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Original Research Article

Extracellular vesicles from the follicular fluid of competent oocytes improve blastocyst yields when supplemented during simulated physiological oocyte maturation in cattle

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ABSTRACT

The objectives of this work were to evaluate: i) the miRNA profile of extracellular vesicles (EVs) isolated from bovine follicular fluid (FF) of follicles enclosing competent (FF-C) and non-competent (FF-NC) oocytes; ii) the effect of incubating bovine cumulus-oocyte complexes (COCs) during a simulated physiological oocyte maturation system (SPOM) with EVs derived from FF-C and FF-NC on developmental capacity, iii) the effect of miR-143, one of the most abundant miRNAs found in EVs isolated from FF-C, during SPOM on oocyte competence. FF was collected from individual follicles of abattoir-derived ovaries and retrospectively categorized based on the developmental outcome of corresponding oocytes: blastocyst (B), cleaved-arrested (C), and uncleaved (U). EVs were isolated from FF pools by ultracentrifugation, characterized by Nanosight, TEM, and Western Blot, and analyzed for miRNA profile. The EVs from competent follicles (B) were larger and more concentrated than those from non-competent (U) ones, and carried distinct miRNA cargo, including higher levels of miR-143. Bovine COCs were matured in SPOM with 0, 1, 10, or 100 $\times$ 10^6 EV/mL corresponding to FF-C or FF-NC. Only EVs from FF-C improved blastocyst rates, with the optimal response at 10 $\times$ 10^6 EV/mL (59.1 $\pm$ 4.1 vs 41.8 $\pm$ 6.4 %). Then, SPOM was supplemented with miR-143 mimic, inhibitor, or corresponding negative controls. While the mimic did not significantly enhance development, inhibition of miR-143 reduced blastocyst rates, suggesting a modulatory role. These findings indicate that EVs from FF-C can enhance oocyte developmental competence during SPOM, while miR-143 alone is not effective.

1. Introduction

Assisted reproductive technologies are currently fundamental in the livestock industry, as they enable the planning and achievement of selective targets in a shorter timeframe. Among these, in vitro embryo production (IVEP) has seen increasing use, representing the most effective tool for accelerating genetic progress through the mother. However, despite improvements made over the years, the in vitro system remains static and suboptimal, not comparable to the natural environment where oocyte maturation, fertilization, and embryonic development occur. The higher embryonic yield from ovulated oocytes compared to in vitro matured oocytes [1] indicates that the in vitro maturation (IVM) environment is the primary factor influencing blastocyst formation. A simulated physiological oocyte maturation system (SPOM) has been developed to mimic the in vivo conditions of oocyte maturation [2]. The SPOM is based on the modulation of intracellular cAMP levels, through pre-maturation with meiotic inhibitors and sequential exposure to gonadotropins, to reduce the asynchrony

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between nuclear and cytoplasmic maturation, improving oocyte developmental competence [2]. Further refining the SPOM protocol could enhance oocyte competence and hence IVEP efficiency, reducing overall embryo production costs.

The oocyte competence is defined as the ability of an oocyte to be fertilized and develop into an embryo [3]. It is mainly affected by the intrinsic quality of the oocyte, which is the key factor determining the blastocyst yield [4,5], and by the follicular environment or the IVM system [6]. The biochemical environment during follicular growth before ovulation influences both the oocyte developmental competence and the follicle steroidogenic capacity in high-yielding dairy cows [7]. Even when fertilization occurs successfully, suboptimal follicular conditions during oocyte growth and maturation can negatively impact embryo viability at later stages [8]. The composition of follicular fluid (FF) is closely linked to the stage of follicular growth and the developmental potential of the oocyte [9,10]. During follicular growth, maternal genes are transcribed, and the synthesized mRNA and proteins accumulate in the oocyte [4,11,12]. As follicular development progresses, fluid accumulates in the antrum, surrounding the oocyte and providing the conditions necessary for its development and maturation [8]. Variations in the follicular environment may affect the oocyte metabolic activity, leading to reduced quality of both the oocyte and the resulting embryo, implantation rate, and pregnancy outcomes [8]. The FF is a complex mixture of electrolytes, RNAs, proteins, amino acids, peptides, sugars, hormones, and growth factors, determinants for oocyte quality [9,13]. Several studies have shown that the metabolite profile of FF may predict bovine oocyte developmental competence [14–16]. The FF contains extracellular vesicles (EVs) that are essential regulators of oocyte competence [17].

The EVs are defined as particles released from cells, delimited by a lipid bilayer, and incapable of self-replication [18]. The EVs in the FF play a critical role in cell communication, facilitating the transfer of messages from the somatic cells to the oocyte. This exchange happens by transzonal projections, enabling EVs and their contents to enter the oocyte [19]. EVs transport coding and non-coding RNAs, like microRNA (miRNAs) that control the expression of genes in their target cells [17]. Previous studies have reported that miRNAs contained into the EVs from FF [20] can serve as non-invasive biomarkers for assessing oocyte fitness and follicular health [21–23]. It has also been demonstrated that in vitro supplementing EVs isolated from the FF improves the IVEP efficiency in cattle [24]. There is evidence, however, that the EV composition varies in relation to several factors, such as the metabolic status in dairy cows [25] and the photoperiod in seasonal species like buffalo [26], affecting oocyte competence. The inclusion of FF from individual cows exhibiting higher oocyte competence or specific isolated miRNAs in the maturation and culture media has been reported to improve embryonic development in cattle [27]. Moreover, it has been demonstrated that the miRNA cargo of EVs isolated from ovarian FF may differ in relation to oocyte competence. However, enriching the IVM medium with the most abundant miRNA from FF-derived EVs corresponding to competent oocytes did not affect embryo development but improved blastocyst cell number [28]. To the best of our knowledge, the effects of EVs or specific competence-associated EV factors have not yet been explored within a SPOM system, which prolongs the bi-directional communication via gap-junctions between cumulus cells and the oocyte, allowing the passage of nutrients and other molecules [2], effectively mimicking what happens in vivo.

The aim of this work was to develop an innovative in vitro maturation (IVM) system for bovine oocytes that closely simulates the follicular environment, with the goal of improving oocyte developmental competence and ultimately enhancing IVEP outcomes. Therefore, the objectives of this work were to evaluate: i) the miRNA profile of EVs isolated from the FF of follicles enclosing oocytes with different developmental competence; ii) the effect of incubating bovine oocytes during SPOM with FF-EVs corresponding to competent (FF-C) and non-competent (FF-NC) oocytes on developmental capacity, i.e. blastocyst outcome; iii) the effect of miR-143, one of the most abundant miRNAs found in EVs isolated from FF-C, during SPOM on oocyte competence.

2. Materials and methods

2.1. Experimental design

The experimental flow is shown in Fig. 1.

