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Soluble and membrane-associated adenylate cyclases participate in capacitation with hyaluronic acid in bovine spermatozoa

Abstract

Hyaluronic acid (HA), a glycosaminoglycan present in the female reproductive tract, mediates sperm motility, maturation and capacitation. Two adenylate cyclases, soluble (sAC) and membrane-associated (mAC), isoenzymes are present in spermatozoa, participating in different signaling mechanisms. The aim of this study was to determine the effect of adenylate cyclase isoenzymes inhibition on bovine sperm processes, such as capacitation, motility, viability, mitochondrial activity and fertilizing capacity, during sperm capacitation with HA. HA was used as a capacitation inducer, while LRE-1 (a specific sAC inhibitor) and 2,5-dideoxyadenosine (2,5-D) were used as sAC and mAC inhibitors, respectively. We evaluated sperm capacitation (chlortetracycline [CTC] technique), viability (trypan blue stain and differential interference contrast), mitochondrial activity (fluorochrome 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide [JC-1]) and motility (computer-assisted sperm analysis). We carried out *in vitro* fertilization (IVF) in IVF- modified synthetic oviductal fluid (mSOF) with frozen-thawed semen. We then cultured presumptive zygotes for 48 hours and analysed the cleavage rate. The addition of sAC or mAC inhibitors, alone or combined, produced a significant decrease in capacitation, mitochondrial activity and motility with respect to sperm samples incubated only with HA and controls ($P < 0.05$). Cleavage rates decreased with the addition of adeylate cyclase (AC) inhibitors compared to HA alone. We observed a greater decrease in cleavage rate with the addition of LRE-1 than with 2,5-D ($P < 0.05$). The combination of both inhibitors decreased the cleavage rate with respect to each inhibitor alone ($P < 0.05$). In bovine spermatozoa, both soluble and membrane-associated adenylate cyclase isoenzymes could participate in the intracellular signaling mechanism and mitochondrial function, which modulate HA capacitation and sperm fertilizing ability.

Keywords: Hyaluronic acid; Adenylate cyclase; Capacitation; *In vitro* fertilization; Cattle.

Study contribution

Research transfer is based on the study and validation of sperm parameters that determine the fertilizing capacity of preserved gametes, which applies to animal production species. Determining the mechanisms involved in sperm capacitation with hyaluronic acid contributes to optimizing *in vitro* fertilization through the use of biotechnology. This is a key factor in multiplying the number of animals for food production.

Abbreviations

2,5-D:	2,5-dideoxyadenosine
AC:	adenylate cyclase
ALH:	amplitude of lateral head displacement
ANOVA:	analysis of variance
ATP:	adenosine triphosphate
BCF:	beat cross frequency
BSA:	bovine serum albumin
cAMP:	cyclic adenosine monophosphate
COCs:	cumulus–oocyte complexes
CTC:	chlortetracycline
ERK:	extracellular signal-regulated kinase
FBS:	fetal bovine serum
FSH:	follicle-stimulating hormone
Gs:	stimulatory heterotrimeric Gs protein
HA:	hyaluronic acid
ICSI:	intracytoplasmic sperm injection
IVC:	in vitro culture
IVF:	in vitro fertilization

JC-1: 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide
LH: luteinizing hormone
mAC: membrane-associated adenylate cyclase
MEM: minimum essential medium
mSOF: modified synthetic oviductal fluid
PI3K: phosphatidylinositol 3-kinase
PM: progressive motility
sAC: soluble adenylate cyclase
SD: standard deviation
TALP: modified Tyrode's medium (TALP medium)
TM: total motility

Introduction

Hyaluronic acid (HA) is a natural glycosaminoglycan localized in the female reproductive tract.(1) It is present in follicular, oviduct and uterine fluids, cervical mucus, cumulus oophorus, and seminal plasma.(2–4) HA interacts with its major cell surface receptor, named CD44.(5) This receptor has been found in bovine oocytes and embryos, and also in boar,(6, 7) human,(8) and bovine sperm.(9) However, the intracellular signaling transduction mechanism has not been elucidated yet in sperm cell. HA plays a significant role in fertilization, since only sperm capable of penetrating the extracellular matrix rich in glycosaminoglycans surrounding the cumulus cells will be able to fertilize the oocyte.(10)

