Journal of Applied Veterinary Sciences, 8 (4): 43-53 (October, 2023)
ISSN: Online: 2090-3308, Print: 1687-4072
Journal homepage : https://javs.journals.ekb.eg

Assessment of Developmental Competence of *Holstein Bulls* Spermatozoa upon Addition of Aloe Vera Raw Extract during *In Vitro* Capacitation

Mona Mahmoud*, Saber Abd-Allah, Abdel-Halim B.R. and Khalil A.A.Y.
Department of Theriogenology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt
***Corresponding Author:** Mona Mahmoud, **E-Mail:** mona.mahmoud21@yahoo.com

ABSTRACT

The study aimed to evaluate the effect of the addition of aloe vera raw extract on the capacitation of bovine spermatozoa during the *in vitro* fertilization process. Hyper activated motility (HAM) and acrosome reaction (AR) of sperm cells, as well as *in vitro* fertilization and cleavage rates are the main parameters used to estimate the aloe vera's effect on bovine spermatozoa's fertilizing ability. In the current study, two different concentrations of aloe vera (5 and 10 µg/ml) were used. Frozen thawed semen spermatozoa were subjected to a swim-up technique to separate the motile spermatozoa and capacitated in modified Sperm Tyrode's albumin lactate pyruvate (S-TALP) medium supplemented with heparin only without aloe vera treatment (positive control), heparin+5µg/ml aloe vera, heparin+10µg/ml aloe vera, and finally the negative control tube, which was supplemented with 5µg/ml aloe vera without adding heparin. Sperm cells were incubated for 90 minutes at $39C^0$ in a 5% CO2 incubator and evaluated every 30 minutes at intervals. Cumulus oophorus complexes (COCs) were matured in a 5% CO2 incubator at $39C^0$ and inseminated *in vitro* with frozen-thawed bull sperm at the above concentrations. The inseminated oocytes were incubated at $39C^0$ in a 5% CO2 incubator for 24 hours and then examined for evidence of fertilization. The current study revealed that the aloe vera concentration of 10µg/ml at incubation time (60 minutes) had the best effect on sperm capacitation and acrosome reactions. The overall percentage of spermatozoa with progressive motility (PM) had declined across all groups as time progressed, with a significantly lower value found at the above concentration, as well as the effect of sperm treated with aloe vera on fertilization rate. The results showed that the proportion of fertilized oocytes was significantly increased at the aloe vera concentration of 10µg/ml compared to the other concentrations (5µg/ml), positive control, and negative control, respectively. **In conclusion**, treatment of bull spermatozoa with 10 µg of aloe vera extract/ml semen was considered the most efficient concentration for enhancement of sperm capacitation as a high IVF rate was obtained in Holstein Bull.

Original Article:
DOI:https://dx.doi.org/10.21608/javs.2023.219422.1249
Received : 22 June, 2023.
Accepted :13 August, 2023.
Published in October, 2023.

Keywords: Aloe Vera; Capacitation; Holstein bull spermatozoa; *In vitro* fertilization.

J. Appl. Vet. Sci., 8(4): 43-53.

INTRODUCTION

Spermatozoa's cellular and molecular structure is altered during the cryopreservation process. Semen handling, cold shock, osmotic and toxic stresses produced by cryoprotectants, and the formation/reshaping of extra- and intracellular ice during freezing and thawing are a few of the mechanisms that contribute to the low sperm quality discovered during semen thawing. Sperm motility, viability, and mitochondrial membrane potential all decrease as a result of all of these traits, as well as plasma membrane rupture, volumetric alterations, and anomalies in the sperm flagellum **(Ros-Santaella *et al*., 2014).**

Overproduction of reactive oxygen species (ROS) during sperm storage might cause oxidative stress (OS), jeopardizing sperm integrity and fertility **(José and Eliana, 2021).** One of the main factors contributing to low sperm quality is oxidative stress, which frequently develops during semen storage and compromises sperm function by causing oxidative damage to proteins, lipids, and nucleic acids **(Aitken, 2017).** Spermatozoa are particularly prone to oxidative damage because of the high concentration

of PUFAs (polyunsaturated fatty acids) in the plasma membrane and the absence of strong antioxidant mechanisms that may protect them from the excessive production of ROS **(Zini *et al.,* 2010).**

Plant extracts have recently become a readily available and natural source of supplements to maintain and improve sperm function. The majority of plant species are high in antioxidants, which can act as ROS scavengers to reduce the deleterious effects of oxidative stress on sperm function **(José and Eliana, 2021).** The extracts are often added to the semen extender and utilized for both cryopreservation and refrigerated storage of sperm. All stages of the sperm cryopreservation process, including pre-freezing cooling/equilibration and post-thawing incubation, can integrate these substances into the diluents. Overall, these natural substances can enhance sperm parameters and fertility; in addition, some plant species can provide defence against harmful germs and serve as ROS scavengers **(José and Eliana, 2021).**

The plant known as Aloe vera (Aloe barbadensis Miller) is a member of the Liliaceae family. This plant is commonly farmed in hot and dry regions in many countries **(Choi and Chung, 2003).** It has stiff grey-green lance-shaped leaves with a clear gel in the core of a mucilaginous pulp **(Rajasekaran *et al.,* 2005).** The aloe vera plant is widely used as a therapeutic and medical agent due to its anti-inflammatory, antimicrobial, anticancer, wound healing, neuroprotective, anti-diabetic, and antioxidant properties **(Kedarnath *et al.,* 2013; Gabriel *et al.,* 2015; Hashemi *et al.,* 2015; Guven *et al.,* 2016; Yuksel *et al.,* 2016).** Sugars, saponins, carotenoids, flavonoids, tannins, anthraquinone, steroids, vitamins, minerals, enzymes, polysaccharides, alkaloids, phenolic compounds, phenols, and organic acids are abundant in aloe vera **(Lopez-Cervantes *et al.,* 2018; Quispe *et al.,* 2018).**

More than 200 different physiologically active components, including vitamins, minerals, enzymes, sugars, anthraquinones or phenolic compounds, lignin, saponins, sterols, amino acids, and salicylic acid, were found in aloe vera, according to **Chauhan *et al.,* (2014)** analysis of the plant's chemistry. The presence of polysaccharides such as pectin, hemicellulose, glucomannan, and acemannan in such components is noted; the bulk of these polysaccharides are glucose and mannose derivatives **(Leung *et al.,* 2004).** These sugars are recognized as significant sperm energy sources **(King *et al.,* 2006).**

When spermatozoa come into contact with the seminal plasma, in the oviduct, or *in vitro*, when cultured in appropriate media, the process of sperm capacitation occurs, involving the removal and change of chemicals that stabilize the sperm membrane **(Andrielle Thainar *et al.,* 2017).** A multitude of characteristics, including capacitating medium and incubation times, influence sperm *in vitro* capacitation. As a result, these variables, according to **Abd-Allah *et al.,* (2017),** would influence motility, viability, and acrosome reaction rates. In this study, we look at the biological effects of aloe vera on the *in vitro* capacitation of bovine spermatozoa without using animals.

