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SEMEN PRESERVATION FOR IRAQ GOITERED GAZELLE BY USING THREE TYPES OF DILUENTS

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Abstract

The study was conducted at Babylon Nature Reserve at Babylon Governorate, Al-Mahawil District, and graduate laboratory in the College of Veterinary Medicine - University of Kufa, from 1/10/2019 to 1/3/2020, to determine Semen preservation for Iraq goitered gazelle by using three types of diluents. 12 male Goitered Gazelle were collected, the best 3 males were evaluated for the purpose of conducting the refrigeration experiment, Electro Ejaculator was used, for the purpose of collecting semen without anesthetizing the animal. Semen samples were distributed evenly over four groups according to the experiment design, where the final concentration of sperm was estimated after dilution to be 200 million sperm/cm^3, the first group is a control treatment that no substance has been added to the diluent, the second treatment contains (5%) of soybean milk, the third treatment was added omega-3 with a concentration of 2% to the diluent, and the last treatment, the two concentrations were added together to the diluent to know the effect of the two concentrations of both substances. The results of the study showed that there was no significant effect of soybean milk treatment on the acrosome integrity, while significantly affected of the percentage of dead and living sperm, general motility and progressive motility.

Key words: soybean milk, omega 3, semen physical, Goitered Gazelle.

Introduction

Deer were considered one of the endangered ruminants around the world, including Iraq (Goossenset *et al.,* 2005). Therefore, the Ghazal Al-Masad Reserve in western Iraq established Anbar in the Rutba District, to protect and proliferate this type of animal, there are other reserves in other governorates (Ashour and Ulaiwy, 2008). Ubgutturosa deer is heavier and the largest of other Asian deer, male weight is usually from 20 to 40 kg, female usually weighed from 18 to 33 kg (Heptner *et al.*, 1988). The first mating age is about 18-20 months under appropriate environmental conditions and proper nutrition (Van Mourik, 1986), long male adult horn (203 - 340 mm) with 16 - 29 rings, females were usually with or without horn, reaching a length of 220 mm (Harrison and Bates, 1991). Sperm cooling preservation was the simplest method that can successfully reduce sperm metabolism, thus prolonging the life of sperm (Colenbrander *et al.*, 2003), as the semen preservation was frozen over half a century ago, for artificial insemination purposes, become widespread worldwide (Calisici, 2010), whereas, the process of semen preservation by refrigeration and freezing was an important technique in breeding animal programs (Sarlos *et al.,* 2002).Freezing semen was an effective technique, but it causes morphological, functional, and vital changes for sperm, especially it affects sperm membranes by destroying their functional potential (Chelucci *et al.*, 2015). Currently animal products are used, such as egg yolks and milk to protect sperm during the freezing process and dissolving semen (Protection of sperm membranes from cold shock and Osmotic stress, which leads to decreased sperm movement and decreased fertility (Papa *et al.*, 2011). To avoid many problems in preserving sperm, especially in rare species by cryogenic or frozen, this study was made for the purpose of selecting the best diluents used to store Goitered Gazelle sperm by cooling.

Materials and Methods

The study was conducted at Babylon Nature Reserve at Babylon Governorate, Al-Mahawil District, and graduate laboratory in the College of Veterinary Medicine, University of Kufa, from 1/10/2019 to 1/3/2020. 12 male Goitered Gazelle were collected, the best 3 males were evaluated for the purpose of conducting the refrigeration experiment, Electro Ejaculator was used, for the purpose of collecting semen without anesthetizing the animal, the

males were placed at house Dimensions of 20 m length x 20 m width (400 m^2), attached by a special net, samples were collected from 4 males.

Samples were diluted field (field) immediately after collection, according to the treatment groups, to keep sperm safe and vital, moved by a sample preserving box of 5°C, to the IVF lab, and the manufacture of frozen bronchi in the Faculty of Veterinary Medicine, University of Kufa.

Semen samples were distributed evenly over four groups according to the experiment design., where the final concentration of sperm was estimated after dilution to be 200 million sperm/cm^3, the first group is a control treatment that no substance has been added to the diluent, the second treatment contains (5%) of soybean milk, the third treatment was added omega-3 with a concentration of 2% to the diluent, and the last treatment, the two concentrations were added together to the diluent to know the effect of the two concentrations of both substances.

Canes were filled for cooling, the semen was examined for three consecutive days after cooling, the studied traits are as follows:

1. Acrosome integrity: Tested by reading the shape of the acrosome under a microscope with a magnification force of 100 using the oil Immersion.

2. Sperm viability: The semen vitality test was performed by mixing semen (whether cool or frozen), with Eosin-nigrosin stain, in a ratio of 1: 3, a smear was then carried out on clean, dry slides at body temperature, leave the slides to dry and then read under the microscope with a magnification strength of 40.

3. General motility: The general motility of all groups was evaluated, with a Computer Assisted Sperm Analysis; CASA device, it is estimated according to the random movement of the sperm.