Sperm motility, maturation and capacitation are partially mediated by HA.(11) Mature spermatozoa can bind to and digest HA, present around the oocyte and cumulus cells, by sperm hyaluronidase.(12, 13) Sperm cells selected by HA show better motility, morphological characteristics and nuclear maturity, also lower DNA fragmentation levels and chromosomal imbalance risk, and presence of an intact acrosome ready to acrosome exocytosis.(14, 15)The fact that sperm capacitation is an entire process mediated by intracellular signaling pathways is well known. Different enzymes such as adenylate cyclase, protein kinase A and protein tyrosine kinases are involved in this process.[16] Two adenylate cyclases, membrane-associated (mAC) and soluble (sAC), isoenzymes are present in spermatozoa, and they participate in different signaling mechanisms.[17]

These enzymes increase intracellular cyclic adenosine monophosphate (cAMP), regulating sperm capacitation in mammals.[18] The cAMP production can be inhibited in mouse sperm by 2,5-D, a specific competitive mAC inhibitor.[19] LRE-1 has been described as a specific inhibitor of sAC, which suppressed cAMP production by a negative allosteric effect.[20]Sperm cell has an active metabolism represented by metabolic pathways such as glycolysis, Krebs cycle, fatty acid β-oxidation and mitochondrial respiratory chain.[21] This oxidative metabolism allows sperm to have energy for hyperactivated motility during capacitation. Previously, it was proved the participation of mAC isoenzyme in the regulation of bovine sperm capacitation induced by HA, analyzing its effect on aerobic and anaerobic metabolism; different metabolic patterns were found compared to heparin, including mitochondrial activity and enzymatic activities (lactate dehydrogenase, creatine phosphokinase, and malate and isocitrate dehydrogenases).[22, 23]

Our hypothesis was that mAC and sAC participate in intracellular signaling mechanisms and in metabolic pathways involved in sperm capacitation induced by HA in bovine species. The aim of this study was to determine the effect of adenylate cyclase isoenzymes inhibition on capacitation, motility, viability, mitochondrial activity, and fertilizing capacity, during sperm capacitation with HA.

Materials and methods

Ethical statement

Male gametes used for this study were provided by straws donated from a bovine insemination center, and female gametes were obtained from bovine ovaries donated by an abattoir. No live animals were used in this study. All the chemicals used were purchased from Sigma Chemical Company (Sigma-Aldrich, St.Louis, USA).

Semen samples

Straws were donated by a bovine insemination center, collected using an artificial vagina from 5 Brangus bulls of proven fertility. Bulls were 4 or 5 years of age and were maintained with uniform nutritional and management conditions throughout the sample extraction. Semen from different animals in each experience was pooled to minimize the bull's individual effect. Diluted semen was slow-cooled (1°C/min) to 5°C and after equilibration straws were frozen in liquid nitrogen (-120°C) for 10 minutes and then dipped in nitrogen for storage at -196°C.[22]

In the laboratory, first straws were thawed for 30–60 seconds at 38°C, then they were cut by each side, and thawed semen were incubated in modified Tyrode's medium (TALP)—TALP medium; 100 mM NaCl, 3 mM KCl, 0.3 mM NaH_2PO_4, 10 mM $NaHCO_3$, 40 mM Hepes, 1.5 mM $MgSO_4$, 22 mM sodium lactate and 1.2 mM sodium pyruvate) for 10 minutes at 38°C. Vigor and progressive motility were evaluated at 38°C using light microscopy after the 10minute thawing. Only sperm samples presenting immediately after thawing at least 60 % progressive motility and 3 or 4 vigor assessment were selected for this study. Samples were centrifuged at 600 g for 5 minutes and re-suspended in TALP medium with the addition of 2.1 mM $CaCl_2$ and 6 mg mL bovine serum albumin (BSA).

Sperm concentration was determined by hemocytometry using a Neubauer chamber in order to adjust all samples to 1.0 × 10^6 spermatozoa mL.[24]

Induction and evaluation of in vitro capacitation

Sperm capacitation was induced by adding HA (1 000 µg mL) or heparin (60 µg mL, positive control) to cumulus-oocyte complex (COCs)-sperm co-incubation at 38°C.[22, 25] Capacitation percentages were determined by CTC technique using an epifluorescence microscope (Jenamed 2, Carl Zeiss, Jena, Germany). An aliquot (25 µL) of the sperm suspension taken from each different treatment was incubated with 25 µL of CTC and buffer solution at 38°C, and immediately fixed with 8 µL of glutaraldehyde solution. Spermatozoa samples were observed between coverslip and slide protected from light, using 25 µL per sample. Three different CTC patterns were recognized: F) non-capacitated sperm, with a fluorescent head; B) capacitated sperm, with a band lacking fluorescence in the post-acrosomal region and AR sperm with a reacted acrosome, with low fluorescence in the whole cell except for a band in the equatorial segment.[26]