MATERIALS AND METHODS

1.Ethical approval

This protocol was approved by Institutional Animal Care and Use Committee, Beni-Suef University (BSU-IACUC) with approval number (002-361).

2. Chemicals and reagents

All reagents and Media were purchased from Sigma-Aldrich (Sant Louis, USA). Chemicals were provided by Dream CO. (Egypt) including:

A- Sodium Chloride extra pure (NaCl) (S-08037).
B- Calcium Chloride Dihydrate extra pure (CaCl2.2H2O) (C-02136).
C- Potassium Chloride (KCL) (90499-500G).
D- Sodium Phosphate Monobasic Di-hydrate extra pure (Sodium Di-hydrogen Orthophosphate) (NaH2PO4) (S-08272).
E- Magnesium Chloride Hexa-hydrate extra pure (MgCl2.6H20) (M-05545).
F- Phenol red indicator (pH indicator) (pH 6.4-8.2 Yellow to Red) (P-06745).
G- Sodium bicarbonate extra pure (Sodium hydrogen carbonate) (NaHCO3) (S-07990).
H- Sodium Lactate 60% solution extra pure (C3H5NaO3) (S-08165, India).
I- Bovine Serum Albumen (BSA) (A-00395).
J- Sodium Pyruvate (C3H3NaO3) (S068110, New Delhi).
K- Ethanol Labchem 70% (C2H5OH).

3. Preparation of aloe vera raw extract

Fresh aloe vera leaves ranging in length from 15 to 25 cm were rinsed in clean water. The fresh leaves were cut into pieces transversely. To avoid gel contamination, the filtrate of aloe vera extract was manually collected after homogenizing the solid gel in the leaf's center. The clear filtrate was kept at 20°C until use **(Subbiah *et al.,* 2005).**

4. Preparation of different concentrations of aloe vera

Aloe Vera was added in two concentrations: 5 µg/ml semen and 10 µg/ml semen.

5. In vitro sperm capacitation

According to **Parrish *et al.,* (1986),** motile spermatozoa were distinguished using the swim-up

technique. Straws of frozen Holstein semen were obtained from the artificial insemination facility in the Beni-Suef Governorate of Egypt. Two semen straws were used in each trial, and the straws were defrosted in a 37°C water bath for 30 seconds. One millilitre of Sperm-TALP medium was prepared in each of the first three sterile tubes, and one millilitre of Sperm-TALP medium without heparin was created in the fourth (negative control). To get the final concentration (5 µg/ml semen), 1.25 µl of aloe vera extract was added to one Eppendorf tube with the post-thawed semen (0.25 ml), and 2.5 µl of aloe vera extract was added to the other Eppendorf tube with the post-thawed semen (0.25 ml) to get the final concentration (10 µg/ml semen).

The four prepared tubes were admitted to four treatments: 50µl of frozen-thawed semen was layered under 1 ml of the medium in tube 1 (positive control; no aloe vera). 50µl of frozen-thawed semen (semen + 1.25µl of aloe vera extract) was layered under 1 ml of the medium in tube 2, 50µl of frozen-thawed semen (semen+ 2.5µl of aloe vera extract) was layered under 1 ml of the medium in tube 3, and 50µl of frozen-thawed semen (semen+ 1.25µl of aloe vera extract) was layered under 1 ml of the medium in tube 4 (negative control; heparin free). All 4 tubes were incubated for 30, 60, and 90 minutes at 39°C and 5% CO2 tension in an inclined position (45-degree angle) in a CO2 incubator. After each incubation period, 200 µl of the uppermost supernatant of each tube (that contains highly motile spermatozoa) was pooled into a centrifuge tube. The 4 tubes were centrifuged at 1800 rpm for 10 minutes, later, the pellet was re-suspended in 1 ml of fertilization medium (F-TALP: Tyrode's albumin-lactate-pyruvate media) to prepare sperm suspension **(Waleed *et al.*, 2018).**

6. Assessment of sperm capacitation

During varied incubation periods, all fractions were examined for the following criteria.

6.1. Assessment of sperm viability

According to **Kitiyanant *et al.*, (2002)**, the trypan blue exclusion method was employed to examine the viability of bovine spermatozoa. It is done by mixing 100 µl of sperm suspension (10^8 cells/ml) with an equivalent volume of Trypan blue 2% and incubating the mixture for 15 minutes at 390 °C.The proportion of viable sperm was measured using a hemocytometer.

The spermatozoa were divided into two groups: live (sperm without color) and dead (sperm with blue color). The percentage of cells was calculated by counting all of the cells at random in the various fields. A minimum of 200 spermatozoa were examined for viability.

6.2. Assessment of the hyper-activation motility (HAM)

Measurements of hyper-activation motility (HAM) were carried out using a modified version of the **Fujinoki *et al.*, (2006)** approach. At 30, 60, and 90 minutes of incubation, it was noticed. The observation was carried out at 37^0C for 2 minutes, and the total number of motile and hyper-activated spermatozoa as well as their counts in each of the 10 fields, were manually counted. A motile tail was observed in normal motile sperm. Following motility activation, the sperm head and flagella begin to beat almost symmetrically, causing the sperm to move along nearly linear trajectories. The spermatozoa pump their tails harder, asymmetrically, and with significant motions in HAM, which is characterized by asymmetrical beating of the flagellum (Fig.3).

6.3. Assessment of acrosome reaction.

The acrosome reaction was assessed using the Coomassie Blue G staining. Sperm cells were fixed in 4% paraformaldehyde for 10 minutes before being centrifuged twice in ammonium acetate at 320 x g for 8 minutes. A smear was made and stained with 0.5% Coomassie Blue (G-250) for 2-3 minutes **(Larson and Miller, 1999).** A total of 100 sperms were examined under a light microscope (x400). Acrosome-intact sperm colored darkly in the apical region of the sperm head while Acrosome-reacted sperm showed very faint or no staining in the acrosome area.

7. The *in vitro* fertilization ability of spermatozoa

7.1. Ovaries collection and transportation

About 150 ovaries were collected totally from apparently Sound cows from the Belefia abattoir and transported to the laboratory According to the procedure of **Abdel-Halim, (2018).**

7.2. . Oocytes recovery

According to **Mogas *et al.*, (1992),** the immature COCs were extracted from the ovarian stroma of small and medium-sized follicles (3-8 mm) using the aspiration technique, and according to **Combelles and Albertini (2003) and Abd-Allah (2009),** they were classified under a stereomicroscope according to their morphological criteria into three grades.