4. Progressive motility: The previous device was used to estimate the natural movement (forward movement) and distinguish it from the other abnormal movements.

Preparation of soybean milk: Soybean milk has been prepared by weighing 10 g of soybeans with a sensitive scale, they were soaked overnight at 100 ml of distilled water, then the water was removed, the soybeans were washed thoroughly washed again with distilled water, add 100 ml of distilled water and heated between 80-100 m 0 for 20 minutes, immediately after heating, it is mixed with a high speed mixer for 5 minutes until the formation of the Slurry, after forming the Slurry it was heated again for 10 minutes with continuous stirring and maintaining the temperature between 80-100°C, to discontinue the activity of lipoxygenase (Nelson et al., 1976; Shurtleff and Aoyagi, 1979, the Slurry was cooled and filtered with a clean gauze cloth, then the centrifuges were carried out at a speed of 4000 rpm for 20 minutes, for complete disposal of solids and set their PH level to 7 (Kasimanickam *et al.*, 2011).

Preparation of semen Extender: Table 1 shows the composition of semen diluted of Goitered Gazelle males per 100 ml according to Baiee *et al.*, (2018).

Results

The physical traits of the semen refrigerated fluid of Goitered Gazelle at the first day

The results of the current study showed table 2 that there was no significant effect (P≤0.05) of soybean milk treatment on the acrosome integrity, whereas, treatment with soybean milk affected the percentage of dead sperm, as it was found that there was significant superiority (P≤0.05) for the second treatment compared to the rest of the treatments, the lowest percentage was in the first

Table 1: The composition of semen diluted of Goitered Gazelle males per 100 ml.

Composition	P1	P2
Tris	2.24g	2.24g
Citric acid	1.48g	1.48g
Fructose	1g	1g
Penicillin-strcptoycin	50000IU	50000IU
Egg yolk	20%	20%
Glycerol	-	12.8%
Distilled H_20	100ml	100ml
Eurycoma longifolia	Dose depended	Dose depended

Table 2: The effect of adding soybean milk and omega 3 on some physical traits of the semen refrigerated fluid of Goitered Gazelle at the first day (mean ± standard error).

Traits Treatments	Acrosome integrity	Dead sperm percentage	Life sperm percentage	General motility	Progressive motility
C	0.13± 98.58	1.47±28.41A	1.47±71.58B	1.16±64.16B	1.12±68.83B
T1	0.15±98.54	0.76±16.16C	0.77±83.87A	0.88±75.83A	0.94±81.25A
T2	0.22±98.04	1.63±29.50B	1.63±70.50A	0.62±67.75A	1.07±73.33A
T3	0.11±98.29	1.45±21.75B	1.45±78.25A	0.96±73.08A	0.83±78.66A
Sig	N.S	0.05	0.05	0.05	0.05

C: control treatment: 0, T1: first treatment: 5% soybeans, T2: second treatment: 2% omega3, T3: third treatment: 5% soybeans + 2% omega3. *Averages with different letters within one row indicate significant differences (P≤0.05) between the treatments.

treatment (T1), the averages for the first, second and third control coefficients were 21.75, 29.50, 16.16 and 27.41, respectively.

As for the percentage of live sperm, it was noted that there was significant superiority (P≤0.05) for the first treatment (T1) compared to the other treatments, the lowest percentage was in the second treatment (T2), the averages for the first, second, and third treatments were 78.25, 70.50, 83.87 and 71.58, respectively.

As for the progressive motility of sperm, it was noted that there was significant superiority (P≤0.05)for the first treatment compared to the rest of the treatments, the lowest percentage was in the second treatment and the averages for the treatments were 73.08, 67.75, 75.83 and 64.16, respectively.

The general motility of sperm showed a significant superiority (P≤0.05) for the first treatment compared to the rest of the treatments, the lowest percentage was in the control treatment, the averages for the first, second, and third treatments were 78.66, 73.33, 81.25 and 68.83, respectively.

The physical traits of the semen refrigerated fluid of Goitered Gazelle at the second day

Table 3 shows that there was no significant effect for treatment with soybean milk and omega 3 on acrosome integrity, while it was found that there were significant differences (P≤0.05) for the second treatment of the percentage of dead sperm percentage compared to the rest of the treatments, the lowest percentage was for the first treatment, the averages for the first, second and third control treatment were 32.66, 39.41, 25.87 and 37.08, respectively.

A significant effect (P≤0.05) was observed for the first treatment with soybean milk in the percentage of live sperm, it was the lowest percentage for the second treatment, the averages for control treatment, first, second, and third were 67.33, 0.58, 75.79 and 64.58, respectively, the third treatment was a significant differences (P≤0.05) on the trait of sperm motility compared to the rest of the treatments, the lowest percentage of treatment was control, the averages for the first, second, and third control coefficients were 71.75,62.50,70.66,59.25, respectively, also, it was found that there was a significant effect (P≤0.05) for the first treatment for the trait of the general motility of sperm, compared to the rest of the treatments and the lowest percentage was for the control treatment, the averages for the first, second and third control treatments were 75.25, 68.00, 75.25 and 66.83, respectively.

