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—Original Article—

Effects of all-*trans* retinoic acid on the *in vitro* maturation of camel (*Camelus dromedarius*) cumulus-oocyte complexes

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Abstract. All-*trans* retinoic acid (RA) is a metabolite of vitamin A and has pleiotropic actions on many different biological processes, including cell growth and differentiation, and is involved in different aspects of fertility and developmental biology. In the current study, we investigated the effects of RA on camel (*Camelus dromedarius*) cumulus-oocyte complex *in vitro* maturation (IVM). IVM medium was supplemented with 0, 10, 20, and 40 μM RA. Application of 20 μM RA significantly reduced the proportion of degenerated oocytes and significantly improved oocyte meiosis and first polar body extrusion compared to the control and other experimental groups. Retinoic acid significantly reduced the mRNA transcript levels of apoptosis-related genes, including *BAX* and *P53*, and reduced the *BAX/BCL2* ratio. In addition, RA significantly reduced the expression of the Transforming growth factor beta (TGFβ) pathway-related transcripts associated with the actin cytoskeleton, *ACTA2* and *TAGLN*; however, RA increased TGFβ expression in cumulus cells. The small molecule SB-431542 inhibits the TGFβ pathway by inhibiting the activity of activin receptor-like kinases (ALK-4, ALK-5, and ALK-7); however, combined supplementation with RA during IVM compensated for the inhibitory effect of SB-431542 on cumulus expansion, oocyte meiosis I, and first polar body extrusion in activated oocytes. The current study shows the beneficial effects of RA on camel oocyte IVM and provides a model to study the multifunctional mechanisms involved in cumulus expansion and oocyte meiosis, particularly those involved in the TGFβ pathway.

Key words: All-*trans* retinoic acid, Apoptosis, Oocytes, SB-431542, Transforming growth factor beta (TGFβ)

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Camels (*Camelus dromedarius*) are xeric animals and, in extremely arid conditions, are better sources of meat and milk than are cattle. However, the low reproductive performance of camels is considered an important factor affecting camel populations. Employing *in vitro* embryo production (IVP) technologies is an efficient means of propagating genetically superior camels; however, compared to other livestock species, camel oocyte *in vitro* maturation (IVM) has been reported to be inefficient [1–4]. One of the main contributing factors is the limited availability of oocytes because the majority of the slaughtered animals are either old, culled for infertility, or very young and have not attained maturity; in addition, there is no standardized protocol for IVP in camels [3, 5]. Therefore, refinement of the camel IVP system and oocyte maturation is necessary, especially when the availability of oocytes is limited [6, 7].

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Retinoic acid (RA) is a metabolite of vitamin A (retinol) that mediates the functions of vitamin A, such as cell growth, development, and differentiation, and has been implicated in reproductive processes including folliculogenesis and embryonic survival [8]. In the last two decades, several reports have revealed the crucial role of the two major retinol metabolites, all-*trans* and 9-*cis* retinoic acid, on oocyte maturation and its developmental competence in different mammalian species, both *in vivo* and *in vitro* [9–21]. However, to the best of our knowledge, there are no reports regarding the effects of RA on camel oocytes. Retinoic acid has multifunctional roles during *in vitro* oocyte maturation; it may promote cytoplasmic maturation through its modulatory effects on the expression of genes encoding gonadotrophin receptors, midkine, cyclooxygenase 2, and nitric oxide synthase in cumulus-granulosa cells [11, 13]. Another potential role for RA is in reducing apoptosis in cumulus-oocyte complexes [13, 20]; in addition, RA may also promote cumulus expansion [14, 16]. Despite the presence of RA receptors in cumulus cells [22], RA may act directly to improve oocyte maturation in the absence of cumulus cells [23], or act as an antioxidant to reduce the levels of reactive oxygen species and oxidative stress during oocyte maturation [11, 24, 25].