7.3. . COCs in vitro maturation

Grades I and II were chosen for in vitro maturation, with Grade III being excluded, and the in vitro maturation phases (IVM) were carried out per the protocol of **Yuanyuan *et al.*, (2020),** with some modifications.

7.4. Assessment of cumulus cells expansion

After 22–24 hours of incubation, the maturation was assessed using a stereomicroscope following the enlargement of the cumulus cells, and the oocytes were divided into three degrees, as stated by **Abd-Allah, (2009).** IVF was performed by randomly dividing IVM oocytes into four groups. A hemocytometer was used to determine the sperm concentration. Aliquots of IVF-TALP with approximately $2_X 10^6$ motile sperm/ml (4000 sperm cells/droplet) were then added to droplets of IVF media with 10–12 mature COCs **(Ward *et al.*, 2003).** The inseminated oocytes were cultured in a CO2 incubator for 24-36 hours, and the formation of a second polar body in a peripheral location served as a marker for fertilization. This was followed by oocyte cleavage up to the level of two cell stages, which served as confirmation **(Hensleigh and Hunter, 1985).** The experiment was replicated 16 times. Throughout 16 trials, a total of 640–760 oocytes were inseminated (160–192 oocytes for each group).

8. Statistical analysis

The data were analyzed using the IMB SPSS version 22 statistical software **(SPSS, 2011).** The differences in progressive motility (PM), hyperactivation motility (HAM), and acrosome reaction (AR) among the different aloe vera concentrations at different incubation durations were analysed using a two-way ANOVA followed by a Fischer's least significant difference (LSD) for multiple comparisons. A one-way ANOVA was used to compare the differences in in vitro fertilization parameters across treatments. The results were given as mean values for each set of data ± standard deviation of the mean, and the level of statistical significance in all analyses was defined at a probability level of $P<0.05$**.**

RESULTS

1. *In vitro* sperm capacitation

1.I. Effects of aloe vera concentrations on the progressive motility (PM) of bovine spermatozoa at different incubation times

Sperm progressive motility% was analyzed at different incubation durations (30, 60, and 90 minutes) after aloe vera supplementation. The overall percentage of spermatozoa with progressive motility had declined across all groups by time progressing with a significantly lower value (1.06 ± 0.92); mean ±SD was found at the aloe vera concentration of (10 µg/ml when compared with other treatments as seen in table (1). So the concentration of 10 µg/ml is the best concentration for sperm capacitation, as the decline in progressive motility% changed to HAM.

Table.1. Effect of supplementation of different concentrations of aloe vera and different incubation times on PM of bull spermatozoa.

Dependent Variable: PM				
Incubation Time	Aloe Vera Treatment	Mean ± SDM	95% Confidence Interval	
			Lower Bound	Upper Bound
Zero minute	Positive Control	57.19 ± 1.97 [a]	56.15	58.22
	5 µg /ml	57.37 ± 2.02 [a]	56.33	58.41
	10 µg /ml	57.31 ± 1.58 [a]	56.27	58.35
	Negative Control	57.37 ± 2.02 [a]	56.33	58.41
30 Minutes	Positive Control	47.19 ± 2.19 [a]	46.15	48.22
	5 µg /ml	41.50 ± 1.46 [b]	40.462	42.53
	10 µg /ml	37.50 ± 2.19 [c]	36.46	38.53
	Negative Control	50.81 ± 2.13 [d]	49.77	51.85
60 Minutes	Positive Control	28.44 ± 2.96 [a]	27.40	29.47
	5 µg /ml	17.06 ± 2.14 [b]	16.02	18.10
	10 µg /ml	9.19 ± 2.10 [c]	8.15	10.22
	Negative Control	38.44 ± 2.15 [d]	37.40	39.47
90 Minutes	Positive Control	25.37 ± 2.55 [a]	24.33	26.41
	5 µg /ml	10.13 ± 2.44 [b]	9.08	11.16
	10 µg /ml	1.06 ± 0.92 [c]	0.02	2.10
	Negative Control	37.44 ± 2.06 [d]	36.40	38.47

1.2. Effects of aloe vera concentrations on the hyperactivated motility (HAM) and acrosome reaction (AR) of bovine spermatozoa at different incubation times

Different stages of the capacitation processes of spermatozoa were analyzed at different incubation durations (30, 60, and 90 minutes). Most of the spermatozoa remained non-hyperactivated in all groups (0.00±.00) at zero minutes. With time progression (at 30 and 60 minutes of incubation), the percentage of hyperactivated spermatozoa increased with a significantly higher value (28.31 ± 2.30); mean ±SD was recorded at the aloe vera concentration of (10 µg /ml) and incubation time of (60 minutes) when compared with other treatments (Fig.1).

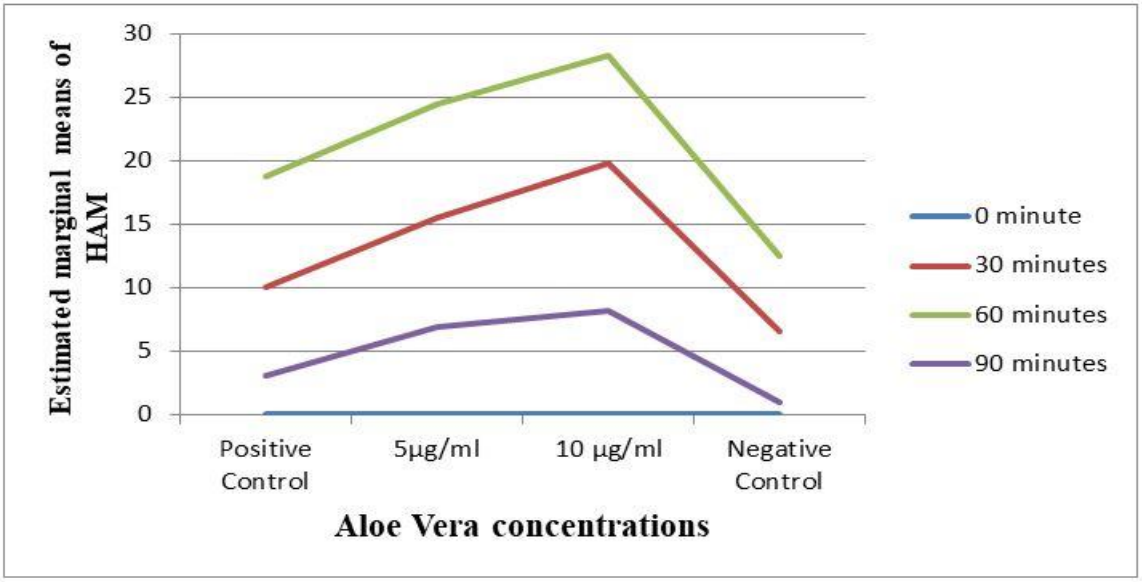

Fig.1: Differences in HAM at different incubation times among different aloe vera concentrations

In the case of acrosome reaction, at the start of incubation, most spermatozoa in all groups were acrosome intact (0.00±0.00). By increasing the time of incubation, the percentage of acrosome-reacted spermatozoa increased with a significantly higher value (33.81 ± 2.16); mean ±SD was found at the aloe vera concentration of (10 µg /ml) and incubation time of (60 minutes) when compared with other groups as seen in Fig. (2). Long incubation for 90 minutes had a bad effect on sperm hyperactivation and acrosome reaction.

