addition of Morin in the IVM media. This preliminary study demonstrates that Morin exhibits antioxidant activity and exerts a dose-dependent effect on porcine oocytes. Moreover, it contributes to the growing body of evidence regarding the role offlavonoids during IVM in mammals. However, further investigations must be done to fully understand the mechanisms involved and to asses the developmental competence of Morin-treated oocytes.

NOTES

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, and formal analysis, Josefina Ghersa, Maria Soledad Lorenzo and Carolina Luchetti**;** software and validation Josefina Ghersa, Maria Soledad Lorenzo and Maria Clara Carou**;** investigation Josefina Ghersa Maria Soledad Lorenzo and Carolina Luchetti**; r**esources and data curation Daniel Marcelo

Lombardo**;** writing-original draft preparation Josefina Ghersa, Maria Soledad Lorenzo**;** writing-review and editing Josefina Ghersa, Maria Soledad Lorenzo, Carolina Luchetti, Maria Clara Carou and Daniel Marcelo Lombardo**;** visualization Daniel Marcelo Lombardo**;** supervision Daniel Marcelo Lombardo; project administration Daniel Marcelo Lombardo; funding acquisition Daniel Marcelo Lombardo. All authors have read and agreed to the published version of the manuscript.

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INSTITUTIONAL REVIEW BOARD STATEMENT
Not applicable for studies not involving humans or animals.

INFORMED CONSENT STATEMENT
Not applicable because this study did not involve humans.

DATA AVAILABILITY STATEMENT
The data can be made available under request.

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CONFLICTS OF INTEREST
The authors declare no conflict of interest.

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