Transforming growth factor beta (TGFβ) superfamily members are involved in oocyte maturation either directly [26] or indirectly

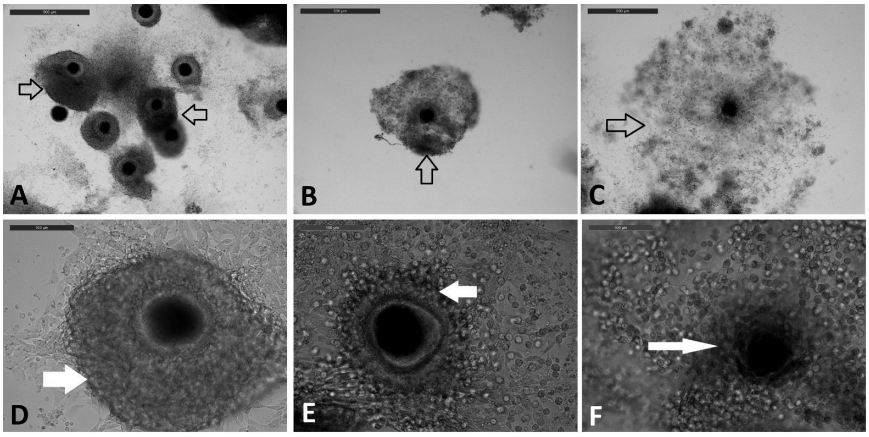

Fig. 1. The degree of cumulus expansion after *in vitro* maturation of camel oocytes. A, B, and C show grade 0, 1, and 2 of cumulus expansion, respectively. Arrows indicate the transparency of intercellular spaces between cumulus cells. D, E, and F show magnified images of grade 0, 1, and 2, respectively. The white arrow in D shows the compactness of cumulus cells after IVM. The arrow in (E) shows the presence of un-expanded corona radiata and cumulus cells surrounding the oocytes. The arrow in (F) shows the fully expanded corona radiata and cumulus cells. A, B, C: Scale bars = 500 μm; D, E, F: Scale bars = 100 μm.

through cumulus expansion and differentiation [27], or through a bidirectional interplay between oocytes and surrounding cumulus cells [28, 29]. Studies have shown the effects of RA on TGFβ pathways in cells other than cumulus-oocyte complexes, such as in osteoblasts [30], smooth muscle cells [31, 32], and mucosal cells [33].

Therefore, in the present study, we investigated the effects of all-*trans* RA on camel cumulus-oocyte complex (COC) IVM by examining cumulus expansion, nuclear maturation, and mRNA transcript levels of apoptosis, gap junction, and TGFβ pathway-related genes involved in cell cytoskeleton integrity, in both oocytes and cumulus cells.

Materials and Methods

Chemicals

Chemicals were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA) unless otherwise stated.

In vitro maturation

Camel ovaries (n = 320) were collected from a local slaughterhouse from October 2017 to March 2018. The ovaries were transported in normal saline solution at 30–33°C to the laboratory within 3–6 h. Antral follicle (2–8 mm in diameter) contents were aspirated with 18-gauge needles connected to 10-ml disposable syringes. Cumulus-oocyte complexes showing an even granulated cytoplasm and enclosed in compact cumulus cells were selected (a total of 1550 eligible COCs were used in the study). They were then washed three times with tissue culture medium-199 (TCM-199) supplemented with 2 mM $NaHCO_3$, 25 mM HEPES, 0.1% bovine serum albumin (BSA), and 5 μg/ml gentamycin sulfate. The COCs were then divided into four groups (20–25 oocytes each) according to the experimental design and cultured in 4-well dishes in 500 μl maturation medium at 38.5°C in a humidified atmosphere with 5% CO_2 for 28 h. The IVM medium comprised of TCM-199 supplemented with 10 μg/ml follicle stimulating hormone, 10 μg/ml luteinizing hormone, 1 μg/ml 17β-estradiol, 20 ng/ml epidermal growth factor, 1 μL/ml insulin-transferrin-selenium, 0.3 μM cysteamine, 0.15 mg/ml L-glutamine, 10% fetal bovine serum (FBS), and 5 μg/ml gentamycin sulfate [6].