In Experiment 1, abattoir-derived cumulus-oocyte complexes (COCs), recovered by aspirating individual 2–4 mm follicles, were in vitro matured, fertilized, and cultured to the blastocyst stage in an individual culture system. Meanwhile, the corresponding FF was diluted 1:20 in PBS, centrifuged, and individually stored at - 80 °C. Retrospectively, at the end of culture, i.e., day 8 (day 0 = IVF), the oocytes were classified into 3 groups: competent, i.e. those that had developed into blastocysts (B), cleaved, i.e. those that had divided but stopped developing (C), and non-competent, i.e. those that have not divided, so uncleaved (U). A total number of 180 follicles was collected, over 8 replicates to obtain 3 pools of 20 follicles corresponding to the three categories of oocytes. The EVs were isolated from the pool of FF of the three categories and characterized for miRNA profile, as described below.

In Experiment 2, to assess the effect of EVs on oocyte competence, abattoir-derived bovine COCs were in vitro matured in the SPOM system with 0 (n = 134), 1 (n = 123), 10 (n = 124), and 100 (n = 124) x 10^6 mL^{-1} EV isolated from FF-C, over four replicates.

Then, to assess whether the beneficial effect was indeed related to the source of EVs, abattoir-derived COCs were in vitro matured with 0 (n = 148), 1 (n = 145), 10 (n = 141), and 100 (n = 147) x 10^6 mL^{-1} EV isolated from FF-NC (Experiment 2b), over four replicates.

In Experiment 3, miR-143, previously detected in higher concentrations in the FF-C, was tested in the SPOM system. Specifically, abattoir-derived bovine COCs were matured in the SPOM system with no supplement (control; n = 194), miR-143 mimic (n = 194), miR-143 inhibitor (n = 189), negative control (NC) of mimic (n = 191), and NC of inhibitor (n = 194), over five replicates.

2.2. Follicular fluid collection

Abattoir-derived bovine ovaries were collected and transported overnight to the laboratory in insulated flasks containing Dulbecco's PBS (14–18 °C), as previously reported [29]. Upon arrival (18–20 h after slaughter), follicles ranging from 2 to 5 mm were aspirated individually using 21-G needles. Follicular fluid samples were collected, and 20 μl were placed in 1.5 mL microtubes previously filled with 380 μl of PBS, while the COCs were isolated for the in vitro production of embryos. Afterward, FF was transferred into a vial and centrifuged at 300×*g* for 10 min at 4 °C to separate the FF and the follicular cells. The FF was centrifuged again at 2000×*g* for 10 min and at 16500×*g* × 30 min, and the supernatant was stored at − 80 °C until further analyses.

2.3. In vitro embryo production (IVEP)

The COCs with a homogenous, evenly granulated ooplasm, with oocytes surrounded by at least three layers of cumulus cells, were transferred to modified Tissue Culture Medium 199 (TCM199) supplemented with 2.2 mg/ml $NaHCO_3$, 0.02 μg/ml sodium-pyruvate, 0.5 mg/mL gentamycin sulphate, 0.4 % fatty acid-free (FAF) bovine serum albumin (BSA) and 0.83 μL/mL Suigonan ® 80/40 (MSD Tiergesundheit). COCs were matured individually in a 100 μL droplet of medium under mineral oil in Well of the Well (WOW) culture dishes (VitaVitro Biotech, Shenzhen Guangdong, China). They were then incubated for 22 h at 39 °C with 5 % CO_2 and backfilled with air. At the end of maturation, the IVM medium with matured COCs was replaced with the IVF Medium® (Stroebech Media, Odense Denmark). Bull semen with proven IVF fertility was thawed in a water bath at 37 °C for 40 s and then

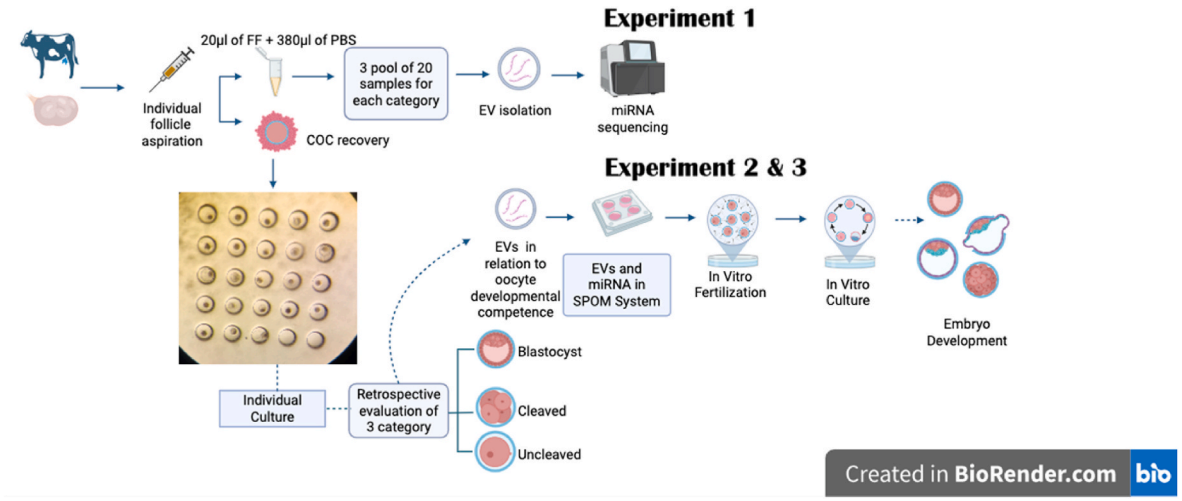

Fig. 1. Visual representation of the experimental flow.

centrifuged for 5 min at 300×*g* with 2 mL ofSemen Wash Medium® (Stroebech Media, Odense, Denmark). The supernatant was discarded, and the remaining 400 µL semen pellet was resuspended in 2 mL of Semen Wash and centrifuged (300×*g* for 5 min). The supernatant was discarded, leaving 100 µL. The final concentration of sperm in IVF droplets was adjusted to 1×10^6 sperm mL^{-1}. Following 18 h of co-culture, cumulus and sperm cells were removed, and presumptive zygotes were washed before transfer to in vitro embryo culture. Embryo culture was performed in a humidified atmosphere with 5 % CO_2 and O_2 in air at 39 °C for up to 8 days in WOW dishes in 100 µL of Synthetic Oviduct Fluid (SOF) medium [30] supplemented with 6 mg/mL fatty acid-free bovine serum albumine (BSA) overlaid with mineral oil, respectively. The cleavage rate was determined on day 4 of culture, whereas the blastocyst rate was determined on day 8 of culture.

2.4. EVs isolation

The EVs were isolated from FF through ultracentrifugation. About 380 µl of diluted FF collected from three pools of 20 follicles each of the oocyte categories (B, C, U) were used for EV isolation. FF was ultracentrifuged at 100.000×*g* (Beckman Coulter OptimaX, Milan, Italy), at 4 °C for 1 h. The pellet was resuspended in filtered PBS and EV aliquots were stored at −20 °C.