Table 3: The effect of adding soybean milk and omega 3 on some physical traits of the semen refrigerated fluid of Goitered Gazelle at the second day (mean ± standard error).

Traits Treatments	Acrosome integrity	Dead sperm percentage	Life sperm percentage	General motility	Progressive motility
C	0.11±98.75	2.99±37.08A	1.72±64.58B	1.36±59.25B	0.90±66.83B
T1	0.17±98.54	0.96±25.87B	1.02±75.79A	1.04±70.66A	1.13±75.25A
T2	0.22±97.70	1.88±39.41A	1.88±0.58B	1.65±62.50B	1.50±68.00B
T3	0.18±98.08	2.42±32.66A	2.42±67.33B	0.75±71.75A	1.00±75.25A
Sig	N.S	0.05	0.05	0.05	0.05

C: control treatment: 0, T1: first treatment: 5% soybeans, T2: second treatment: 2% omega3, T3: third treatment: 5% soybeans + 2% omega3. *Averages with different letters within one row indicate significant differences (P≤0.05) between the treatments.

Table 4: The effect of adding soybean milk and omega 3 on some physical traits of the semen refrigerated fluid of Goitered Gazelle at the third day (mean ± standard error).

Traits Treatments	Acrosome integrity	Dead sperm percentage	Life sperm percentage	General motility	Progressive motility
C	0.35±96.91	1.47±36.20A	1.46±63.70B	1.55±56.66B	1.42±61.58C
T1	0.31±97.45	1.25±29.50B	1.42±71.33A	1.88±60.83A	1.65±68.41B
T2	0.26±96.62	1.63±42.16A	1.63±57.83C	1.20±55.75B	0.79±62.33C
T3	0.42±96.50	2.05±35.50A	2.05±64.50B	1.72±65.91A	1.42±72.83A
Sig	N.S	0.05	0.05	0.05	0.05

C: control treatment: 0, T1: first treatment: 5% soybeans, T2: second treatment: 2% omega3, T3: third treatment: 5% soybeans + 2% omega3. *Averages with different letters within one row indicate significant differences (P≤0.05) between the treatments.

The physical traits of the semen refrigerated fluid of Goitered Gazelle at the third day

Table 4 shows that there was no significant effect (P≤0.05) of soybean milk treatment on acrosome integrity of all treatments, while there was a significant effect of omega 3 treatment in the percentage of dead sperm, where the second and third treatment and control were superior (P≤0.05) compared to the first treatment, the averages for the first, second and third control and treatments were 35.50, 42.16, 29.50 and 36.20, respectively.

As for the live percentage of the first treatment, there was a significant effect (P≤0.05) compared to the rest of the treatments, the lowest percentages for the second treatment were and the averages were the control coefficients and the first, second and third treatment

64.50, 57.83, 71.33, 63.70, respectively.

The third treatment for the trait of the individual motility of sperm, with a significant effect (P≤0.05) compared to the rest of the treatments, it was the lowest percentage for the second treatment, the averages for the control, the first, second and third treatment were 65.91, 55.75, 60.83 and 56.66, respectively.

The third treatment was significance effect (P≤0.05) compared to the rest of the treatment on the general motility, the lowest percentage of treatment was control, the averages for the control treatment and the first, second and third treatment were 72.83,62.33,68.41,61.58, respectively.

Discussion

The experiment was conducted to find the effect of adding soybean milk and omega-3 on the physical traits of cooled semen of Goitered Gazelle, no significant differences in the safety profile of acrosome in all three-day treatments, agreed with Anel-López *et al.,* (2012), through his studies on sperm selected in two ways electrical stimulation and by epididymal testis, for the integrity of the membrane and DNA, after centrifuging the samples from the red deer, there is no difference between treatments within the same type of sample.

The study by Garde *et al.,* (2003) conducted on three species of deer, to keeping the semen cooled, such as Gazella cuvieri, G. Dama mhorr, and G. dorcas neglect using three diluents (Triladyl, Test and Tris-trehalose), where the movement of sperms for the three types of deer using the three diluents (77-70%), the integrity of the sperm membrane was higher in the diluted Triladyl by 63% and medium with Test and its ratio 51% and lower Tris-trehalose and its proportion 45%.

Significant difference was observed for different concentrations of soybean milk by 5% in all treatments for the studied characteristics for three days from semen cooling, vegetable-based diluents are better at preserving semen than animal diluents like egg yolk, it was evaluated for bulls, rams, goats, and buffaloes, because it is more susceptible to bacterial contamination, leads to the production of internal toxins (Stradaioli *et al.,* 2007).

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