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Using Aqueous Extract of Maca (*Lepidium meyenii*) to Study its Effectiveness on Short-Term Storage Ram Semen Dilution and Fertility

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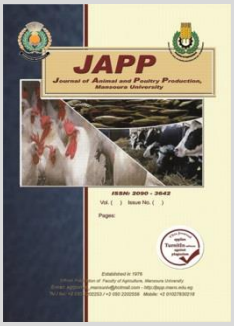

ABSTRACT

Studying aims to evaluate sperm characteristics using aqueous extract of maca added to ram semen extender. Five media of ram semen extenders as M0, M1, M2, M3 and M4 contained different levels of aqueous extract of maca at 0, 5, 10, 20 and 40 µg/mL respectively, were storage at 5˚C for 5th days,. Then, sperm characteristics as progressive sperm motility, livability, abnormality and intact acrosome and lipid peroxidation (LPO) concentration were estimated. Also, a fertility trial using forty ewes (*n=20* in each) were inseminated by M0 (as control) and M3 (as the best sperm parameters). The aqueous extract of maca 20 µg/mL improved previous sperm characteristics and reduced both abnormal sperm and lipid peroxidation (LPO) compared with other extenders during storage at 5˚C for 5th days. Also, an improvement observed in total pregnancy rate of ewes using M3 (79.17%) compared with M0 (65.38%) after chilling at 5˚C for 2nd days. The results indicate that the aqueous extract of maca maintains spermatozoa characteristics during chilling at 5ºC for 5th days especially 20µg/mL which maintains fertile ability after the 2nd days of chilling.

Keywords: Maca, semen extender, preservation, fertility.

INTRODUCTION

Maca is a plant with great potential as an adaptogenic and appears to be promising as a nutraceutical in the prevention of several diseases and the scientific evidence showed effects on sexual behavior, fertility, mood, memory, osteoporosis, metabolism and the treatment of some tumor entities (Pino-Figueroa *et al.*, 2010). Maca belongs to the brassica (mustard) family which the most relevant plants related to mustard, turnip, black mustard, cabbage, garden cress, water cress (Clément *et al.*, 2010[a]). A dry maca contains 10.2% proteins, 59% carbohydrates, 2.2% lipids, 8.5% fibre, free fatty acids (linoleic, palmitic, and oleic acids), 40.1% saturated fatty acids and 52.7% unsaturated fatty acids according to (Gonzales, 2012). Also, the ingredients of maca are benefited in metabolites by alkaloids are only found in maca (Clément *et al.*, 2010[b]). Hence, Maca has a positive effect on reducing oxidative stress due to an excess of reactive oxygen species (ROS) then, the oxidative stress significantly damages sperm functions such as motility, fluidity of the sperm plasma membrane and the integrity of DNA due to lipid peroxidation induced by ROS (Del Prete *et al.*, 2022).

Therefore, a feasible new strategy by using aqueous extract of maca to improve ram sperm function during short-term preservation. Hence, this work studies the effect of adding different concentrations of maca aqueous extract at 0, 5, 10, 20 and 40 µg /mL extender on parameters quality of ram semen dilution during short-term storage at 5°C for five days. Also, the conception rate after two days of short-term storage of semen was studied.

MATERIALS AND METHODS

The local and experimental period

This work was carried out in the EL-Serw Research Station, Animal Production Research Institute (*APRI*), Agricultural Research Center, Ministry of Agriculture, Egypt. This study was started from June to September 2022 for semen collection and fertility trial was occurred in the September breeding season in 2022.

Experimental Animals

Semen samples were collected from five rams of the Rahmani breeds, at old age 2.5-3.0 years and weight 75-80 kg.

All rams were received a standard commercial ration twice daily at 8.00 am and 3.00 pm and fresh water was available through experimental times. They had routine vaccinations and shared the same environment.

Preparation of aqueous extract of maca

Five grams of maca powder were mixed with 100 mL of distilled water and automatically stirred in a water bath at 70ºC for 3 hours. The mixture was centrifuged at 4000 *rpm* for 10 min and extraction repeated into a water bath at 70ºC for 2 h again.