2.5. Nanoparticle tracking analysis (NTA)

EVs' size and concentration were determined by Nanoparticle tracking analysis (NTA) with the NanoSight NS300 system (Malvern Technologies, Malvern, UK) configured with 532 nm laser. All samples were diluted in filtered PBS (1:1000 vol/vol) in order to obtain an ideal particle per frame value (20–100 particles/frame). Three videos of 60 s were captured for each preparation and analyzed with NTA software version 3.2. From each video, the mean, mode, and median EVs size were used to calculate samples concentration expressed in nanoparticles/ml.

2.6. Western Blot

Isolated EVs from the three FF categories (B, C, U) were solubilized in Laemmli buffer supplemented with reduction agent, and incubated for 5 min at 95 °C, then loaded onto a SDS-PAGE (4–20 %, Mini-Protean TGX Precast protein gel, Bio-Rad) for protein one-dimensional separation. The separated proteins were transferred onto a nitrocellulose membrane using the Trans-Blot Turbo system (Bio-Rad). The membrane was then blocked with 5 % (w/v) BSA in T-TBS (tris-buffered saline: 150 mM NaCl, 20 mM TrisHCl, pH 7.4, and 0.5 % Tween 20) for 1 h to saturate nonspecific sites. Following the blocking step, the membranes were incubated overnight at 4 °C with anti-Alix (1:1000, Santa Cruz, CA, USA), anti-TSG101 (1:1000, Novus Bio, Centennial, CO, USA), anti-CD9 (1:1000, BD Pharmingen, San Jose, CA, USA), anti-CD63 (1:1000; BD Pharmingen, San Jose, CA, USA) and anti-Calnexin (1:2000, Sigma-Aldrich, Milwaukee, WI, USA). The next day, membranes were washed with T-TBS and incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:3000, Jackson ImmunoResearch, Tucker, GA, USA) for 1 h at room temperature. After subsequent washes, the Bio-Rad Clarity Western ECL Substrate was applied, and the signal was detected using a Chemidoc XRS + imaging system (BioRad).

2.7. The ultrasensitive single-molecule array (Simoa)

Beads were conjugated with a mix of antibodies targeting tetraspanins (CD9, CD63, and CD81) following the Quanterix Homebrew kit instructions, using the recommended buffers as described below. For the conjugation, 150 µl of carboxylate paramagnetic beads (2.8×10^9 particles/ml) were washed three times with 300 µl of Bead Wash Buffer (Quanterix, phosphate buffer with detergent). After each washing step, the beads were centrifuged and placed on a magnetic separator for 1 min to aspirate the supernatant. The beads were then washed three additional times with 300 µl of Bead Conjugation Buffer (Quanterix, 50 mM MES buffer, pH 6.2).

2.8. Transmission electron microscopy TEM

One hundred microliters of EV pellet isolated by ultracentrifugation were fixed for 1 h with 2 % paraformaldehyde and 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffered solution at pH 7.4 at room temperature. Samples were postfixed on ice using 1 % OsO_4, 1.5 % potassium ferrocyanide in 0.1 M cacodylate solution, for 1 h in dark conditions. Samples were washed with distilled water and stained with 0.5 % uranyl acetate solution at 4 °C overnight. Samples were dehydrated using a series of ethanol/water mixtures (30, 50, 70, 80, 90, 96 %) for 5 min each and washed thrice with absolute ethanol (10 min each). Finally, samples were infiltrated with the ethanol solution containing the resin (Araldite-Epon) (1:1 vol) for 2 h, then with two incubations with 100 % Epon resin for 1h. Samples were finally polymerized at 60 °C for 48 h. Sections of 70 nm were collected on 300-mesh uncoated copper grid using an Ultracut E microtome (Reichert, Austria) and observed with a Zeiss LEO 912 ab Energy Filtering TEM operating at 120 kV. Digital images were acquired using a CCD-BM/1 K system operating with the iTEM (Olympus Soft Imaging Solutions).

2.9. RNA extraction

Total RNA was isolated from FF for each replicate (n = 3) and condition (B, C, U) by the NucleoSpin miRNA kit (Macherey–Nagel, Germany), using the protocol in combination with TRIzol lysis (Invitrogen). The Agilent 2100 instrument (Santa Clara, CA, USA) was used to determine the quality and quantity of RNAs, which were stored at −80 °C until use.

2.10. Library preparation and miRNA sequencing

Small RNA libraries were obtained with the TruSeq Small RNA Library Preparation kit (Illumina, San Diego, CA, USA), according to the manufacturer's instructions, with 14 cycles of amplification. Libraries were pooled together and purified with the Pippin-Prep instrument (Sage Science, MA, USA). Concentration and quality checks of libraries were pooled and sequenced on a single lane of Illumina Novaseq X (San Diego, CA, USA).

2.11. Data analysis

Illumina raw sequences were analyzed with the nf-core/smrnaseq v1.0.0 pipeline (doi: 10.1038/s41587-020-0439-x and 10.5281/zenodo.1400710). Bos taurus miRNAs from MirBase v22.1 were used to support known miRNA detection. Differentially expressed miRNAs (DEmiRNAs) between groups were calculated with The EdgeR Bioconductor package version 3.6 (Bioconductor, https://bioconductor.org/packages/release/bioc/html/edgeR.html) [31].

2.12. EVs supplementation during simulated physiological oocyte maturation

Starting from the initial concentration of EVs, serial dilutions in TCM 199 were carried out to reach the final concentrations of 100, 10, and 1 $\times 10^6$ ml^{-1} EV. Approximately 40 abattoir-derived bovine COCs were in vitro matured in 400 μl of medium in four-well plates (Nunc™, Thermo Fisher Scientific, Waltham, MA, USA) in a SPOM system at 38.5 °C in a humidified atmosphere with 5 % CO_2. The SPOM system consists of 2 h of pre-IVM, i.e. TCM 199 buffered with sodium bicarbonate and supplemented with 0.4 % BSA, 1 mM dbcAMP and 500 μM IBMX, followed by standard 22 h IVM, i.e. TCM 199 buffered with sodium bicarbonate and supplemented with 0.4 % BSA, 0.5 μg/ml FSH, 5 μg/ml LH, 1 μg/ml estradiol [2]. Both pre-IVM and IVM media were supplemented with0 (control), 1, 10, and 100 $\times 10^6$ ml^{-1} EV. Matured oocytes were fertilized with frozen/thawed sperm from a bull already tested for IVF in Tyrode Albumine Lactate Pyruvate (TALP) medium, added with 0.2 mM penicillamine, 0.1 mM hypotaurine, and 0.01 mM heparin, under the same incubation conditions used for maturation. After about 20 h of co-incubation with the spermatozoa, the presumed zygotes were stripped of the cumulus cells and cultured in vitro in SOF medium [32] added with amino acids and BSA. Cleavage rate (2-cell division) and the percentage of blastocysts on day 7 and day 8 (with day 0 = day of fertilization) were evaluated.

