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Article

Study the properties of ram semen preserved at 5°C for different periods after adding different levels of acai fruit extract.

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ABSTRACT
This study was conducted to know the effect of adding an aqueous extract of Acai fruit (Euterpe oleracea Martius) to the semen extender of Awassi rams on semen Parameters after storage at cooling. This study was carried out in the animal field and laboratories of the Department of Animal Production-College of Agricultural Engineering University of Baghdad from November 15, 2021, to April 25, 2022. Three local Awassi rams were used in this experiment. Their ages were between 2.5 - 3 years, and they weighed Between 50-58 kg. Semen was collected from them using an artificial vagina to obtain one ejaculate from each ram per week. The samples were divided using Tris extender: 1.0, 1.5, 2.0, 2.5 mg Acai extract / 1 ml Tris in addition to the control group. The necessary tests were performed to evaluate the semen in terms of estimating individual motility, the percentage of sperm viability, the percentage of abnormal sperms, the integrity of the plasma membrane and the acrosome integrity. There was a significant increase ($P<0.05$) in the percentage of sperm viability during the T3 treatment periods (48 and 72 hours) compared to the control group C for the same two periods; also there were no significant differences between all groups in the two periods (0 and 24 hours) of preservation in comparison with the control treatment C. Moreover T3 recorded a significant decrease ($P < 0.05$) in the percentage of sperm abnormality after 24 hours of preservation when compared with the control group C in the same period, while there were no significant differences between groups T1, T2, T3 and T4 compared with the control group C in the periods 0, 48 and 72 hours.
Keywords: ram semen, acai fruit, plant extracts

INTRODUCTION

The addition of plant extracts, which is a cheap and natural source, to the diluted semen in order to preserve, enhance and protect the function of sperm while preserving the semen by preservation and freezing [1]. Some plant species are active sources of antioxidants, which can act as reactive oxygen species (R.O.S.) scavengers to mitigate the harmful effects of oxidative stress on sperm vitality and function [2,3,4]. Acai (*Euterpe oleracea* Martius) is one of the plants that grows in the Amazon basin in northern Brazil and has broad medicinal uses [5]. It has gained significant interest in animal reproduction due to its chemical composition, rich in

polyphenols [6]. Polyphenols are a broad group of compounds, including anthocyanins, proanthocyanidins, and flavonoids, active antioxidants that ultimately demonstrate the potential to remove free radicals and mitigate the harmful effects associated with oxidative stress [7]. When adding acai fruit extract to semen extenders, due to its chemical composition rich in polyphenols, including anthocyanins, which bind directly to hydroxyl groups, this property allowed the electrons of hydrogen atoms to neutralize reactive oxygen species (R.O.S.) and thus eliminate the oxidative stress of sperm in preserved semen and freezing [8,9]

MATERIALS AND METHODS

This study was conducted in the animal field and reproductive physiology and biotechnologies laboratory affiliated with the Department of Animal Production / College of Agricultural Sciences / University of Baghdad from November 15, 2021, to April 25, 2022. Three rams of the local Awassi breed were used and trained to collect semen using the Artificial Vagina (A.V.). The rams were aged between 2.5 - 3 years, and their weight ranged between 50 - 58 kg. Semen collection begins in the early morning, once a week. The samples were mixed and diluted at a ratio of 1:20 with a diluent prepared according to Moce *et al.* [10] The concentrations of the aqueous extract of acai fruits according to the treatments. The acai extract was added to the diluted semen of five treatments, which include a control group (C), T1, T2, T3 and T4 that received 0, 1.0, 1.5, 2.0 and 2.5 mg acai/ 1ml of an extender. Microscopic examinations were performed. The mass activity was estimated according to the method of Evans and Maxwell [11]. The percentage of sperm viability was estimated according to the method of Swanson and Beardon [12]. The percentage of abnormal sperms was estimated according to Hancock's [13] methods. Hypo Osmatic Swelling Test [14]. The sperm acrosome integrity was estimated using Gentian Violet Solution-Eosin Solution [13]

RESULTS

Percentage of individual motility of the sperms.

Table (1) showed that there was no significant effect on the percentages of individual sperm motility for Awassi rams between treatments T1, T2, T3 and T4 with the control group at durations (0, 24, 48, 72) hours after preservation at 5cº. However, the treatments T1, T2, T3 and T4 were mathematically superior to the control group for all periods.