RA and SB-431542 supplementation

A 50 mM stock of all-*trans* RA (R2625, Sigma-Aldrich) dissolved in dimethyl sulfoxide (DMSO) was stored at −20°C until further use. For the experiments, the all-*trans* RA stock was diluted in IVM medium to 0, 10, 20, and 40 μM. The solvent with DMSO at a final concentration of < 0.02% was used as the vehicle control. SB-431542 at 20 μM [34] was supplemented either individually or combined with RA as explained in the experimental design.

Evaluation of cumulus cell expansion and oocyte nuclear maturation

After IVM, the COCs were categorized according to the degree of cumulus expansion as follows: grade 0, no expansion was observed; grade 1, partially expanded cumulus; and grade 2, completely expanded cumulus with clear intercellular spaces around the oocytes as shown in Fig. 1 [7]. For oocyte degeneration and oocyte nuclear assessment (the meiotic transition from metaphase I, anaphase I, telophase I, and metaphase II; Fig. 2), cumulus cells were separated from oocytes by pipetting in TCM-199 supplemented with 1 mg/ml hyaluronidase and washed three times in TCM-199 supplemented with 10% FBS. The oocytes were then stained with 5 μg/ml bisbenzimide (Hoechst 33342) for 5 min to visualize the nuclei and polar body extrusions using an inverted microscope equipped with epifluorescence [6].

Parthenogenesis of mature oocytes and embryo culture

Oocytes were stripped from surrounding cumulus cells by pipetting in TCM-199 supplemented with 1 mg/ml hyaluronidase and washed three times in TCM-199 supplemented with 10% FBS. Oocytes extruding the first polar body were selected and activated in TCM-199

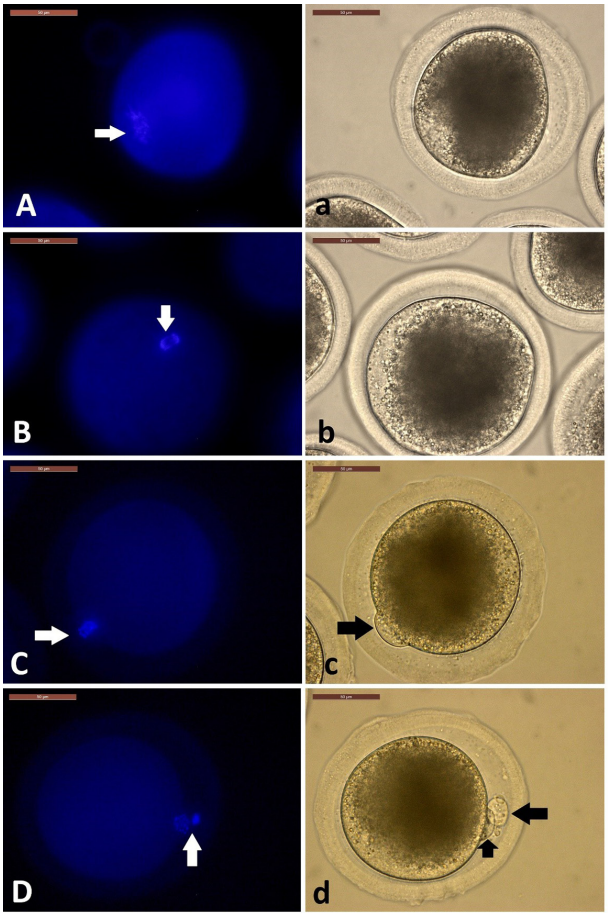

Fig. 2. Phases of meiosis in *in vitro* matured camel oocytes. Oocytes were stained with Hoechst and visualized by fluorescence microscopy. (A) metaphase I; (B) anaphase I; (C) telophase I; (D) first polar body extrusion and metaphase II. a, b, c, and d are the corresponding brightfield images of A, B, C, and D, respectively. Arrows in (c) indicate the start of ooplasmic cytokinesis. Arrows in (d) show the polar body extrusion and its location beside the metaphase II plate. Scale bar = 50 µm.