2.13. Supplementation of mimics and inhibitors

Mimics and inhibitors of miR-143 (Qiagen, Hilden, Germany) were supplemented at 1 μM to the SPOM system as described before [2]. In parallel, NC of mimic (sequence 5′→3′: UGAGAUGAAGCACUGUAGCUCG) and inhibitor (sequence 5′→3′: GAGCTACAGTGCTTCATCTC) were supplemented to the SPOM system. The final experimental design included a control group supplemented with an equal volume of RNA-free water. *In vitro* embryo production was carried out as previously described.

3. Statistical analysis

With regard to the experiments aimed at assessing the effect of EVs and miR-143 during SPOM, statistical analysis was carried out using SPSS (29.0.1) for Windows 10 (SPSS Inc., Chicago, IL). The normal distribution and the homogeneity of variance of data were verified using the Shapiro-Wilk and Levene's tests, respectively. Data were log-transformed when necessary. Cleavage and blastocyst rates were compared among groups by analyses of variance (ANOVA) using a generalized linear mixed model. The significance level was set at $P < 0.05$.

4. Results

4.1. Experiment 1 - EV isolation and characterization

The NTA EV profile is shown in Fig. 2A. Western Blot verified the presence of specific EV internal markers such as Alix and TSG101, but it was not able to detect external protein markers such as CD9, and CD63, probably due to low sensitivity (Fig. 2B).

Simoa assay was used to quantify the three widely expressed transmembrane proteins: the tetraspanins on the EVs (CD9, CD63, and CD81; Fig. 2C) in samples from three groups. Using Simoa to measure these three EV markers, we quantified the presence of these tetraspanins on the surfaces of EVs that were consistent with the concentration of EVs by NTA (Table 1). Finally, the observation by electron microscope revealed that the preparations contained EVs (Fig. 2D).

As shown in Table 1, the EVs isolated from the B group showed higher size ($P < 0.05$) and concentration ($P < 0.01$) than those from the U group, while no differences were observed in the C group.

The FF isolated from follicles enclosing oocytes with different developmental competence contained EVs that showed differences in miRNA cargo (Supplementary table). Two DEmiRNAs (bta-miR-143 and bta-miR-21-5p; FDR< 0.05) were found between competent oocytes (B) and non-competent oocytes (U), showing an opposite pattern, with a higher expression of bta-miR-143 and lower expression of bta-miR-21-5p in the B group. Another miRNA, bta-miR-19a, was overexpressed in the B group compared to the C group. The comparison between C and U groups showed two DEmiRNAs with an opposite pattern: bta-miR-19a and bta-let-7f were respectively higher and lower in the C group.

4.2. Experiment 2 - effects of EVs on oocyte developmental competence

Regarding EVs isolated from the FF-C, no differences in cleavage rate were recorded among groups (79.4 ± 2.8, 82.9 ± 5.0, 82.6 ± 2.7, and 80.0 ± 2.0 %, respectively, for 0, 1, 10, and 100 $\times 10^6$ mL^{-1}). However, the addition of 10 $\times 10^6$ mL^{-1} EV during SPOM increased ($P < 0.01$) blastocyst outcome, both in relation to COCs (41.8 ± 6.4, 37.1 ± 3.8, 59.1 ± 4.1, and 34.2 ± 4.9, respectively for 0, 1, 10, and 100 $\times 10^6$ mL^{-1}), and cleaved (52.7 ± 5.2, 45.0 ± 4.2, ±72.0 ± 6.4, and 43.1 ± 7.0, respectively for 0, 1, 10, and 100 $\times 10^6$ mL^{-1}), as shown in Fig. 3A.

Regarding EVs isolated from the FF-NC, no differences in cleavage rate were recorded among groups (80.4 ± 2.1, 82.5 ± 1.4, 82.5 ± 1.7, and 78.2 ± 1.5, respectively, for 0, 1, 10, and 100 $\times 10^6$ mL^{-1}). Likewise, the addition of EVs during IVM did not increase blastocyst outcome, both in relation to COCs (45.8 ± 5.7, 41.1 ± 5.3, 34.9 ± 3.4 and 34.6 ± 2.7, respectively for 0, 1, 10, and 100 $\times 10^6$ mL^{-1}), and cleaved (57.1 ± 7.2, 49.8 ± 6.3, 42.5 ± 4.4, and 44.6 ± 4.4, respectively for 0, 1, 10, and 100 $\times 10^6$ mL^{-1}) as shown in Fig. 3B.

4.3. Experiment 3 -effects of miR-143 mimics and inhibitors on oocyte developmental competence

No differences in cleavage rate were observed among the groups (Table 2). Supplementation of miR-143 mimic in the SPOM did not increase blastocyst rates compared to the control. However, the COCs