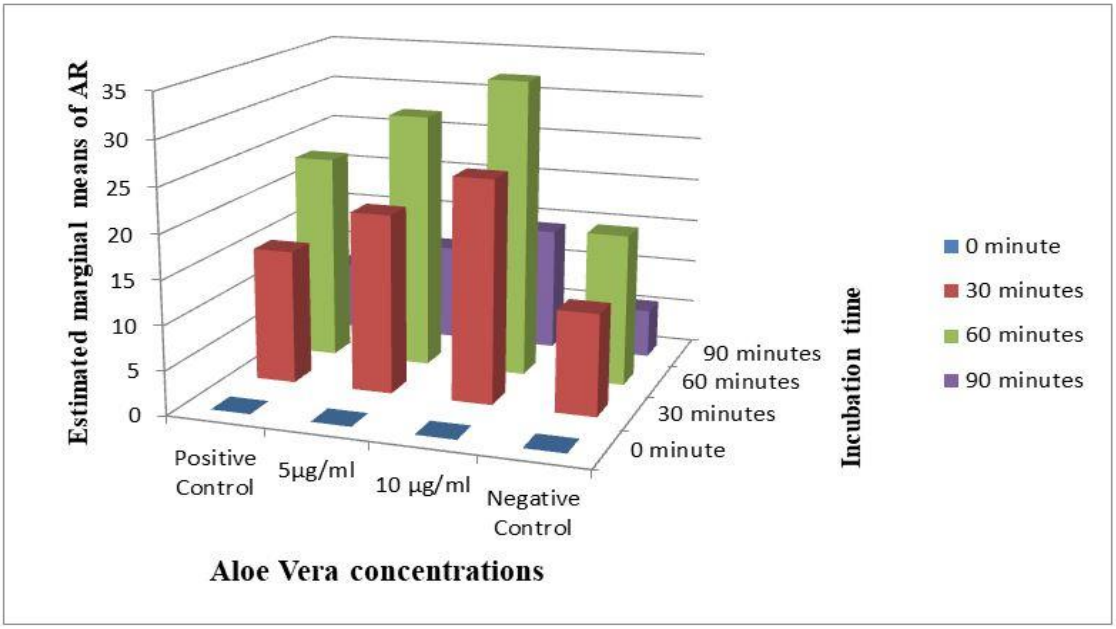

Fig.2: Differences in AR at different incubation times among different aloe vera concentrations

2. *In vitro* fertilization

The current study found that treatment of spermatozoa with aloe vera before fertilization was beneficial for fertilization. It showed that aloe vera concentration of (10 µg/ml) had a significant impact on the number of viable zygotes, with the highest value obtained (8.50 ± 2.36) when compared to other treatments. Concerning the effect of aloe vera on the development rate, it was found that the addition of aloe vera (10 µg/ml) resulted in a significantly higher value for cultured zygotes with male and female pronuclei, second polar body, and oocytes cleaved to two cell stages (0.50 ± 0.81; 3.94 ± 2.67; 0.31 ± 0.60) respectively than other groups. Furthermore, The data also revealed that aloe vera has a substantial influence on the number of non-developed (degenerated) zygotes, with the lowest value (3 ± 1.26) seen at aloe vera concentration of (10 µg /ml) when compared to other treatments. Table (2) summarized the outcomes of *in vitro* fertilization based on the results of hyper-activated motility (HAM) and acrosome reaction (AR) of spermatozoa treated with aloe vera extract.

Table 2: Effect of supplementation of different concentrations of aloe vera in capacitation media on *in vitro* fertilization parameters of bovine oocytes

IVF Parameters	Aloe Vera Treatment	Mean ± SDM	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
Trypan blue exclusion test (Live Zygotes)	Positive Control	2.75 ± 1.34 ^{a}	2.04	3.46
	5 µg/ml	6.63 ± 2.27 ^{b}	5.41	7.84
	10 µg/ml	8.50 ± 2.36 ^{c}	7.24	9.76
	Negative control	1.06 ± 0.85 ^{d}	0.61	1.52
NO. of Zygotes Cultured	Positive Control	2.75 ± 1.34 ^{a}	2.04	3.46
	5 µg/ml	6.63 ± 2.27 ^{b}	5.41	7.84
	10 µg/ml	8.50 ± 2.36 ^{c}	7.24	9.76
	Negative control	1.06 ± 0.85 ^{d}	0.61	1.52
NO. of Zygotes Developed With 2PN	Positive Control	0.06 ± 0.25 ^{a}	-0.07	0.20
	5 µg/ml	0.13 ± 0.34 ^{a}	-0.06	0.31
	10 µg/ml	0.50 ± 0.81 ^{b}	0.06	0.94
	Negative control	0.00 ± 0.00 ^{a}	0.00	0.00
NO. of Zygotes Developed With 2nd Pb	Positive Control	0.81 ± 0.91 ^{ac}	0.33	1.30
	5 µg/ml	1.69 ± 1.35 ^{a}	0.97	2.41
	10 µg/ml	3.94 ± 2.67 ^{b}	2.51	5.36
	Negative control	0.31 ± 0.47 ^{c}	0.06	0.57
NO. of Zygotes Developed With 2 cell stage	Positive Control	0.00 ± 0.00 ^{a}	0.00	0.00
	5 µg/ml	0.13 ± 0.34 ^{ab}	-0.06	0.31
	10 µg/ml	0.31 ± 0.60 ^{b}	-0.01	0.63
	Negative control	0.00 ± 0.00 ^{a}	0.00	0.00
Non-Developed Zygotes	Positive Control	8.75 ± 1.88 ^{a}	7.75	9.75
	5 µg/ml	4.88 ± 1.45 ^{b}	4.10	5.65
	10 µg/ml	3 ± 1.26 ^{c}	2.33	3.67
	Negative control	10.44 ± 2.15 ^{d}	9.29	11.59
Non-Identified Zygotes	Positive Control	1.88 ± 0.88 ^{a}	1.40	2.35
	5 µg/ml	4.69 ± 1.58 ^{b}	3.85	5.53
	10 µg/ml	3.75 ± 1.61 ^{c}	2.89	4.61
	Negative control	0.75 ± 0.77 ^{d}	0.34	1.16

Data are expressed as means ± SDM.