supplemented with 10% FBS and 5 µM ionomycin for 5 min [6, 7]. Activated oocytes (15 oocytes per treatment, five replicates) were washed three times in TCM-199 with 10% FBS, transferred to 50 µl microdrops of 4 mM 6-dimethylaminopurine (6-DMAP), overlaid with mineral oil, and cultured for 4 h in an atmosphere of 5% CO_2, 5% O_2, and 90% N_2 at 38.5°C. Activated oocytes were then washed in the culture medium and distributed in freshly prepared KSOMaa culture medium (one oocyte/5 µl) covered with mineral oil, and cultured in an atmosphere of 5% CO_2, 5% O_2, and 90% N_2 at 38.5°C. Cleavage and subsequent embryo development were evaluated on days 2 and 7 of IVC, respectively. The cleavage proportion was calculated as a percentage of the number of oocytes showing first polar body extrusion. Blastocyst development was calculated as the percentage of cleaved embryos.

Relative quantification of cumulus and oocyte mRNA transcript levels

Cumulus cells were stripped from the oocytes and washed with centrifugation at 3000 rpm for 2 min in phosphate-buffered saline (PBS). Oocytes (n = 20, three replicates) were washed in PBS. The experiment was repeated three times. Total RNA was extracted from cumulus cells and oocytes using an RNA extraction Kit (iNtRON Biotechnology, Daegu, South Korea) according to the manufacturer's instructions. A NanoDrop™ 2000 spectrophotometer (Thermo Fisher, Waltham, MA, USA) was used to estimate RNA concentration and purity. Pulsed reverse transcription (RT) was performed on the extracted RNA, according to Saadedin *et al*. [35]: 80 cycles of 2 min at 16°C, 1 min at 37°C, 0.1 s at 50°C, followed by final inactivation at 85°C for 5 min. Individual RT reactions were performed using a SuperScript™ III First-Strand Synthesis System (Thermo Fischer) according to the manufacturer's instructions, using 30 ng of RNA and random hexamers in a 20 µl reaction volume. Relative quantification of mRNA transcripts was performed using real-time PCR (ViiA 7, Applied Biosystems, Foster City, CA, USA). The reactions contained 100 ng cDNA, 1 µM forward primer, 1 µM reverse primer, and 1 × SYBR® Green Premix (Applied Biosystems). The reactions were run in triplicate and *GAPDH* was used as a reference gene. The fold change and relative quantities of apoptosis-related (*P53*, *BAX*, and *BCL2*), gap junction *CX43*, and TGFβ pathway-related (*TGFB*, *TAGLN*, and *ACTA2*) transcripts were calculated using the $2^{-\Delta\Delta Ct}$ method [36]. Reactions without cDNA template or reverse transcriptase resulted in no amplification. Cumulus cells and oocytes of the control group were set as the arbitrary group to calculate the fold change in mRNA expression. Thermal cycling conditions were 95°C for 10 min, followed by 40 cycles of 95°C for 10 sec, 60°C for 20 sec, and 72°C for 40 sec. PCR products were fractioned on 1.5% agarose gel using a 1 kb DNA ladder (iNtRON Biotechnology) as a reference and stained with RedSafe™ (iNtRON Biotechnology). Table 1 shows the primer sequences, annealing temperatures, and approximate product sizes of the amplified fragments of the candidate genes.

Experimental design

Experiment 1 was conducted to investigate four different concentrations of RA (0 [control], 10, 20, and 40 µM) on COC *in vitro* maturation, cumulus expansion, and oocyte developmental competence after parthenogenetic activation. For IVM, a total of 500 IVM oocytes were used, 25 oocytes per treatment with 5 replicates. For parthenogenetic activation, a total of 300 activated oocytes were used, 15 activated oocytes per treatment with 5 replicates. Moreover, the relative quantification of mRNA transcripts of apoptosis-related genes, the *CX43* gene, and TGFβ-related cell integrity pathway genes was evaluated between control and 20 µM RA-supplemented oocytes (20 oocytes per group with 3 replicates).

Experiment 2 was conducted to investigate the ameliorative or compensatory effects of RA on TGFβ inhibitor (SB-431542)-treated COCs. A total of 500 COCs were distributed between four groups (25 COCs each group, 5 replicates): control, 20 µM SB-431542 (SB), 20 µM RA, and RA + SB. Cumulus expansion and nuclear status were assessed in each group after 28 h of IVM.