The solution was filtrated and placed in dark vials and stored in a refrigerator at 5ºC to use in semen extenders. The analysis of the powder and maca aqueous extract was described by Del Prete *et al.* (2022) in Table (1).

In addition, the analysis of dry maca powder as amino acids and minerals (Gonzales, 2012) were in Table (2).

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Table 1. Analysis of the powder and maca aqueous extract.

Items	Analysis of maca types	
	Maca Powder (µg/L)	Aqueous Extract of maca (µg/L)
5-oxo-6E,8E-octadecadienoic acid (Macaen)	69.53	17.89
N-(3-hydroxy-benzyl)-2Z-fivecarbon acrylamide	614.29	157.99
N-benzyl-5-oxo-6E,8E-octadecadienamide (MI 7)	46.08	61.81
N-benzyloctadecanamide (MI 16)	53.96	28.89
Macalines or Lepilidines	59.03	13.31
Methyltetrahydro hydridecarboline carboxylic acid	47.17	3.63
1-dibenzyl-2-propane-4,5-dimethylimidazilium	19.52	1.25

Table 2. Analysis of amino acids and minerals presented in dry maca powder.

Items	Levels of amino acid in dry maca powder (mg/g protein)
Leucine	91.0
Arginine	99.4
Phenylalanine	55.3
Lysine	54.3
Glycine	68.3
Alanine	63.1
Valine	79.3
Isoleucine	47.4
Glutamic acid	156.5
Serine	50.4
Aspartic acid	91.7
Levels of minerals in dry maca powder (mg/ 100 g dry matter of maca powder)	
Iron	16.6
Calcium	150.0
Copper	5.9
Zinc	3.8
Potassium	2050.0

Semen collection

The raw semen was collected by artificial vagina as 2 ejaculates/ram/weekly for up to five weeks. Immediately after collection semen was taken to the laboratory and kept in the water bath at 37°C for evaluation superficially (volume, color, smell and density) and microscopically (mass motility, individual motility, viability, abnormal, sperm cells concentration and acrosomal integrity). Then, ejaculates free of urine, water, blood or feces and had volume from 0.5 to 2.0 mL, minimum semen concentration at 2.0-3.0 x 10^9 spermatozoa/mL were used. Also, semen ejaculates had total motility higher than 80%; abnormal sperm less than 15% and integrity acrosome more than 85% were used in the experiment.

Preparation of Tris semen extenders with aqueous extract of maca

The pooled samples were split into 5 aliquots in clean tubes as M0, M1, M2, M3 and M4. Each aliquot was diluted at room temperature with Tris-based extender before cooling within the diluted at a rate of 1(semen): 6 (extender) to reach a final concentration of 200 × 10^6 sperm/mL. The M0, M1, M2, M3 and M4 media contained aqueous extract of maca up to 0, 5, 10, 20 and 40 µg /mL, respectively. The five extended semen tubes were subjected to gradual cooling from 37 ºC to 5 ºC and storage in chilling up to 5 days. The Tris-based semen extender was provided by dissolving 3.634 g of Tris, 1.99 g of citric acid, 0.5 g of fructose, 15 mL of egg yolk, and antibiotics (1000 IU/mL of penicillin, and 2 mg/mL of streptomycin) in double distilled water and the volume was made up to 100 mL.

Processing of semen evaluation

Progressive sperm motility

A light microscope with a heated stage was used to determine the progressive motility. For this purpose, a small drop of semen was added into this solution (NaCl 0.9%) and mixed to ensure homogenization. The drop of mixture was placed on the slide was covered and raised to 37°C on the heated stage of the microscope. Then, the motility was visually determined at a 100× magnification. The motility estimates were expressed as percentage with the examination of five different sites of slide.