Values with different superscript letters in the same parameter are significantly differing at $P < 0.05$.

2nd PB = second polar bodies; 2 PN = two pronuclei; 2 cell = fertilized oocytes (2 cell stage).

Non-Developed zygotes = degenerated; Non-Identified zygotes = Alive zygotes but their development is unclear to me.

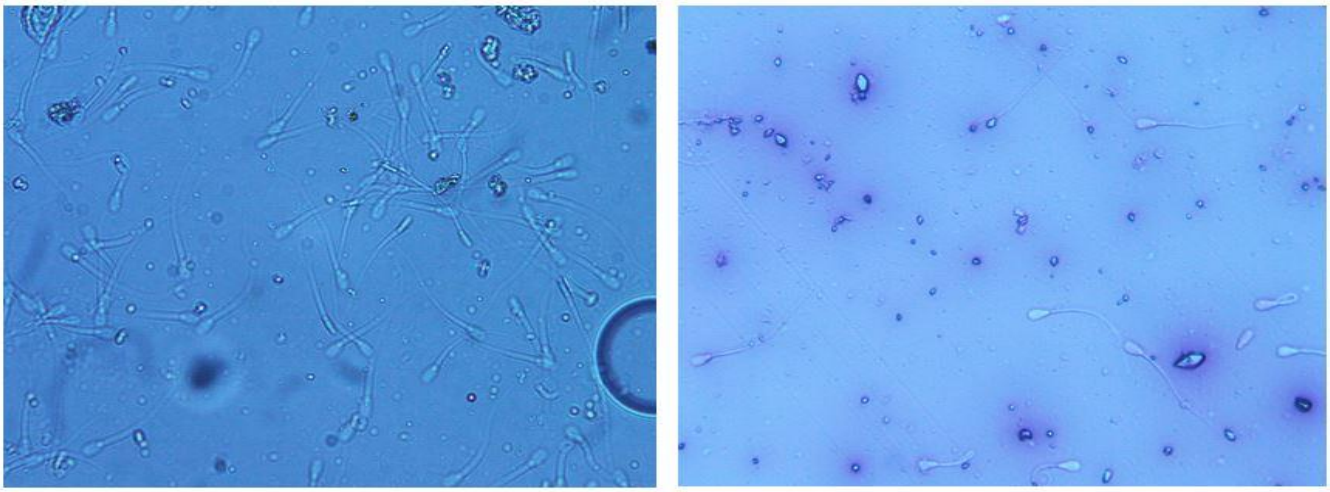

Fig.3: Capacitated bovine spermatozoa, notice the Hyper-activated motile bovine spermatozoa under microscope. Hyper activated sperm (open arrows) shows asymmetrical flagellate bends and with large bent tail while Non-hyper activated sperm (filled arrows) display a straight tail (x 400).

DISCUSSION

Numerous studies have focused on improving sperm quality by *in vitro* treatment of frozen-thawed semen with antioxidants that induce their positive effect on sperm parameters by suppressing ROS formation and reducing the detrimental effects of OS, resulting in increased sperm motility, viability, capacitation, and acrosome reaction **(Garagozloo and Atiken, 2011; Pinto *et al.*, 2020).** It is thought that mild and low OS induce hyperactivation, motility, and capacitation via increased tyrosine phosphorylation, hence promoting fertilizing potential **(Lamirandee *et al.*, 1997).**

Aloe Vera is a medicinal plant that can be used as a natural antioxidant, according to studies conducted in vivo and *in vitro*, as it has a strong potential to reduce oxidative stress and fat oxidation **(Taukoorah and Fawzi, 2016; Sacan *et al.*, 2017; Behmanesh *et al.*, 2018*).*** Aloe vera extracts are shown to reduce lipid peroxidation and DNA fragmentation by free radicals in a different study conducted by **Debnath *et al.*, (2018). Zareie *et al.*, (2021)** stated that the antioxidant activity of this plant depended on its total phenolic content. The integrity and fertility of semen are significantly influenced by vitamin C in aloe vera gel, which accounts for 65% of seminal plasma's overall antioxidant capacity. As a result, Aloe Vera's antioxidant properties enable it to protect spermatozoa from free radicals **(Naeem *et al.*, 2021).** To the best of our knowledge, no research has been done to investigate the role of aloe vera addition on the capacitation and acrosome reaction of frozen-thawed bovine spermatozoa.

In this study, supplementation of the capacitation medium with different aloe vera concentrations significantly enhanced hyperactivation and acrosome reaction as well as the fertilizing capacity of frozen-thawed semen, but the greatest values were observed in the case of frozen-thawed semen treated with 10µg/ml Aloe Vera concentration and incubated for 60 minutes for HAM and AR, and consequently, IVF parameters when compared with the other different concentrations and incubation periods, while progressive motility significantly decreased with incubation time progression. The positive effects of aloe vera were clarified by improving sperm functions and a change in sperm motility pattern from progressive to HAM, which is a movement pattern characterised by asymmetrical flagellar beating (**Mortimer and Mortimer, 1990; Demott and Suarez, 1992)** and may be critical to fertilization success **(Ho and Suarez, 2001).** It is unknown exactly how aloe vera extract improves sperm quality at this time. It has been reported that this plant contains folic acid and zinc, both of which function as antioxidants and can improve the quality of sperm by lowering semen apoptosis **(Pfeifer *et al.*, 2001; Shahraki *et al.*, 2014).**

Ghotbabadi *et al.*, (2012) and **Quispe *et al.*, (2018)** have also shown that aloe vera extract contains phenols. Phenolic compounds are widely distributed in plants and have the direct ability to remove free radicals. As a result, the antioxidant properties of aloe vera's phenolic components may be responsible for the improvement in sperm quality. Phenolic antioxidants function as metal chelators or free radical terminators **(Shahidi *et al.*, 1992).** The antioxidant characteristics of phenolic compounds can be attributed to their capacity to donate hydrogen ions **(Wojdyło *et al.*, 2007).** Additionally, it contains a variety of polysaccharides **(Leung *et al.*, 2004)**,

which, in addition to providing energy, are known to have certain extracellular cryoprotective properties and support the stability of the sperm membrane **(Hu *et al.*, 2009).**

The findings of our study come to a consensus with the results of the study conducted by **Zareie *et al.*, (2021),** who reported a significant improvement in total motility, progressive motility, and membrane integrity when using 20 µg/ml of aloe vera extract in the extender compared to control treatment. Additionally, according to **Yong *et al.*, (2017),** the use of aloe vera as a cryoprotectant for freezing fish semen improved sperm viability and motility. Besides, **Jasem and Nasim (2011)** found that aloe vera can promote male fertility by improving sperm quality and may be useful in the development of medications to boost male fertility. Furthermore, our results coincide with **Fakhrildin and Sodani (2014)** observation that the antioxidative phenolic compound in aloe vera has a positive effect on sperm parameters, including a highly significant rise in the percentage of sperm motility, viability, concentration, agglutination, normal sperm morphology, and hypo-osmotic swelling test results (HOS test).