Table 1. Primers used for quantitative PCR

Gene	Forward primer (5'→3')	Reverse primer (5'→3')	Product size (bp)*	Accession No.
BAX	AGATCATGAAGACAGGGGCC	GCGATCATCCTCTGCAACTC	190	XM_010996357.1
BCL-2	CAGGCTCAACGTCGAATCAG	TAGGTGGGGCTTGGCAATTA	151	XM_010979993.1
P53	AGCTCCTCTCCACCACAAAA	GTGAGCCTTGTTTTCCCCTG	159	XM_010996514.1
ACTA2	CAGGGCTGTTTTCCCATCTA	TTTTGCTTTGTGCTTCATCG	104	XM_010996278
TAGLN	CTCAGCCCAACTTCTCATCC	CCAACAAGGACAGTGGACCT	148	XM_010981405
TGFB1	TGGCTGTCCTTTGATGTCAC	CCCTGCGTTAATGTCCACTT	135	XM_010985237
CX43	TCAGGTGGACTGCTTCCTCT	TCTTTCCCTTGACACGATCC	149	XM_010979171
GAPDH	TGCTGAGTACGTTGTGGAGT	TCACGCCCATCACAAACATG	134	XM_010990867

* The melting curve for each primer was evaluated by ViiA^{TM}7 apparatus-associated software and the product size was confirmed by gel electrophoresis of PCR products on 1.5% agarose gels using a 1 kb DNA ladder as a reference.

Table 2. Effects of different concentrations of all-*trans* retinoic acid (RA) on camel oocyte *in vitro* maturation and subsequent parthenogenetic embryo development

		Control	RA (10 μM)	RA (20 μM)	RA (40 μM)
No. of IVM oocytes		125	125	125	125
First polar body extrusion (%)		65 (52.95 ± 4.2) [b]	65 (53.01 ± 3.3) [b]	86 (69.43 ± 4.6) [a]	64 (52.72 ± 2.01) [b]
No polar body (%) *	Metaphase I	5 (2.9 ± 1.0)	5 (3.7 ± 0.8)	6 (4.12 ± 1.1)	6 (4.03 ± 1.3)
	Anaphase I	5 (2.5 ± 0.9)	5 (3.63 ± 0.9)	5 (3.54 ± 1.2)	6 (4.03 ± 1.3)
	Telophase I	6 (3.19 ± 1.1)	5 (3.1 ± 1.0)	5 (3.9 ± 0.9)	5 (3.8 ± 0.9)
Degenerated oocytes (%)		44 (38.46 ± 2.1) [a]	45 (36.56 ± 2.4) [a]	23 (19.01 ± 1.9) [b]	44 (35.42 ± 1.5) [a]
No. of activated oocytes		75	75	75	75
Cleavage [#] (%)		45 (60.52 ± 3.2)	44 (58.43 ± 3.1)	45 (61.05 ± 2.1)	44 (59.8 ± 3.2)
Blastocyst [†] (%)		5 (12.33 ± 2.7)	6 (13.21 ± 2.1)	6 (13.5 ± 1.3)	5 (12.16 ± 2.0)

* No polar body; morphologically normal with no completion of first meiosis and still in metaphase I, anaphase I, or in pro-telophase I as illustrated in Fig. 2. [#] Cleavage ratio was calculated as the percentage of > 2-cell stage resulting from parthenogenetically activated first polar body oocytes. [†] Blastocyst ratio was calculated as the percentage of cleaved embryos.

Statistical analysis

COCs were randomly assigned to the designed experimental groups, and the experiments were repeated at least five times. Oocyte maturation values, degree of cumulus expansion, and embryo development (cleavage and blastocyst) from each trial are presented as means ± SEM, and values were analyzed using one-way analysis of variance (ANOVA). Quantitative PCR values were also compared by ANOVA. The *BAX*/*BCL2* values were calculated for all replicates and are presented as the means ± SEM and compared using ANOVA. Differences were considered significant at $P < 0.05$.