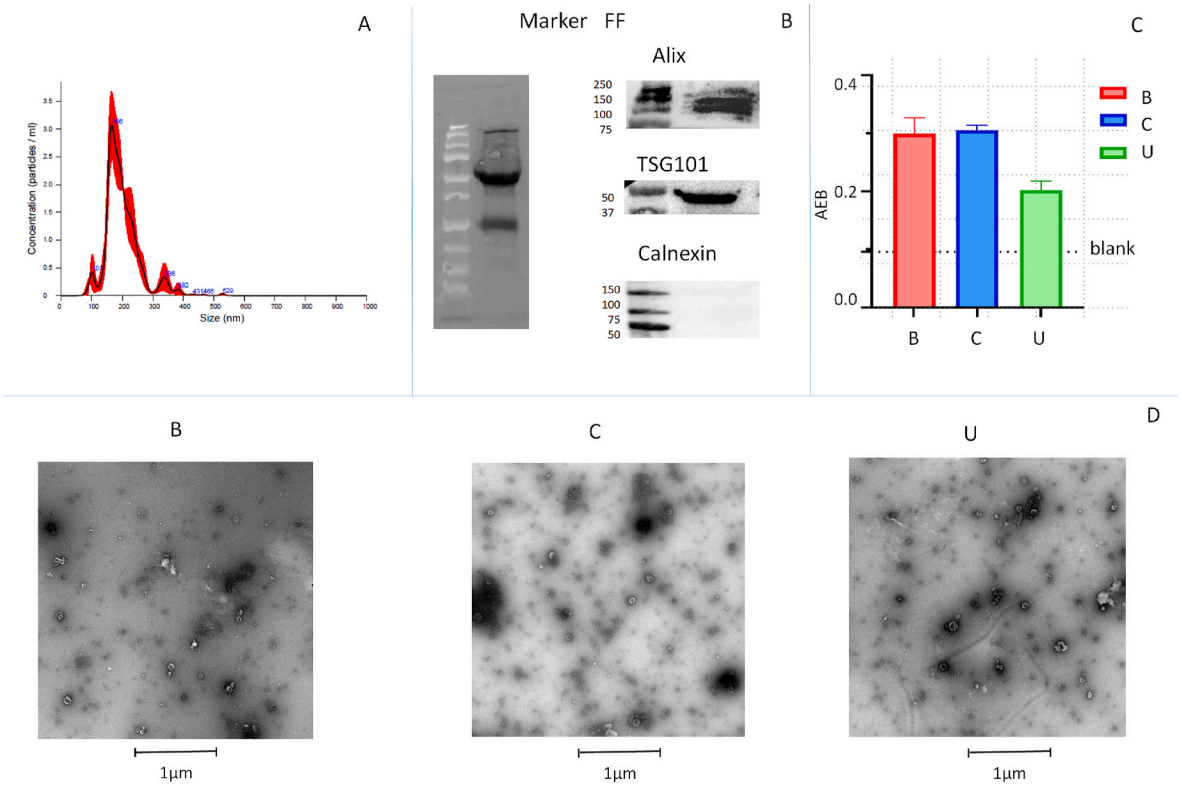

Fig. 2. EV characterization with A) Nanoparticle Tracking Analysis (NTA) profile EVs B) Western blot profile for internal marker Alix,TSG101 and Calnexin as a marker of cell contamination. C) SIMOA analysis using a combination of tetraspanins (CD9, CD63 and CD81), D) Transmission Electron Microscopy that revealed typical morphologies characteristic of vesicle (scale bar: 1 µm). U: EVs isolated from FF enclosing oocytes that did not cleave, B: EVs isolated from FF enclosing oocytes giving blastocyst, C: EVs isolated from FF enclosing oocytes that cleaved but stopped development.

Table 1
Size distribution and concentration of EVs isolated from follicular fluid of follicles corresponding to oocytes with different developmental competence. Data are presented as Mean ± SEM.

Groups	Particle size (nm)	Particle/mL (E+11)
Blastocysts	213.4 ± 3.1^{a}	4.0 ± 0.2^{A}
Cleaved	208.9 ± 1.2^{ab}	3.9 ± 0.6^{AB}
Uncleaved	202.1 ± 2.3^{b}	2.8 ± 0.1^{B}

^{a,b} Values with different superscripts are significantly different; $P < 0.05$.
^{A,B} Values with different superscripts are significantly different; $P < 0.01$.

matured in the presence of miR-143 yielded higher ($P < 0.05$) blastocyst rates than those exposed to the inhibitor, both in relation to COCs and cleaved oocytes.

5. Discussion

This study investigates the association between the miRNA cargo of FF-derived EVs isolated from individual bovine follicles and the developmental competence of corresponding COCs. To achieve this goal, the miRNA cargo of FF-derived EVs isolated from small antral follicles enclosing oocytes of three categories was compared: those that developed into blastocysts (B), those that cleaved but did not develop into blastocysts (C), and those that remained uncleaved (U), as retrospectively evaluated at the end of individual culture (day 8). For this study, we selected small antral follicles (2- mm) as EVs from their FF, which are linked to oocyte competence [24]. The rationale of the work was that the identification of FF competence-associated factors and their incorporation into the SPOM would be a combined approach to better mimic the follicular environment in vitro and enhance oocyte competence and embryo development. Our results showed that the miRNA profile of FF-derived EVs differs in relation to competence and that supplementing the SPOM system with EVs corresponding to FF-C improves embryo yields.

The first indication that the follicular environment differs with the developmental competence of the enclosed oocytes was the higher concentration and the greater size of EVs isolated from follicles corresponding to COCs that developed into blastocysts compared to the uncleaved ones. This interesting finding poses the question of whether these features are determinants or collateral effects of enhanced developmental capacity. In other words, does the greater quantity and size of EVs in the FF make the follicular environment more suitable for the COCs, or do competent COCs release more and greater EVs in the FF? The improved oocyte competence observed by exposing COCs to FF-derived EVs corresponding to FF-C (Experiment 2) would support the first hypothesis. However, it has been previously reported that the conditioned medium from bovine zygotes developing into blastocysts contains EVs in greater concentration and size than that from arrested embryos [33]. Likewise, the content of FF-derived EVs differs with embryo development [33].

Unexpectedly, only a few differently expressed miRNAs (DEmiRNAs) were detected among EVs from different FF categories, in contrast to previous studies [27,28]. Notably, the B group exhibited significantly higher expression levels of miR-143 and lower levels of miR-21-5p than the U group. Additionally, miR-19a expression was higher in the B group than in the C and U groups. However, the DEmiRNAs associated with oocyte competence identified in this study differ from those reported in previous research [27,28]. The discrepancies with the previous studies may be due to the variations in oocyte sources, sample preparation protocols, and EVs isolation techniques.

The miR-143 is among the top 10 abundantly expressed miRNAs in bovine ovarian tissues, suggesting its potential role in ovarian functions [34]. Furthermore, miR-143 is among the four most highly expressed

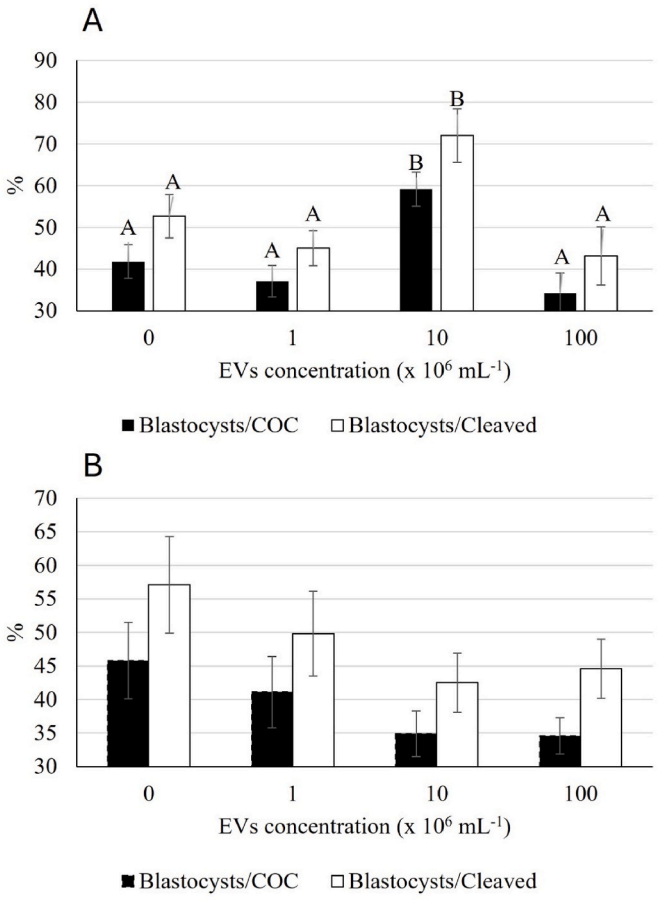

Fig. 3. Percentages of blastocysts (mean ± SEM) obtained in relation to cumulus-oocyte complexes (COC) and cleaved oocytes matured in the presence of different concentrations of EVs isolated from the follicular fluid enclosing competent oocytes (A) and non-competent oocytes (B).
[A,B] Values with different superscripts are significantly different; $P < 0.01$.

Table 2
Effects of MiR-143 mimic supplementation during SPOM on cleavage and blastocyst rates in relation to COCs (BL/COCs) and cleaved oocytes (BL/Cleaved).