In line with our results, **Padamveer *et al.*, (2020)** showed a significant difference in post-thaw % progressive motility, living spermatozoa, acrosomal integrity, sperm abnormalities, and (HOST) positive spermatozoa between the control and aloe vera groups.

On the contrary, our findings contradict **Mina, (2019)** findings that aloe vera leaf extract inhibits animal sperm motility. This disagreement could be related to the differing conditions of experimentation.

CONCLUSION

Based on the findings of this study, it can be concluded that bovine spermatozoa incubated with Aloe Vera raw extract (10µg/ml) for 60 minutes showed increased sperm hyper-motility and acrosome reaction when compared to other treatments, positive and negative controls, and produced the highest percentage of fertilized oocytes. This could be related to aloe vera's ability to increase sperm viability parameters. So this study is considered a promise for dissolving many problems associated with IVF due to the bad quality of the sperm.

Competing interes

There is no conflict of interests of any sort between authors or elsewhere.

REFERENCES

ABD-ALLAH, S.M. 2009. Successful cryopreservation of buffalo ovaries using in situ oocyte cryopreservation. Veterinaria Italiana, 45(4), 507-512.

ABD-ALLAH, S.M., RALAN, M., SULIMAN, H., ESSAM, T., and SALEM, H.F., 2017. Preparation of progesterone nanoparticles and evaluation of its effect on the capacitation of Bovine spermatozoa used in the in Vitro Fertilization. International Journal of Drug Delivery, 9(4), 125-131. http://www.arjournals.org/index.php/ijdd/article/view/2224/pdf.

ABDEL-HALIM, B.R. 2018. Protective effect of Chitosan nanoparticles against the inhibitory effect of linoleic acid supplementation on maturation and developmental competence of bovine oocytes. Theriogenology, 114,143-148. https://doi.org/10.1016/j.theriogenology.2018.03.032

AITKEN, R.J. 2017. Reactive oxygen species as mediators of sperm capacitation and pathological damage. Molecular Reproduction and Development, 84, 1039-1052. https://doi.org/10.1002/mrd.22871

ANDRIELLE THAINAR MENDES CUNHA, FARIA, O.A.C., and GUIMARÃES, A.L.S., 2017. Bovine Sperm Capacitation: Physiological Changes and Evaluations. JSM In vitro Fertilization, 2(1), 1011. https://www.jscimedcentral.com/jounal-article-info/JSM-Invitro-Fertilization/Bovine-Sperm-Capacitation%3A-Physiological-Changes-and-Evaluations--6007#

Behmanesh, M.A., Najafzadehvarzi, H., and Poormoosavi, S.M., 2018. Protective Effect of Aloe Vera Extract against Bisphenol A Induced Testicular Toxicity in Wistar Rats. Cell Journal (Yakhteh), 2, 278-283. https://doi.org/10.22074%2Fcellj.2018.5256

CHAUHAN, S., GUPTA, K.C., and AGRAWAL, M., 2014. Application of Biodegradable Aloe Vera gel to control post-harvest decay and longer the shelf life of Grapes. International Journal of Current Microbiology and Applied Sciences, 3,632-642. http://www.ijcmas.com/

CHOI, S., and CHUNG, M.H., 2003. A review on the relationship between Aloe Vera components and their biologic effects. In Semin integ Med, 1, 53-62. https://doi.org/10.1016/S1543-1150(03)00005-X

COMBELLES, C., and ALBERTINI, D., 2003. Assessment of oocyte quality following repeated gonadotropin stimulation in the mouse. Biology of Reproduction, 68,812-821. https://doi.org/10.1095/biolreprod.102.008656

DE LAMIRANDE, E., LECLERC, P., and GAGNON, C., 1997. Capacitation as a regulatory event that primes spermatozoa for the acrosome reaction and fertilization. Molecular Human Reproduction, 3,175-94. https://doi.org/10.1093/molehr/3.3.175

DEBNATH, T., GHOSH, M., LEE, Y.M., NATH, N.C.D., LEE, K.G., and LIM, B.O., 2018. Identification of phenolic constituents and antioxidant activity of Aloe barbadensis flower extracts. Food and Agricultural Immunology, 29, 27-38. https://doi.org/10.1080/09540105.2017.1358254

DEMOTT, R.P., and SUAREZ, S.S., 1992. Hyper activated sperm progress in the mouse oviduct. Biology of Reproduction, 46, 779-85. https://doi.org/10.1095/biolreprod46.5.779

FAKHRILDIN, M.B. and SODANI, F.I.J. 2014. Effect of Aloe Vera on its in vitro human sperm parameters for asthenozoospermic patients. Journal of Thi-Qar Science, 5(1), 8-13. https://www.researchgate.net/publication/318795994_Effect_of_Aloe_vera_extracts_on_in_vitro_human_sperm_parameters_for_asthenozoospermic_patients

FUJINOKI, M., SUZUKI, T., TAKAYAMA, T., SHIBAHARA, H., and OHTAKE, H., 2006. Profiling of proteins phosphorylated or dephosphorylated during hyper activation via activation on hamster spermatozoa. Reproductive Medicine and Biology, 5(2), 123-135. https://doi.org/10.1007/BF03016148

GABRIEL, N.N., QIANG, J., MA, X.Y., HE, J., XU, P., and LIU, K., 2015. Dietary Aloe Vera improves plasma lipid profile, antioxidant, and hepatoprotective enzyme activities in GIFT-tilapia (Oreochromis niloticus) after Streptococcus iniae challenge. Fish Physio Bio, 41, 1321-1332. https://doi.org/10.1007/s10695-015-0088-z

GARAGOZLOO, P., and AITKEN, R.J., 2011. The role of sperm oxidative stress in male infertility and the significance of oral antioxidant therapy. Human Reproduction, 26, 1628-40. https://doi.org/10.1093/humrep/der132