Soluble adenylate cyclase and membrane-associated adenylate cyclase inhibition

LRE-1 (100 µM) and 2,5-D (100 µM) were used as sAC and mAC inhibitors, respectively, and were incubated in samples for 60 minutes.[19, 20] Six treatments were performed with frozen-thawed semen in TALP medium at 38°C: control (no capacitation inducers or inhibitors included), heparin, HA, HA + LRE-1, HA + 2,5-D, and HA + LRE-1 + 2,5-D.

Evaluation of sperm viability

Percentages of live sperm were determined by vital trypan blue stain using differential interference contrast microscopy (Jenamed 2, Carl Zeiss, Jena, Germany). An aliquot

(25 μL) of the sperm suspension taken from each different treatment was incubated with an equal volume (25 μL) of trypan blue in TALP medium for 15 minutes at 38°C, then centrifuged at 600 g for 10 minutes to remove excess stain and fixed with 8 μL of glutaraldehyde solution. Spermatozoa samples were observed between coverslip and slide, using 25 μL per sample. The patterns observed in bovine sperm are: 1) Live intact: transparent sperm with intact acrosome, 2) live non-intact: transparent sperm with damaged acrosome, 3) dead intact: blue sperm with intact acrosome and 4) dead non-intact: blue sperm with damaged acrosome.[27]

Evaluation of sperm mitochondrial activity

Mitochondrial membrane potential was measured by the fluorochrome JC-1 (2 μM). Spermatozoa samples were observed between coverslip and slide protected from light, using 25 μL per sample. Sperm patterns were evaluated using an epifluorescence microscope with 510–570 nm filters (Carl Zeiss Jenamed 2, Jena, Germany). Results were expressed as the percentage of spermatozoa with inactive mitochondria (low membrane potential, green color in the intermediate piece) or with active mitochondria (high membrane potential, red-orange color in the middle piece). Two hundred spermatozoa per sample were observed.[28, 29]

Evaluation of sperm motility

Sperm motility was analyzed using computer-assisted sperm analysis (ISAS V. 1.2 Prosier).[30–32] Each capture was performed with negative phase contrast and 10× objective, frame rate of 25 Hz and particle area of 35–100 μ^2. For each determination, between 10 and 20 fields were analyzed, always carried out by the same operator, using

15 µL of sperm suspension between slide and coverslip at 38°C. The following parameters were considered in this study as indicators of motile sperm: total motility (TM, %), progressive motility (PM, %), amplitude of lateral head displacement (ALH, µm) and beat cross frequency (BCF, Hz).

Recovery and in vitro *maturation of cumulus-oocyte complexes*

Bovine ovaries were collected from an abattoir within 30 minutes after slaughter and kept warm (30°C) during the 2-hour transport to the laboratory. Ovaries were washed with physiological saline containing 100 000 IU/L penicillin and 100 mg/L streptomycin. COCs were recovered by aspiration of antral follicles (3–5 mm diameter) and only oocytes surrounded by a compact and multilayered cumulus oophorus were used.[(33)] COCs were cultured in medium 199 (Earle's salts, L-glutamine, 2.2 mg/L sodium bicarbonate; GIBCO, Grand Island, NY, USA) supplemented with 5 % (v/v) fetal bovine serum (FBS; GIBCO), 0.2 mg/L porcine FSH (Folltropin-V; Bioniche, Belleville, Ontario, Canada), 2 mg/L porcine LH (Lutropin-V, Bioniche) and 50 mg/L gentamycin sulfate under mineral oil at 39°C for 22 hours in a humidified atmosphere with 5 % CO_2.[(34)]