	N	Cleavage (%)	BL/COCs (%)	BL/Cleaved (%)
Control	194	80.8 ± 1.4	40.8 ± 5.4^{ab}	50.4 ± 6.7^{ab}
MiR-143 mimic	194	79.1 ± 3.6	48.4 ± 0.4^{a}	61.9 ± 3.0^{a}
MiR-143 Inhibitor	189	82.6 ± 2.7	35.3 ± 1.4^{b}	42.9 ± 2.4^{b}
Negative Control mimic	191	79.4 ± 3.2	43.2 ± 3.4^{ab}	54.1 ± 2.3^{ab}
Negative Control inhibitor	194	82.6 ± 3.8	44.0 ± 1.6^{ab}	53.9 ± 3.6^{ab}

[a,b] Values within columns with different superscripts are significantly different; $P < 0.05$.

miRNAs in the bovine FF [35]. A potential role for miR-143 in modulating oocyte competence is confirmed by several studies. A high expression of miR-143 has been detected through the various stages of preantral bovine follicular growth, suggesting an association with oocyte development [36]. Moreover, miR-143 was found to change in relation to season and competence in buffalo [26]. MiR-143 was also found in EVs isolated from granulosa cells' conditioned media [37]. In the present study, the miR-143 and the miR-21-5p showed an opposite pattern, with higher expression of miR-143 and lower expression of miR-21-5p in FF-C. Interestingly, an opposite pattern of these miRNAs has been previously described in bovine granulosa cells, in relation to the follicular growth, with higher expression of miR-143 and lower expression of miR-21-5p in subordinate follicles compared to preovulatory follicles [34]. Likewise, an increase of miR-143 and a decrease of miR-21-5p were found at day 7 of the bovine estrous cycle during the early luteal phase [38]. In agreement with our results, other authors reported a lower expression of miR-21 in healthy compared to atretic follicles [39]. The miR-19a was overexpressed in the B group compared to the C group, suggesting a role in oocyte competence. This is confirmed by previous studies in humans that reported a downregulation of miR-19a in conditions associated with reduced oocyte competence, such as patients with polycystic ovary syndrome [40], and older women [41]. Likewise, miR-19b, which has an identical seed region, was highly abundant in the FF of young cows compared to old cows [42]. Finally, miR-7f was overexpressed in the C vs the U group. This is in line with the pattern of this miRNA in pre-ovulatory follicles according to season and, hence, oocyte competence, in buffalo [26]. A few members of the let7 family have also been reported to vary in the sheep ovary in relation to season [43]. In addition, members of the let-7 miRNA family are involved in granulosa cell programmed death and follicular atresia in the pig [44]. Moreover, the miR-let-7f was demonstrated to modulate the expression of CYP19AI transcript in cultured buffalo granulosa cells [45]. In conclusion, it was demonstrated that the follicles enclosing bovine oocytes with different developmental ability exhibit variations in EV concentration and size, as well as in the profile of a few miRNAs, such as miR-143, MiR 21, MiR-19a, and MiR-let-7f, demonstrating that these miRNAs may be potential biomarkers of oocyte competence.

This work hypothesized that the addition of FF-derived EVs isolated from FF-C, or miR-143 alone, to a SPOM system would enhance bovine oocyte developmental competence. Our results demonstrated that maturing bovine COCs in the presence of the EVs derived from FF-C increases blastocyst yield. However, supplementing miR-143 to the IVM system did not improve oocyte developmental capacity. The improved blastocyst yields recorded from oocytes matured with 10×10^6 ml^{-1} competent EVs confirm the hypothesis that including FF-C-derived EVs during SPOM improves oocyte competence. Moreover, this assumption is confirmed by the lack of beneficial effect of supplementing FF-NC-derived EVs. Our results agree with those of a previous study in which supplementing the IVEP system with EVs derived from the FF of small follicles increased embryo yields in cattle [24]. The beneficial effects of EVs observed in this study are likely due to their pivotal role in intra-follicular communication [10]. It is known that to obtain a developmentally competent fertilizable oocyte and beyond, communication between the oocyte and its surrounding somatic cells is indispensable [46]. This communication, mediated by either direct communication via gap junctions or transzonal projections or indirectly through endocrine, paracrine, and autocrine signaling factors [17], is prolonged and improved by using the SPOM system [2], which may allow the passage of nutrients, molecules and EVs in a more physiological way. It was demonstrated that EVs are taken up by granulosa and cumulus cells during IVM, and their presence in the transzonal projections [24] suggests an important role in shuttling bioactive molecules that facilitate oocyte maturation [17]. Moreover, EVs have been shown to influence cellular functions like granulosa cell proliferation and cumulus expansion in vitro [17,47], further emphasizing their role as mediators of intercellular communication within the follicle. In conclusion, the improved developmental capacity of oocytes matured in the presence of FF-C-derived EVs confirms the important role played by EVs as mediators of cellular communication within the follicular environment.

To investigate the mechanism behind the beneficial effects of FF-C-derived EVs, we conducted a functional experiment to assess the impact of miR-143, the most abundant miRNA associated with FF-C, on blastocyst yield when supplemented into the SPOM system. MiR-143 mimic supplementation in the SPOM system had no effect on oocyte developmental competence, as indicated by blastocyst yields similar to

the controls. However, we observed a reduced blastocyst rate when the miR-143 inhibitor was present during IVM. Previous studies highlighted a pivotal role of miRNA-143 in regulating ovarian function and oocyte competence [17,26,35,36]. A similar outcome was observed in a previous study, where supplementation of miRNA-34c, the most abundant miRNA in FF-C-derived EVs, was added to the IVM medium [28]. Therefore, we speculate that a single miRNA may not be sufficient to replicate the complex signaling required for optimal oocyte development. To summarize, the results suggest that the approach to identify a specific competence-associated miRNA to be included in the in vitro system is less efficient than providing a mix of bioactive compounds contained in the EVs that can act synergically. In addition, although a different miRNA profile of the EVs contained in the FF was associated with oocyte competence, it is not possible to rule out that the beneficial effect described in vitro was due to bioactive molecules other than miRNAs, such as lipids, proteins, cytokines etc, known to be carried by EVs [17,24].

6. Conclusions

In conclusion, differences in concentration and size, as well as in miRNA cargo, were found at the level of EVs isolated from follicles associated with oocytes of different competence. In particular, the different expressions of miR-143, miR-21-5p, miR-19a, and miR-let-7f suggest these miRNAs as potential biomarkers of bovine oocyte competence. It was demonstrated that incubation of bovine COCs with 10×10^6 mL $^{-1}$ EVs derived from the FF-C during SPOM increases blastocyst rates, while supplementation with EVs derived from the FF-NC does not affect embryo yields. However, supplementing miR-143 during SPOM did not result in improved development compared to the control, although reduced blastocyst rates in the presence of its inhibitor were recorded. Therefore, maturing bovine COCs in SPOM with the EVs isolated from FF-C is a more valid way to mimic the follicular environment in vitro and improve oocyte competence.