Results

Effect of RA on COC in vitro maturation

Supplementation of RA at 20 μM elicited a significant increase in the proportion of oocytes showing an extruded first polar body (69.43% ± 4.6%) compared to the control and other experimental groups (Table 2). Moreover, supplementing RA at 20 μM significantly decreased the proportion of degenerated oocytes (19.01% ± 1.9%) compared to the control and other experimental groups (Table 2). No differences were found between the experimental groups in cumulus expansion, cleavage percentage, and blastocyst percentage.

Effects of RA on cumulus and oocyte mRNA transcript levels

Retinoic acid (20 μM) application significantly reduced the expression of *BAX*, *BCL2*, and *P53* in both cumulus cells and oocytes compared to the control group (Fig. 3). However, RA elicited no effect on the expression of *CX43* in cumulus cells and oocytes (Fig. 4). Moreover, RA application significantly reduced *ACTA2* and *TAGLN* expression in both cumulus cells and oocytes, whereas *TGFB* expression was significantly increased in cumulus cells with RA supplementation (Fig. 5).

Effects of RA supplementation on SB-431542-treated COCs

The lowest first polar body extrusion rate was found in the SB-431542 group (11.2% ± 2.5%), which significantly inhibited cumulus expansion (Table 3). Notably, SB-431542 inhibited the meiosis I/meiosis II transition and significantly increased the proportion of metaphase I and anaphase I oocytes compared to the control group (Table 3). However, RA supplementation significantly mitigated the inhibitory effects of SB-431542 on nuclear maturation and the meiosis I/meiosis II transition (Table 3).

Discussion

Several studies have revealed the crucial role of all-*trans* retinoic

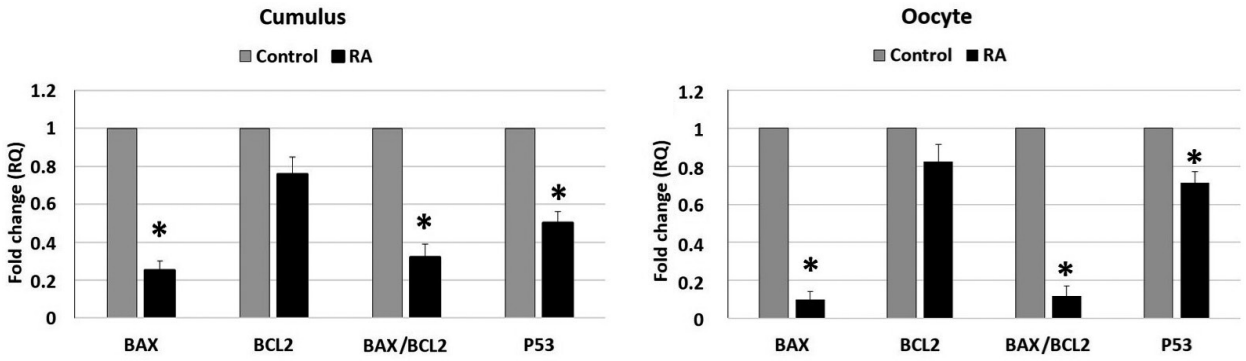

Fig. 3. Effect of all-*trans* retinoic acid (RA) on the relative quantification (RQ) of *BAX*, *BCL2*, and *P53* mRNA transcripts in camel cumulus cells and oocytes after IVM. The fold change (RQ) in mRNA transcripts was normalized to *GAPDH* levels for each treatment. Relative mRNA expression levels in the control group were arbitrarily set as 1. Fold change of the *BAX*/*BCL2* ratio was calculated for cumulus cells and oocytes and is presented as the mean ± SEM. Asterisks (*) indicate significant differences ($P < 0.05$) compared to control.