In vitro *fertilization*

In vitro fertilization (IVF) was performed using a pool of thawed semen then centrifuged at 500 g for 5 minutes in TALP and finally resuspended in the same medium to a final concentration of 2×10^6 motile spermatozoa/L. Co-incubation of COCs and sperm was performed under mineral oil at 39°C in a humidified atmosphere with 5 % CO_2 for 20 hours. Six repetitions were performed for each sperm treatment, not simultaneously. Putative zygotes were denuded by repeated pipetting and placed in *in vitro* culture (IVC) medium, consisting of modified synthetic oviductal fluid (mSOF) supplemented with 30 mL/L amino acid minimum essential medium (MEM) (GIBCO), 10 mmol/L non-essential amino acid MEM (GIBCO), 2 mmol L-glutamine, 6 g/L BSA and 5 % (v/v) FBS (GIBCO), under mineral oil at 39°C in a humidified atmosphere with 90 % N_2:5 % CO_2:5 % O_2 for 24 hours. The proportion of cleaved embryos 48 hours after IVF was evaluated by the number of embryos that presented two or more blastomeres.[34]

Experimental design and statistical analysis

To evaluate sperm parameters, six treatments were performed with frozen-thawed semen in TALP medium at 38°C, each one with 8 replicates: control without capacitation inducers or inhibitors included, heparin, HA, HA + LRE-1, HA + 2,5-D, and HA + LRE-1 + 2,5-D. In all these cases the assays were started from an aliquot of thawed and re-suspended sperm sample, then each treatment was incubated with the corresponding capacitation inductor and adenylate cyclase inhibitor, for 15 minutes (for heparin sample) or 60 minutes (for HA samples) of incubation, according to optimal concentration and incubation time of specific inductors mentioned before.[22, 25] Capacitation, motility, viability and mitochondrial activity were determined for each sample.

To evaluate sperm fertilizing capacity, spermatozoa treated with capacitation inductors and inhibitors were added to the respective wells in the culture plate and then co-incubated with matured COCs. Six treatments, as described above, were evaluated and the proportion of cleaved embryos was recorded for each one. Sperm parameter data were expressed as mean ±SD. Normal distribution was confirmed by a Shapiro-Wilk normality test. Differences among groups were analyzed by ANOVA, while Tukey test was used as a post-ANOVA analysis to compare means (STATISTIX 7. 2000, Analytical

Software for Windows, Version 7.0; Analytical Software, Tallahassee, Florida, USA). Cleavage rates were compared using a Chi-square analysis for non-parametric data (homogeneity of proportions).

Results

Sperm parameters

In order to determine the effect of adenylate cyclase isoenzymes inhibition in bovine sperm capacitation induced by HA, different sperm parameters were evaluated: sperm capacitation, motility, viability, mitochondrial activity and fertilizing capacity. We found no significant differences between HA and heparin (**Tables 1** and **2**) in any of the sperm parameters studied and both capacitation inducers disclosed similar embryo cleavage rates (**Table 3**).The use of mAC or the sAC inhibitors, alone or combined, in HA-treated samples (HA + LRE-1, HA + 2,5-D, and HA + LRE-1 + 2,5-D) produced a significant decrease in the percentages of capacitated spermatozoa and spermatozoa with positive mitochondrial membrane potential (mitochondrial activity) with respect to HA ($P < 0.05$), without affecting sperm viability (**Table 1**).

Evaluation of sperm motility revealed that TM, PM, ALH and BCF decreased when HA samples were added with the mAC or the sAC inhibitors, alone or combined (HA + LRE-1, HA + 2,5-D, and HA + LRE-1 + 2,5-D), with respect to heparin (considered in this case as the negative control) and HA ($P < 0.05$, **Table 2**).

In vitro *fertilization*

Percentages of embryo cleavage decreased with the addition of the AC inhibitors (HA + LRE-1 and HA + 2,5-D), compared to HA ($P < 0.05$, **Table 3**). However, the decrease in cleavage rate was different according to the inhibitor used. A greater descent in cleavage rate was observed with the addition of LRE-1, the sAC inhibitor, with respect to 2,5-D, the mAC inhibitor (P < 0.05, **Table 3**). Additionally, the combination of both inhibitors (HA + LRE-1 + 2,5-D) decreased the cleavage rate with respect to HA + LRE-1 and HA + 2,5-D (P < 0.05, **Table 3**).