GHOTBABADI, F.S., ALIZADEH, A., ZADEHBAGHERI, M., KAMELMANESH, M.M., and SHAABANI, M., 2012. Phytochemical composition of the essential oil, total phenolic content, antioxidant and antimicrobial activity in Iranian Satureja sahendica Bornm. At different ontogenesis conditions. Journal of Medicinal Plants Research, 6, 3525-3534. http://dx.doi.org/10.5897/JMPR11.374

GUVEN, M., GOLGE, U.H., ASLAN, E., SEHITOGLU, M.H., ARAS, A.B., AKMAN, T., and COSAR, M., 2016. The effect of aloe Vera on ischemia—Reperfusion injury of sciatic nerve in rats. Biomed Pharma, 79, 201-207. https://doi.org/10.1016/j.biopha.2016.02.023

HASHEMI, S.A., MADANI, S.A., and ABEDIANKENARI, S., 2015. The review on properties of Aloe Vera in healing of cutaneous wounds. BioMed Research International, 2015, 714216. https://doi.org/10.1155/2015/714216

HENSLEIGH, H.C., and HUNTER, A.G., 1985. IVM of bovine cumulus enclosed primary oocytes and their subsequent IVF and cleavage. Journal of Dairy Science, 68, 1456-1462. https://doi.org/10.3168/jds.S0022-0302(85)80983-8

HO, H.C., and SUAREZ, S.S., 2001. Hyper activation of mammalian spermatozoa: function and regulation. Reproduction, 122,519-26. https://doi.org/10.1530/rep.0.1220519

HU, J.H., LI, Q.W., ZHANG, T., and JIANG, Z.L., 2009. Effect of Gynostemma Pentaphyllum Polysaccharide on boar spermatozoa quality following freezing-thawing. Cryobiology, 59, 244–249. https://doi.org/10.1016/j.cryobiol.2009.04.008

JASEM, E., and NASIM, J., 2011. Spermatogenic activity Aloe Vera in adult male rat. Pharmacology online, 2, 886-889. https://www.researchgate.net/publication/265152041_Spermatogenic_activity_of_Aloe_vera_in_adult_male_rats

JOSÉ LUIS ROS-SANTAELLA and ELIANA PINTUS., 2021. Plant Extracts as Alternative Additives for Sperm Preservation. Antioxidants, 10, 772.https://doi.org/10.3390/antiox10050772

KEDARNATH KAMBLE KAVERI, M., CHIMKOD VISHWANATH, B., and PATIL, C.S., 2013. Antimicrobial Activity of Aloe Vera Leaf Extract. International Journal of Applied Biology and Pharmaceutical Technology, 4, 286.

KING, S.S., SPEISER, S.A., JONES, K.L., APGAR, G.A., and WESSELS, S.E., 2006. Equine spermatozoal motility and fertility associated with the incorporation of d-(+)-mannose into semen extender. Theriogenology, 65, 1171–1179. https://doi.org/10.1016/j.theriogenology.2005.08.002

KITIYANANT, Y., CHAISALEE, B., and PAVASUTHIPAISIT, K., 2002. Evaluation of the acrosome reaction and viability in buffalo spermatozoa using two staining methods: the effects of heparin and calcium ionophore A23187. International Journal of Andrology, 25(4), 215-222. https://doi.org/10.1046/j.1365-2605.2002.00350.x

LARSON, J.L., and MILLER, D.J., 1999. Simple histochemical stain for acrosomes on sperm from several species. Molecular Reproduction and Development, 52(4), 445-449. https://doi.org/10.1002/(SICI)1098-2795(199904)52:4%3C445::AID-MRD14%3E3.0.CO;2-6

LEUNG, M.Y.K., LIU, C., ZHU, L.F., HUI, B., and FUNG, K.P., 2004. Chemical and biological characterization of a polysaccharide biological response modifier from Aloe Vera L. var chinensis (Haw) Berg. Glycobiology, 14, 501-510. https://doi.org/10.1093/glycob/cwh050

LOPEZ-CERVANTES, J., SÁNCHEZ-MACHADO, D.I., CRUZ-FLORES, P., MARISCALDOMÍNGUEZ, M.F., DE LA MORA-LÓPEZ, G.S., and CAMPAS-BAYPOLI, O.N., 2018. Antioxidant capacity, proximate composition, and lipid constituents of Aloe Vera flowers. Journal of Applied Research on Medicinal and Aromatic plant, 10, 93-98. https://doi.org/10.1016/j.jarmap.2018.02.004

MINA MAHMOODI POUR, 2019. In vitro Spermicidal Effects of Hydroalcoholic Crude Aloe Vera Leaf Extract on Animal Sperm Motility. International Conference on Biomedical Sciences (ICBMS19), ISBN 978-600-98459-5-8. https://www.greennetwork-events.com/all_images/file_193.pdf

MOGAS, T., MARTINO, A., PALOMO, M.J., and PARAMIO, M.T., 1992. Effect of method of recovery on the number and type of oocytes obtained for IVM. Journal of Reproduction and Fertility, 9, 53.

Mortimer, S.T., and Mortimer, D., 1990. Kinematics of human spermatozoa incubated under capacitating conditions. Journal of Andrology, 11, 195-203. https://doi.org/10.1002/j.1939-4640.1990.tb03228.x

NAEEM ERFANI MAJD, MOHAMMAD REZA TABANDEH, SHIMA HOSSEINIFAR, and MAHIN SADEGHI, 2021. Protective Effect of Aloe Vera Gel against Cisplatin-Induced Testicular Damage, Sperm Alteration and Oxidative Stress in Rats. International Journal of Fertility and Sterility, 15 (3), 210-218. https://doi.org/10.22074/ijfs.2020.134691

PADAMVEER SINGH, SANJAY AGARWAL, HARJYOTE SINGH, PAWAN KUMAR VERMA, A. K. and PANDEY SHARAD KUMAR., 2020. Antioxidant Effects of Aloe Vera as Semen Additive in Cryopreservation of Cattle Bull Semen. International Journal of Current Microbiology and Applied Sciences, 9(9), 1625-1635. https://doi.org/10.20546/ijcmas.2020.909.202

PARRISH, J.J., SUSKO-PARRISH, J.L., LIEBFRIED-RUTLEDGE, L., CRISTER, E.S., EYESTONE, W.H., and FRIST, N.L., 1986. Bovine in vitro fertilization with frozen-thawed semen. Theriogenology, 25, 591- 600. https://doi.org/10.1016/0093-691X(86)90143-3