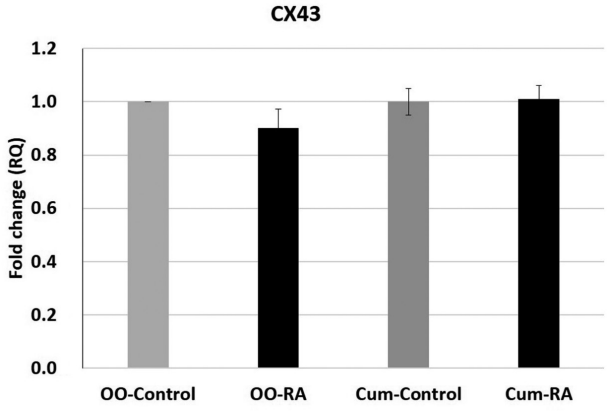

Fig. 4. Effects of all-*trans* retinoic acid (RA) on the relative quantification (RQ) of *CX43* mRNA transcripts in camel cumulus cells (Cum) and oocytes (OO) after IVM. The fold change (RQ) in *CX43* transcript levels was normalized to *GAPDH* levels in cells for each treatment. Data are presented as means ± SEM. Relative mRNA expression levels in the control group were arbitrarily set as 1.

acid on *in vitro* oocyte maturation and developmental competence in different livestock species [11, 13, 14, 17, 18, 20]. However, to the best of our knowledge, there are no studies on the effects of RA on camel oocytes.

Retinoic acid supplementation significantly decreased the proportion of degenerated oocytes (Table 2), which might be attributed to the decreased expression of *BAX*, *BAX*/*BCL2*, and *P53* observed in the current study (Fig. 3). This result coincides with the decreasing effects of RA on apoptosis-related genes in canine and caprine oocytes [13, 20].

However, RA supplementation had no effect on the expression of *CX43*, which encodes a gap junction protein, in both oocytes and cumulus cells (Fig. 4). This result contradicts those obtained by Best *et al*. 2015 and Pauli *et al*. 2010 [37, 38] who showed that retinoids upregulate *CX43* expression. In these two studies, the authors investigated the effects of retinoids on primary cumulus granulosa cells without oocytes in the culture conditions. This major difference in experimental design between the studies may indicate a bidirectional paracrine effect between oocytes and cumulus cells to stabilize *CX43* expression mediated by RA.

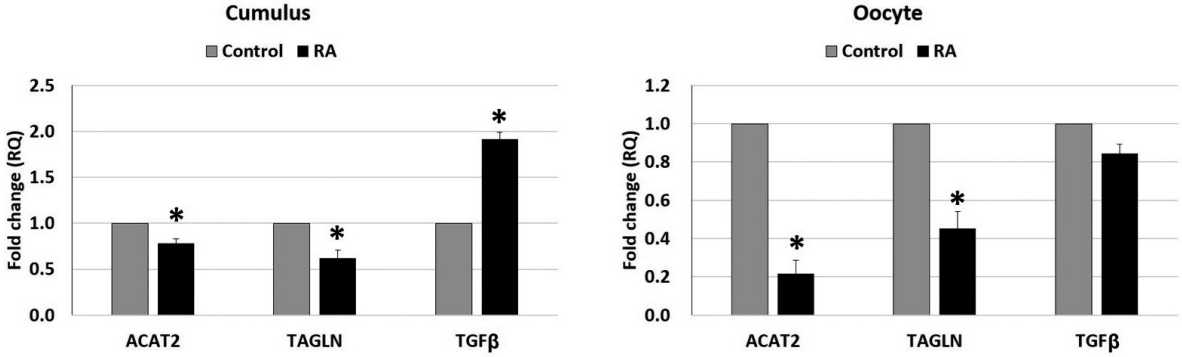

Fig. 5. Effects of all-*trans* retinoic acid (RA) on the relative quantification (RQ) of *ACTA2*, *TAGLN*, and *TGFβ* mRNA transcripts in camel cumulus cells and oocytes after IVM. The fold change (RQ) in mRNA transcript levels was normalized to *GAPDH* levels in cells for each treatment. The mRNA expression levels in the control group were arbitrarily set as 1. Data are presented as means ± SEM. Asterisks (*) indicate significant differences ($P < 0.05$) between transcripts.