CRediT authorship contribution statement

R. Esposito: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **F. Piscopo:** Writing – review & editing, Methodology, Investigation, Conceptualization. **C. Del Prete:** Writing – review & editing, Writing – original draft, Data curation. **M.A. Kosior:** Writing – review & editing, Supervision, Investigation, Data curation. **C. Benedetti:** Writing – review & editing, Investigation. **M. Schreiber:** Writing – review & editing, Investigation. **E. Capra:** Methodology, Investigation, Data curation. **A. Lange-Consiglio:** Supervision, Methodology, Investigation, Data curation. **R. Frigerio:** Writing – review & editing, Investigation. **B. Lazzari:** Methodology, Investigation, Data curation. **M. Hoelker:** Writing – review & editing, Supervision, Methodology, Conceptualization. **B. Gasparrini:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.theriogenology.2025.117629.

References

[1] Sugimura S, Richani D, Gilchrist RB. Follicular guidance for oocyte developmental competence. Anim Reprod n.d.;15:721–725. https://doi.org/10.21451/1984-3143-AR2018-0035.

[2] Sugimura S, Yamanouchi T, Palmerini MG, Hashiyada Y, Imai K, Gilchrist RB. Effect of pre-*in vitro* maturation with cAMP modulators on the acquisition of oocyte developmental competence in cattle. J Reprod Dev 2018;64:233–41. https://doi.org/10.1262/jrd.2018-009.

[3] Sirard M-A, Richard F, Blondin P, Robert C. Contribution of the oocyte to embryo quality. Theriogenology 2006;65:126–36. https://doi.org/10.1016/j.theriogenology.2005.09.020.

[4] Lonergan P, Rizos D, Gutierrez-Adan A, Fair T, Boland M. Oocyte and embryo quality: effect of origin, culture conditions and gene expression patterns. Reprod Domest Anim 2003;38:259–67. https://doi.org/10.1046/j.1439-0531.2003.00437.x.

[5] Rizos D, Ward F, Duffy P, Boland MP, Lonergan P. Consequences of bovine oocyte maturation, fertilization or early embryo development in vitro versus in vivo: implications for blastocyst yield and blastocyst quality. Mol Reprod Dev 2002;61:234–48. https://doi.org/10.1002/mrd.1153.

[6] Lonergan P, Fair T. Maturation of oocytes in vitro. Annu Rev Anim Biosci 2016;4:255–68. https://doi.org/10.1146/annurev-animal-022114-110822.

[7] Britt JH. Impacts of early postpartum metabolism on follicular development and fertility. AABP Proceedings 1991:39–43. https://doi.org/10.21423/aabppro19916706.

[8] Leroy JLMR, Rizos D, Sturmey R, Bossaert P, Gutierrez-Adan A, Van Hoeck V, et al. Intrafollicular conditions as a major link between maternal metabolism and oocyte quality: a focus on dairy cow fertility. Reprod Fertil Dev 2012;24:1. https://doi.org/10.1071/RD11901.

[9] Revelli A, Piane LD, Casano S, Molinari E, Massobrio M, Rinaudo P. Follicular fluid content and oocyte quality: from single biochemical markers to metabolomics. Reprod Biol Endocrinol 2009;7:40. https://doi.org/10.1186/1477-7827-7-40.

[10] Sohel MdMH, Hoelker M, Noferesti SS, Salilew-Wondim D, Tholen E, Looft C, et al. Exosomal and non-exosomal transport of extra-cellular micrornas in follicular fluid: implications for bovine oocyte developmental competence. PLoS One 2013;8:e78505. https://doi.org/10.1371/journal.pone.0078505.

[11] Vassena R, Mapletoft RJ, Allodi S, Singh J, Adams GP. Morphology and developmental competence of bovine oocytes relative to follicular status. Theriogenology 2003;60:923–32. https://doi.org/10.1016/S0093-691X(03)00101-8.

[12] Van Den Hurk R, Zhao J. Formation of mammalian oocytes and their growth, differentiation and maturation within ovarian follicles. Theriogenology 2005;63:1717–51. https://doi.org/10.1016/j.theriogenology.2004.08.005.

[13] Kussano NR, Franco MM, Dode MAN. Biochemical profiling of the follicular environment to predict oocyte competence in cattle. PLoS One 2024;19:e0298316. https://doi.org/10.1371/journal.pone.0298316.

[14] Alves BG, Alves KA, Lúcio AC, Martins MC, Silva TH, Alves BG, et al. Ovarian activity and oocyte quality associated with the biochemical profile of serum and follicular fluid from Girolando dairy cows postpartum. Anim Reprod Sci 2014;146:117–25. https://doi.org/10.1016/j.anireprosci.2014.02.019.

[15] Alves GP, Cordeiro FB, Lima CB de, Annes K, Santos ÉC dos, Ispada J, et al. Follicular environment as a predictive tool for embryo development and kinetics in cattle. Reprod Fertil Dev 2019;31:451–61. https://doi.org/10.1071/RD18143.

[16] Matoba S, Bender K, Fahey AG, Mamo S, Brennan L, Lonergan P, et al. Predictive value of bovine follicular components as markers of oocyte developmental potential. Reprod Fertil Dev 2014;26:337. https://doi.org/10.1071/RD13007.

[17] Tesfaye D, Hailay T, Salilew-Wondim D, Hoelker M, Bitseha S, Gebremedhn S. Extracellular vesicle mediated molecular signaling in ovarian follicle: implication for oocyte developmental competence. Theriogenology 2020;150:70–4. https://doi.org/10.1016/j.theriogenology.2020.01.075.

[18] Welsh JA, Goberdhan DC, O'Driscoll L, Théry C, Witwer KW. MISEV2023: an updated guide to EV research and applications. J Extracell Vesicles 2024;13:e12416. https://doi.org/10.1002/jev2.12416.

[19] Macaulay AD, Gilbert I, Caballero J, Barreto R, Fournier E, Tossou P, et al. The gametic synapse: rna transfer to the bovine oocyte1. Biol Reprod 2014;91. https://doi.org/10.1095/biolreprod.114.119867.

[20] Nejabati HR, Roshangar L, Nouri M. Follicular fluid extracellular vesicle miRNAs and ovarian aging. Clin Chim Acta 2023;538:29–35. https://doi.org/10.1016/j.cca.2022.11.003.

[21] Wang Q, Sun Q-Y. Evaluation of oocyte quality: morphological, cellular and molecular predictors. Reprod Fertil Dev 2007;19:1. https://doi.org/10.1071/RD06103.

[22] Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007;9:654–9. https://doi.org/10.1038/ncb1596.

[23] Cortez MA, Welsh JW, Calin GA. Circulating micrornas as noninvasive biomarkers in breast cancer. In: Ignatiadis M, Sotiriou C, Pantel K, editors. Minimal residual disease and circulating tumor cells in breast cancer. Berlin, Heidelberg: Springer; 2012. p. 151–61. https://doi.org/10.1007/978-3-642-28160-0_13.