	Time (Hour)			
	0	24	48	**72**
C	73.50±2.34 A	62.62±2.86 A	48.62±2.77 A	**36.62±3.17** **A**
T1	75.62±2.22 A	65.62±2.87 A	53.62±3.26 A	**42.37±3.96** **A**
T2	76.62±2.37 A	67.25±2.98 A	55.12±3.39 A	**45.25±4.25** **A**
T3	78.00±1.77 A	70.12±2.08 A	58.37±3.30 A	**49.75±4.34** **A**
T5	78.00±1.55 A	68.37±2.57 A	56.62±3.49 A	**47.37±4.37** **A**

Table 1. Effect of adding different concentrations of aqueous extract of acai fruit to Tris extender on the percentage of sperm individual motility of Awassi rams after different periods of preservation(mean ± standard error).

Averages that carry different letters within a single column (between treatments) differ significantly between them. NS: not significant.

Percentage of live sperms

Table (2) showed that there were no significant differences in the percentage of sperm viability of Awassi rams of groups T1, T2, T3 and T4 at the periods (0 and 24 hours) when semen was preserved at 5c^{o} compared with the control group C, while there was a superior Significant ($P < 0.05$) for treatment T3 compared to control group C for both periods (48 and 72 hours). There were no significant differences between treatment T3 and treatment T2, T3 and T4 for the same two periods.

	Time (Hour)			
	0	24	48	**72**
C	76.50±2.26 A	65.37±2.67 A	51.24±2.63 B	**39.00±3.27 B**
T1	78.50±2.14 A	68.25±2.67 A	56.50±3.29 AB	**44.50±4.22 AB**
T2	80.12±1.99 A	70.00±2.64 A	57.87±3.30 AB	**46.12±4.13 AB**
T3	81.62±1.62 A	71.62±2.14 A	61.50±3.40 A	**52.12±4.20 A**
T4	80.75±1.59 A	71.12±2.22 A	59.87±3.29 AB	**50.12±4.29 AB**
P VALUE	N.S.	N.S.	*	*

Table 2. Effect of adding different concentrations of aqueous extract of acai fruit to Tris extender on the percentage of sperm viability of Awassi rams after different periods of preservation(mean ± standard error). Averages that carry different letters within a single column (between treatments differ significantly between them. * (P <0.05), NS: not significant.

Percentage of abnormality

Table (3) The results indicated that there were no significant differences in the percentages of abnormal sperms in the semen of Awassi rams between groups T1, T2, T3 and T4 in comparison with the control group C at the periods (0, 48, 72) hours of preservation at 5 °C. However, the results indicated a significant decrease ($P<0.05$) for treatment T3 compared with control group C after 24 hours of preservation.

Percentage of plasma membrane integrity of sperms (HOST)

Table (4) the results presented that there was no significant effect on the percentages of plasma membrane integrity of Awassi rams sperm between groups T1, T2, T3 and 4 T compared with the control group C at the periods (0, 24, 48, 72hours) of preservation at 5°C. Although, the treatments T1, T2, T3, and T4 were arithmetic superior to the control group C in all periods.

	Time (Hour)			
	0	24	48	**72**
c	77.00±2.34 A	64.12±2.79 A	52.62±2.77 A	**39.37±3.68 A**
T1	78.50±2.13 A	68.87±2.36 A	57.00±3.01 A	**45.12±4.24 A**
T2	80.87±1.88 A	70.12±2.92 A	58.00±2.90 A	**45.62±4.10 A**
T3	81.87±1.79 A	73.00±2.27 A	61.62±3.12 A	**50.75±4.09 A**
T5	80.62±1.83 A	71.00±2.27 A	59.75±3.23 A	**49.87±4.15 A**
P VALUE	N.S.	N.S.	N.S.	**N.S.**

Table 4. Effect of adding different concentrations of aqueous extract of acai fruit to Tris extender on the percentage of plasma membrane integrity of sperms of Awassi rams after different periods of preservation(mean ± standard error). Averages that carry different letters within a single column (between treatments) differ significantly between them. NS: not significant.

Percentage of acrosome integrity.

The results presented in Table (5) indicated that there were no significant differences between groups T1, T2, T3 and T4 compared to the control group C in the percentages of acrosome integrity of Awassi rams' sperm at the durations (0, 24, 48 and 72 hours) after preservation at 5° C. Although the T1, T2, T3, and T4 treatments were arithmetically superior to the control group C in all periods.