Table 1. Capacitation, viability and mitochondrial activity in thawed sperm

	Capacitation (%)	**Viability (%)**	**Mitochondrial activity (%)**
Control	8.00 ±1.15^{b}	55.67 ±5.99^{a}	45.00 ±7.78^{ab}
Heparin	20.89 ±6.25^{a}	46.00 ±8.29^{a}	63.67 ±5.57^{a}
HA	23.60 ±5.90^{a}	43.20 ±6.77^{a}	56.33 ±8.52^{a}
HA + LRE-1	4.67 ±2.31^{b}	43.33 ±7.97^{a}	38.25 ±10.82^{b}
HA + 2,5-D	6.00 ±1.63^{b}	44.20 ±14.36^{a}	34.75 ±11.05^{b}
HA + LRE-1 + 2,5-D	3.33 ±1.15^{b}	34.87 ±12.28^{a}	38.00 ±7.07^{b}

Capacitation, viability and mitochondrial activity (positive mitochondrial membrane potential) percentages were expressed as mean ±SD (n = 8). Control: no capacitation inducer was used. Heparin: heparin used as a capacitation inducer. HA: hyaluronic acid. LRE-1: soluble adenylate cyclase inhibitor. 2,5-D: 2,5-dideoxyadenosine, membrane-associated adenylate cyclase inhibitor. Different superscripts indicate significant differences between treatments within each column (P < 0.05).

Table 2. Sperm motility

	Total motility (%)	**Progressive motility (%)**	**ALH (µm)**	**BCF (Hz)**
Control	26.42 ±10.09[a]	20.69 ±10.63[a]	2.45 ±0.53[a]	7.30 ±1.59[a]
Heparin	28.27 ±6.82[a]	19.75 ±4.42[a]	2.64 ±0.41[a]	12.87 ±2.63[a]
HA	27.74 ±4.30[a]	17.72 ±6.00[a]	2.51 ±0.85[a]	7.48 ±2.99[a]
HA + LRE-1	1.46 ±1.03[b]	0.53 ±0.28[b]	0.12 ±0.05[b]	0.32 ±0.09[b]
HA + 2,5-D	6.47 ±1.94b	2.62 ±1.11b	0.40 ±0.30b	1.43 ±0.72b
HA + LRE-1 + 2,5-D	1.71 ±1.18[b]	1.03 ±0.31[b]	0.17 ±0.06[b]	0.67 ±0.32[b]

Total and progressive motility (%), amplitude of lateral head displacement (ALH, µm) and beat-cross frequency (BCF, Hz) were expressed as mean ±SD (n = 8). Control: no capacitation inducer was used. Heparin: heparin used as a capacitation inducer. HA: hyaluronic acid. LRE-1: soluble adenylate cyclase inhibitor. 2,5-D: 2,5-dideoxyadenosine, membrane-associated adenylate cyclase inhibitor. Different superscripts indicate significant differences between treatments within each column ($P < 0.05$).

Table 3. Cleavage rates produced from *in vitro* matured oocytes

	Number of matured oocytes used for IVF	**Number of cleaved embryos (%)**
Control	93	44 (47.30)^{b}
Control (heparin)	93	69 (74.00)^{a}
HA	104	66 (63.40)^{a}
HA + LRE-1	85	26 (30.60)^{c}
HA + 2,5-D	88	40 (45.40)^{b}
HA + LRE-1 + 2,5-D	87	7 (8.05)^{d}

Cleavage rates produced from *in vitro* matured oocytes. fertilized with non-capacitated sperm (control) and sperm capacitated with heparin (control heparin), hyaluronic acid (HA), hyaluronic acid + LRE-1, hyaluronic acid + 2,5-dideoxyadenosine (2,5-D) or hyaluronic acid + 2,5-dideoxyadenosine + LRE-1. N = 550 oocytes, 5 replicates. Numbers in parentheses are percentages. Different superscripts indicate significant differences between treatments within each column ($P < 0.05$).

Discussion

There has been an increasing recognition regarding the role of HA in reproductive biology and clinical procedures.[35] HA is essential for *in vivo* fertilization since only sperm capable of penetrating the extracellular matrix, rich in glycosaminoglycans surrounding the cumulus cells, are able to fertilize the oocyte.[10] Furthermore, the interaction of hyaluronan receptors on sperm plasma membrane with HA *in vitro* is considered a noninvasive and effective sperm selection method, leading to the test known as hyaluronan binding assay.[15, 36] A report describes a hydrogel-based approach to select bull spermatozoa, a crucial step for successful assisted reproductive techniques, because spermatozoa retain their viability, motility and acrosome integrity.[37]