PFEIFER, H., CONRAD, M., ROETHLEIN, D., KYRIAKOPOULOS, A., BRIELMEIER, M., BORNKAMM, G.W., and BEHNE, D., 2001. Identification of a specific sperm nuclei selenoenzyme necessary for protamine thiol cross-linking during sperm maturation. The FASEB Journal, 15, 1236-1238. https://doi.org/10.1096/fj.00-0655fje

PINTO, S.C.C., ALMEIDA, D.S., ALVES, M.B.R., FLOREZ-RODRIGUEZ, S.A., ABREU JÚNIOR, G.S., ALVES, N., CELEGHINI, E.C.C., LASKOKI, L.M., and SOUZA, F.A. 2020. Does supplementation of vitamin C, reduced glutathione or their association in semen extender reduce oxidative stress in bovine frozen semen? Arquivo Brasileiro de Medicina Veterinária e Zootecnia, *72*, 9–17. https://doi.org/10.1590/1678-4162-11293

QUISPE, C., VILLALOBOS, M., BORQUEZ, J., and SIMIRGIOTIS, M., 2018. Chemical Composition and Antioxidant Activity of Aloe Vera from the Pica Oasis (Tarapac´a, Chile) by UHPLC-Q/Orbitrap/MS/MS. Journal of Chemistry, 1-12. https://doi.org/10.1155/2018/6123850

RAJASEKARAN, S., SIVAGNANAM, K., and SUBRAMANIAN, S., 2005. Antioxidant effect of Aloe Vera gel extract in streptozotocin-induced diabetes in rat. Pharm Rep, 57, 90-96.

ROS-SANTAELLA, J.L., DOMÍNGUEZ-REBOLLEDO, A.E., and GARDE, J.J., 2014. Sperm flagellum volume determines freezability in red deer spermatozoa. PLoS ONE, 9, e112382. https://doi.org/10.1371/journal.pone.0112382

SACAN, O., AKEV, N., and YANARDAG, R., 2017. In vitro inhibitory effect of Aloe Vera (L.) Burm. f. leaf extracts on the activity of some enzymes and antioxidant activity. Indian Journal of Biochemistry and Biophysics, 54, 82-89. http://nopr.niscpr.res.in/handle/123456789/42894

SHAHIDI, F., WANASUNDARA, P.K., and JANITHA, P.D., 1992. Phenolic antioxidants. Critical Reviews in Food Science and Nutrition, 32, 67-103. https://doi.org/10.1080/10408399209527581

SHAHRAKI, A., SHAHKARI MOJAHED, A., and AFSHAR-GOLI, J., 2014. The effects of hydroalcoholic extract of Aloe Vera gel on spermatogenesis of adult male rats. International Journal of Biosciences, 5(7), 158-165. http://dx.doi.org/10.12692/ijb/5.7.158-8

SPSS. (2011). Statistical Package for Social Science. Computer Program. Version 22.

SUBBIAH, R., KARURAN, S., and SORIMUTHU, S., 2005. Antioxidant effect of Aloe Vera gel extract in streptozotocin induced diabetes in rats. Institute of Pharmacology Polish Academy of Sciences, 57, 90-96. https://pubmed.ncbi.nlm.nih.gov/15849382

TAUKOORAH, U., and Fawzi MAHOMOODALLY, M., 2016. Crude Aloe Vera Gel Shows Antioxidant Propensities and Inhibits Pancreatic Lipase and Glucose Movement in vitro. Journal of Advanced Biomedical and Pharmaceutical Sciences, 2016, 3720850. https://doi.org/10.1155/2016/3720850

WALEED, F.A. MAREI, WAEL A. KHALIL, ANIL, P.G. PUSHPAKUMARA, MOSTAFA, A. EL-HARAIRY, AHMED, M.A. ABO EL-ATTA, CLAIRE WATHESD, D. and ALI FOULADI-NASHTAE, 2018. Polyunsaturated fatty acids influence offspring sex ratio in cows. International Journal of Veterinary Science and Medicine, 6, S36-S40. https://doi.org/10.1016/j.ijvsm.2018.01.006

WARD, F., RIZOS, D., BOLAND, M.P., and LONERGAN, P., 2003. Effect of reducing sperm concentration during IVF on the ability to distinguish between bulls of high and low field fertility: work in progress. Theriogenology, 59, 1575-1584. https://doi.org/10.1016/S0093-691X(02)01202-5

WOJDYŁO, A., OSZMIAŃSKI, J., and CZEMERYS, R., 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chemistry, 105, 940-949. https://doi.org/10.1016/j.foodchem.2007.04.038

YONG, S.Y., NGUANG, S.I., and KARI, A., 2017. Comparison between the effect of egg yolk based extender and Aloe Vera (Aloe Barbadensis)-based extender on red tilapia (Oreochromis Niloticus) sperm quality. Journal of fundamental and applied sciences, 9, 813-820. http://dx.doi.org/10.4314/jfas.v9i2s

YUANYUAN HE, YILE WANG, HENGDE ZHANG, YONG ZHANG and FUSHENG QUAN, 2020. Alpha- lipoic acid improves the maturation and the developmental potential of goat oocytes in vitro. Reproduction in Domestic Animals, 56, 545-554. https://doi.org/10.1111/rda.13892

YUKSEL, Y., GUVEN, M., KAYMAZ, B., SEHITOGLU, M.H., ARAS, A.B., AKMAN, T., TOSU, M., and COSAR, M., 2016. Effects of aloe Vera on spinal cord Ischemia–Reperfusion injury of

rats. Journal of Investigate Surgery, 29, 389-398. https://doi.org/10.1080/08941939.2016.1178358

ZAREIE, K., FARSHAD, A., ROSTAMZADEH, J., AZIMI, G., and ARIYAN. F., 2021. Freezability of Goat Epididymal Sperm using Aloe Vera Extract and Trehalose in Diluents. Austin Journal of Veterinary Science & Animal Husbandry, 8(2), 1078. http://www.austinpublishinggroup.com/

ZINI, A., SAN GABRIEL, M., and LIBMAN, M., 2010. Lycopene supplementation in vitro can protect human sperm deoxyribonucleic acid from oxidative damage. Fertility and Sterility, 94, 1033–6. https://doi.org/10.1016/j.fertnstert.2009.04.004

How to cite this article:
Mona Mahmoud, Saber Abd-Allah, Abdel-Halim B.R., and Khalil A.A.Y., 2023. Assessment of Developmental Competence of *Holstein Bulls* Spermatozoa upon Addition of Aloe Vera Raw Extract during *In Vitro* Capacitation. Journal of Applied Veterinary Sciences, 8 (4): 43-53.
DOI: **https://dx.doi.org/10.21608/javs.2023.219422.1249**