Table 3. Effects of combined supplementation of all-*trans* retinoic acid (RA) with the TGFβ pathway inhibitor SB-431542 (SB) on camel oocyte *in vitro* maturation

		Control	SB	RA	RA + SB
No. of oocytes		125	125	125	125
First polar body (%)		66 (53.6 ± 3.1) [b]	14 (11.2 ± 2.5) [c]	90 (71.9 ± 4.1) [a]	66 (52.9 ± 3.4) [b]
No polar body (%) *	Metaphase I	5 (3.8 ± 0.8) [b]	50 (40.08 ± 1.1) [a]	4 (2.9 ± 0.6) [b]	5 (4.2 ± 1.0) [b]
	Anaphase I	5 (3.1 ± 0.9) [b]	13 (10.0 ± 1.9) [a]	4 (3.1 ± 0.7) [b]	5 (3.8 ± 0.9) [b]
	Telophase I	5 (3.29 ± 1.1)	4 (3.0 ± 1.0)	4 (2.36 ± 0.9)	4 (2.32 ± 0.7)
Degenerated oocytes (%)		44 (36.21 ± 1.7) [a]	44 (35.72 ± 0.8) [a]	23 (19.74 ± 1.0) [b]	45 (36.78 ± 1.1) [a]
Cumulus expansion degree [$]		2.0 ± 0.0 [a]	0.0 ± 0.0 [c]	2.0 ± 0.0 [a]	1.0 ± 0.0 [b]

* No polar body; morphologically normal with no completion of first meiosis and still in metaphase I or anaphase I. The nuclear status and meiosis steps are shown in Fig. 2. [$] The degree of cumulus expansion was calculated by observation of the five IVM replicates; representative images are shown in Fig. 1.

Additionally, RA supplementation increased *TGFβ* expression in cumulus cells (Fig. 5). The increase in *TGFβ* expression enhances oocyte development, steroidogenesis, and aromatization by cumulus granulosa cells, and upregulate essential transcripts required for oocyte meiosis [27, 39–41]. Paradoxically, RA significantly reduced *TAGLN* and *ACTA2* expression in oocytes and cumulus cells. Xiao *et al.* [42] observed that *TAGLN* downregulation was associated with human oocyte maturation. These interesting results coincide with the paradoxical effects of RA on the TGFβ pathways in somatic cells, in addition to ovarian or germ cells. It has been shown that RA upregulates TGFβ expression in intestinal cells [43], osteoblasts [30], and bronchial epithelium [44]; however, RA inhibits TGFβ signaling and restricts the expression of smooth muscle genes like *ACTA2* and *TAGLN* in distal lungs to prevent ectopic and excessive cell differentiation [31, 32]. *TAGLN* and *ACTA2* are TGFβ-inducible genes and are required for actin cytoskeleton organization and cellular differentiation [45]; however, their function in oocytes, cumulus cells, and ovarian cells is unknown and requires further investigation [42].

To investigate the effects of RA on the TGFβ pathway, we used SB-431542 to specifically inhibit the activin receptor-like kinase activity of ALK-4, ALK-5, and ALK-7 [46]. This molecule was previously shown to specifically inhibit cumulus expansion through the inhibition of SMAD2/3 phosphorylation and downregulation of FSH-stimulated GDF9-, activin A-, activin B-, and oocyte-induced cumulus expansion [26, 34, 47, 48]. Our results are in agreement with the results of the aforementioned studies and show that SB-431542 application induced complete arrest of cumulus expansion and meiosis I (Table 3; Fig. 2). Interestingly, RA mitigated the inhibitory effect of SB-431542, improving cumulus expansion and activating the oocytes to complete meiosis I and first polar body extrusion (Table 3). This result indicates the importance of alternative pathways, other than activin receptor-like kinases (ALK) 4/5/7, that can be stimulated by RA in cumulus-oocyte bidirectional communication.

The current study showed the beneficial effects of all-*trans* RA on camel oocyte *in vitro* maturation in terms of a reduction in degenerated oocytes, an increase in first polar body extrusion, and a reduction in the expression of apoptosis-related transcripts. Moreover, RA overcame the SB-431542-mediated inhibition of ALK 4/5/7 and improved oocyte meiosis and cumulus expansion, indicating a molecular compensatory action for RA.

Conflict of interest: The authors declare that they have no conflicts of interest.

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