[24] Da Silveira JC, Andrade GM, Del Collado M, Sampaio RV, Sangalli JR, Silva LA, et al. Supplementation with small-extracellular vesicles from ovarian follicular fluid during in vitro production modulates bovine embryo development. PLoS One 2017;12:e0179451. https://doi.org/10.1371/journal.pone.0179451.

[25] Hailay T, Hoelker M, Poirier M, Gebremedhn S, Rings F, Saeed-Zidane M, et al. Extracellular vesicle-coupled miRNA profiles in follicular fluid of cows with

divergent post-calving metabolic status. Sci Rep 2019;9:12851. https://doi.org/10.1038/s41598-019-49029-9.
[26] Capra E, Lazzari B, Russo M, Kosior MA, Valle GD, Longobardi V, et al. Seasonal effects on miRNA and transcriptomic profile of oocytes and follicular cells in buffalo (Bubalus bubalis). Sci Rep 2020;10:13557. https://doi.org/10.1038/s41598-020-70546-5.
[27] Aoki S, Inoue Y, Hara S, Itou J, Shirasuna K, Iwata H. Micrornas associated with the quality of follicular fluids affect oocyte and early embryonic development. Reprod Med Biol 2024;23:e12559. https://doi.org/10.1002/rmb2.12559.
[28] Benedetti C, Pavani KC, Gansemans Y, Azari-Dolatabad N, Pascottini OB, Peelman L, et al. From follicle to blastocyst: microRNA-34c from follicular fluid-derived extracellular vesicles modulates blastocyst quality. J Animal Sci Biotechnol 2024;15:104. https://doi.org/10.1186/s40104-024-01059-8.
[29] Nagao Y, Harada Y, Yamaguchi M, Igarashi A, Ooshima Y, Kato Y. Antioxidant treatment during preservation of bovine ovaries increased the development potential of embryos. Zygote 2010;18:315–21. https://doi.org/10.1017/S0967199409990384.
[30] Holm P, Booth PJ, Schmidt MH, Greve T, Callesen H. High bovine blastocyst development in a static in vitro production system using sofaa medium supplemented with sodium citrate and myo-inositol with or without serum-proteins. Theriogenology 1999;52:683–700. https://doi.org/10.1016/S0093-691X(99)00162-4.
[31] Robinson MD, McCarthy DJ, Smyth GK. Edger : a bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 2010;26:139–40. https://doi.org/10.1093/bioinformatics/btp616.
[32] Tervit HR, Whittingham DG, Rowson LEA. Successful culture in vitro of sheep and cattle ova. Reproduction 1972;30:493–7. https://doi.org/10.1530/jrf.0.0300493.
[33] Pavani KC, Meese T, Pascottini OB, Guan X, Lin X, Peelman L, et al. Hatching is modulated by microRNA-378a-3p derived from extracellular vesicles secreted by blastocysts. Proc Natl Acad Sci USA 2022;119:e2122708119. https://doi.org/10.1073/pnas.2122708119.
[34] Gebremedhn S, Salilew-Wondim D, Ahmad I, Sahadevan S, Hossain MM, Hoelker M, et al. Microrna expression profile in bovine granulosa cells of preovulatory dominant and subordinate follicles during the late follicular phase of the estrous cycle. PLoS One 2015;10:e0125912. https://doi.org/10.1371/journal.pone.0125912.
[35] Pasquariello R, Manzoni EFM, Fiandanese N, Viglino A, Pocar P, Brevini TAL, et al. Implications of miRNA expression pattern in bovine oocytes and follicular fluids for developmental competence. Theriogenology 2020;145:77–85. https://doi.org/10.1016/j.theriogenology.2020.01.027.
[36] Krisher RL, editor. Oocyte physiology and development in domestic animals. first ed. Wiley; 2013. https://doi.org/10.1002/9781118538074.
[37] Andrade G, Meirelles F, Perecin F, Da Silveira J. Cellular and extracellular vesicular origins of miRNAs within the bovine ovarian follicle. Reprod Domest Anim 2017;52:1036–45. https://doi.org/10.1111/rda.13021.
[38] Salilew-Wondim D, Ahmad I, Gebremedhn S, Sahadevan S, Hossain MM, Rings F, et al. The expression pattern of micrornas in granulosa cells of subordinate and dominant follicles during the early luteal phase of the bovine estrous cycle. PLoS One 2014;9:e106795. https://doi.org/10.1371/journal.pone.0106795.
[39] Donadeu FX, Mohammed BT, Ioannidis J. A miRNA target network putatively involved in follicular atresia. Domest Anim Endocrinol 2017;58:76–83. https://doi.org/10.1016/j.domaniend.2016.08.002.
[40] McCallie B, Schoolcraft WB, Katz-Jaffe MG. Aberration of blastocyst microRNA expression is associated with human infertility. Fertil Steril 2010;93:2374–82. https://doi.org/10.1016/j.fertnstert.2009.01.069.
[41] Battaglia R, Vento ME, Ragusa M, Barbagallo D, La Ferlita A, Di Emidio G, et al. Micrornas are stored in human mii oocyte and their expression profile changes in reproductive aging. Biol Reprod 2016;95:131. https://doi.org/10.1095/biolreprod.116.142711. 131.
[42] Nagata S, Inoue Y, Sato T, Tanaka K, Shinozawa A, Shirasuna K, et al. Age-associated changes in miRNA profile of bovine follicular fluid. Reproduction 2022;164:195–206. https://doi.org/10.1530/REP-22-0036.
[43] Iorio MV, Visone R, Di Leva G, Donati V, Petrocca F, Casalini P, et al. Microrna signatures in human ovarian cancer. Cancer Res 2007;67:8699–707. https://doi.org/10.1158/0008-5472.CAN-07-1936.
[44] Papamentzelopoulou M, Mavrogianni D, Dinopoulou V, Theofanakis H, Malamas F, Marinopoulos S, et al. Detection of RUNX2 gene expression in cumulus cells in women undergoing controlled ovarian stimulation. Reprod Biol Endocrinol 2012;10:99. https://doi.org/10.1186/1477-7827-10-99.
[45] Bettegowda A, Patel OV, Lee K-B, Park K-E, Salem M, Yao J, et al. Identification of novel bovine cumulus cell molecular markers predictive of oocyte competence: functional and diagnostic implications1. Biol Reprod 2008;79:301–9. https://doi.org/10.1095/biolreprod.107.067223.
[46] Matzuk MM, Burns KH, Viveiros MM, Eppig JJ. Intercellular communication in the mammalian ovary: oocytes carry the conversation. Science 2002;296:2178–80. https://doi.org/10.1126/science.1071965.
[47] Uzbekova S, Almiñana C, Labas V, Teixeira-Gomes A-P, Combes-Soia L, Tsikis G, et al. Protein cargo of extracellular vesicles from bovine follicular fluid and analysis of their origin from different ovarian cells. Front Vet Sci 2020;7. https://doi.org/10.3389/fvets.2020.584948.