	Time (Hour)			
	0	24	48	**72**
C	77.50±1.87 A	67.62±1.93 A	53.75±3.35 A	**41.25±2.94 A**
T1	79.00±1.59 A	70.75±1.87 A	58.00±3.75 A	**46.75±3.52 A**
T2	79.12±2.00 A	70.37±2.26 A	59.25±3.35 A	**48.62±4.03 A**
T3	81.62±1.77 A	72.25±2.05 A	60.25±3.09 A	**52.00±3.54 A**
T5	80.75±1.81 A	71.25±2.10 A	60.12±2.10 A	**51.75±3.46 A**
P VALUE	N.S.	N.S.	N.S.	**N.S.**

Table 5. Effect of adding different concentrations of aqueous extract of acai fruit to Tris extender on the percentage of acrosome integrity of sperms of Awassi rams after different periods of preservation(mean ± standard error). Averages that carry different letters within a single column (between treatments) differ significantly between them. NS: not significant

DISCUSSION

Percentage of individual motility.

The addition of different concentrations of the aqueous extract of Acai fruits to the diluted semen did not have any significant effect on the percentage of individual motility of sperm for all treatments and in the studied preservation periods at a temperature of 5 °C as shown in Table (1). At the same time, there was an arithmetical superiority for the 3T treatment at the durations (0, 24, 48 and 72 hours) of preservation compared with the control group C and the same periods. The reason may be attributed to the addition of the extract of the Acai plant, which contains sugars such as glucose and fructose, which are a source of energy and metabolism of sperm, which gives the highest rate of movement of sperm, as indicated by Luz et al. [15]

Percentage of sperm viability.

The addition of acai fruit extract at a concentration of (2 mg / 1 ml) to the diluted semen led to significant differences ($P<0.05$) in the percentages of live sperm as shown in Table (2), as the T3 treatment was superior in both preservation periods (48 and 72 hours) of preservation compared to the control group C in the two mentioned periods, while there were no significant differences for all treatments T1, T2, T3 and T4 when compared to the control group C in the two periods (0 and 24 hours) of preservation, and since Increasing the level of reactive oxygen species generated during the preservation of semen at low temperatures leads to damage to the plasma membrane of the sperm and thus affects the duration of survival and movement of the sperm [16] so adding antioxidants to the semen thinner reduces the formation of free radicals [17] Therefore, the reason for maintaining the vitality of the sperm is due to the rich content of anthocyanins in the Acai fruit, which is the most effective and active compound. It has a role in scavenging free radicals and stopping the oxidation of unsaturated fatty acids as hydrogen peroxide, thus stopping the formation of aldehydic compounds such as (M.D.A.), which are toxic And with a mutagenic effect [18]

Percentage of abnormality.

In Table (3) the results showed that adding Acai fruit extract at a concentration (20 mg / 01 ml) to the diluted semen and kept at 5 °C caused a significant difference ($P<0.05$), where the percentage of deformed sperm decreased in the T3 treatment after 24 hours of preservation compared to the control treatment in the same period, while there was no significant effect of all treatments in the periods of preservation (0, 48 and 72) in comparison with the control group. The reason for the decrease in the percentage of morphological defects may be due to the presence of antioxidants such as quercetin, ferulic acid and gallic acid present in the aqueous extract of acai, which protects the plasma membrane of sperm from damage by the lipid oxidation process [19] and consequently a decrease in the numbers of abnormal sperms [15]

Percentage of plasma membrane integrity.

The addition of acai fruit extract had no significant effect on the percentages of plasma membrane integrity of Awassi rams among all treatments T1, T2, T3 and T4 at (0, 24, 48, 72) hours after preservation at 5°C as shown in Table (4) While the results in the same Table showed clear arithmetic superiority of the T3 treatment at (0, 24, 48, 72) hours compared to the control treatment at the same periods. (20) where it works by eliminating free radicals (peroxide and hydroxyl radicals) and thus disrupting the lipid oxidation process in the plasma membrane of Sperms [15]

Percentage of acrosome integrity of the sperms.

From Table (5), although there are no significant differences between all treatments T1, T2, T3 and T4 in the studied periods, there is an arithmetical superiority of the treatment T3 at the durations (0, 24, 48 and 72 hours) over all treatments as a result of adding acai fruit extract at a concentration (2mg/1 ml) compared to the

control group and for the same periods. This may be attributed to the high susceptibility of the components of the Acai fruit extract to substances with antioxidant properties such as polyphenols, including anthocyanins, which act as a free radical scavenger, reducing agent, hydrogen donor, and metal chelation such as iron, thus preventing the oxidation of unsaturated fatty acids that abound in the plasma membrane [21].

CONCLUSIONS

The current study showed that there were no significant differences in the percentage of individual motility, the integrity of the plasma membrane, or the acrosome integrity of the sperm for all treatments and at all periods (0, 24, 48 and 72 hours), noting that there is an arithmetical superiority recorded by the T3 treatment compared to the control group C for all periods for the studied traits.

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