It has been studied that spermatozoa selected using HA showed an increase in IVF rates in several species such as human, equine and buffalo.[31, 38–40] In the bovine, HA synthesis during cumulus mucification contributes to sperm penetration and fertilization of bovine oocytes, most likely by facilitating the processes of capacitation and acrosome reaction.[41] It has been reported that mature sperm have receptors capable of binding to HA and the zona pellucida, and these sperm have been shown to have normal morphology and reduced levels of chromosomal aneuploidy and fragmented DNA. HA-binding could be used as a selection tool for functional sperm in ICSI treatments.[13]

It has been proposed that HA-CD44 interaction may stimulate intracellular signaling through extracellular signal-regulated kinase (ERK), phosphatidylinositol 3-kinase (PI3K), Rac, and Ras in muscle and mesenchymal cells.[42, 43] Furthermore, in mammalian spermatozoa the presence and compartmentalization of an sAC and an mAC were described, and the involvement of a stimulatory heterotrimeric Gs protein was also observed.[44] However, little is known about the role of HA in intracellular mechanisms associated with sperm capacitation in the bovine species, including sperm AC. In previous studies, it was determined the mAC participation in HA-induced capacitation in cryopreserved bovine spermatozoa.[22] It was also observed that mAC inhibition decreased cleavage rates in bovine sperm capacitation induced by HA.[45]

It is also important to consider that mitochondrial respiration is able to produce a great amount of energy necessary for sperm motility,[46] which is crucial for the penetration of the cumulus cells and the oocyte’s zona pellucida. In the present study, we used JC-1 as an indicator of mitochondrial activity, considering that it measures mitochondrial membrane potential changes in mammalian spermatozoa.(47) A positive correlation between this high membrane potential (JC-1), membrane integrity and increasing motility in bull sperm has been reported.(48) HA induces capacitation with a lower mitochondrial membrane potential than other capacitation inductors, like heparin, in cattle.(45) Oxygen consumption is another parameter to evaluate mitochondrial activity, and it was also lower in HA-capacitated sperm than in heparin samples.(22, 27)

In accordance, creatine kinase shuttle activity(26) increased in HA-capacitated sperm in order to compensate the lower mitochondrial activity detected.(23) In order to study if AC regulation is involved in bovine sperm mitochondrial function, both AC inhibitors were used to evaluate their effect on mitochondrial activity, observing a lower mitochondrial membrane potential in sperm samples capacitated with HA. This effect may be involved in a lower energy supply by mitochondria, which would be related to the reduction in motility and capacitation status observed in our study. So, we propose that mitochondrial function would be modulated by sAC and mAC as key enzymes during HA bovine sperm capacitation.

In the studied conditions, heparin and HA maintained similar sperm motility parameters with respect to their respective controls, observing similar capacitation percentages with both inducers. According to a previous work, HA does not improve post-thaw sperm motility in bull semen.(32) Furthermore, it is important to consider that cyclic AMP, the product of AC, modulates different aspects of sperm function required for fertilizing capacity. Cyclic AMP is fundamental for sperm capacitation, including motility activation, changes in motility pattern and the ability to undergo acrosome reaction.(49) Moreover, in the absence of sAC, sperm have reduced intracellular concentration of ATP, not allowing hyperactivation and also not allowing successful IVF.[50, 51] Although some studies presented evidence of its presence in mammalian male gametes,[18, 44, 52–54] others reported its absence. In this study, the inhibition of sAC and mAC in bovine sperm decreased motility and capacitation percentages, suggesting the participation of both isoenzymes in these sperm parameters in presence of HA.

As regards the fertilizing capacity of bovine sperm with HA, the use of each inhibitor alone diminished cleavage and the combination of both inhibitors produced even lower rates. Interestingly, this synergistic effect was not observed in the other sperm parameters evaluated before IVF, so this effect could be related to oocyte-sperm interaction. Accordingly, the presence of CD44 receptors in mature oocytes, pre-implantation embryos and endometrium was related to the beneficial effect of hyaluronan.[55, 56]

Conclusions

Our results indicate that, in bovine spermatozoa, both soluble and membrane-associated adenylate cyclase isoenzymes are involved in the intracellular signaling mechanism and mitochondrial function which modulate HA capacitation and sperm fertilizing ability.

Data availability

All relevant data are within the manuscript and its supporting information files.

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Conflicts of 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. The authors have no conflict of interest to declare in regard to this publication.

Author contributions

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