Live and abnormal spermatozoa

In order to determine the ratio of live spermatozoa; a drop from raw or diluted semen, was mixed with a few drops of Eosin–Nigrosin stain put in test tube. A drop of mixture was placed on a preheated (37°C) clean and dry slide. Then, thin smears were prepared and dried in a very short time. After drying, the smear was examined at a 400× magnification under the light microscope. A total of 400 spermatozoa were examined in a smear to observed live sperm (head of live spermatozoa appeared transparent or clear). Meanwhile died spermatozoa suffered damage on the plasma membrane, caused increased permeability, dyes will enter the cell and head of spermatozoa look reddish. The same slide was used to investigate the percentage of abnormal spermatozoa by counted 400 sperm in five microscopic fields of view with a microscope (400×).

Intact acrosome

Semen sample 50 µL was added to a 500 µL formalin citrate solution (96 mL 2.9 % sodium citrate, with 4 mL 37 % formaldehyde) and mixed carefully. A small drop of the mixture was placed on a microscope slide and a total of 200 spermatozoa were counted in five different microscopic fields for each sample using microscope (1000×). Spermatozoa that showed normal apical ridge in acrosome region were assessed as intact acrosomes.

Lipid peroxidation (LPO) concentration

The LPO was estimated by measuring the level of malondialdehyde acid after centrifuged samples at 4500 *rpm* for 15 min and were stored at -20ºC until analysis. Then, the commercial kit LPO-586 (Oxis Research, Burlingame, CA, US) has range curve from 0.5 to 4.0 µM with sensitivity at 0.5µM was used.

Fertility trial

The artificial insemination (AI) was done by semen extended with control media and the best optimal aqueous extract of maca which was stored at 5ºC for up to two days. The results indicated that the best preservation media is M3 with 20 µg/mL of aqueous extract of maca. Then, the fertility trial as conception rate was carried out between M0 (as control) and M3 (as the best treated extender media) used 40 Rahmani ewes (*n*=20 ewes/extender media). At beginning of breeding season (in September month), the estrous cycles of either ewes in M0 or M3 were checked twice a day with a time interval of about 12 hours using a teaser ram. If ewe comes to estrous cycle, it will inseminate

by 1.0 ml of semen dilution which has short-term storage up to two days. At time of AI the semen dilution from M0 media (for control ewes) or M3 media (for treated ewes) was warmed before used. The cervical insemination was applied two times after 12 and 24 hours of estrous cycle onset by helping of vaginal speculum and penlight. The vaginal speculum lubricated with glycerol and inserted gently into ewe vagina to open it. Then, the gun of the extended semen does was slowly deposited as deep as possible into the front of Os-cervix. The ewe considered pregnant when passed two oestrous cycles without appearing any heating again after 20 days. If ewe returns to oestrus cycle, it will service again using the same technique of AI. Then, fertility trial was calculated as pregnancy rate percentage as follows:

$$\frac{\text{Total number of ewes conceived}}{\text{Total number of ewes' inseminated}} \times 100$$

Statistical Analysis

Statistical were performed by ANOVA followed by the Duncan *post hoc* test to determine significant differences in all the parameters among all maca trial using the SPSS/PC computer program (SPSS Statistics version 2020). The conception rate results were analyzed by the chi-square X^2 test. The test in a completely randomized design as the following model:

$$Y_{ijK} = \mu + M_i + T_j + e_{ij}$$

Yijk = observation.
μ = overall mean.
M_i = fixed effect of extension media (i=M0, M1, M2, M3 and M4).
T_j= time of storage at 5°C (j=1, 2, 3, 4 and 5 days).
e_{ij}= residual error.

RESULTS AND DISCUSSION

Results

Effect of different concentrations of maca aqueous extract on percentage of progressive sperm motility

Observation has insignificant differences in the percentage of progressive sperm motility among treatment media at 0 day. The difference values observed at passed one day of short-term storage at 5°C. At the 2nd days of chilling the significantly (P<0.05) higher values of progressive motility obtained in M1, M2 and M3 up to 73.52, 74.21 and 79.59% than 71.12 and 72.49% in M0 and M4, respectively. Accordingly, it is cleared that M3 media being superior to all other media in short-term storage in progressive sperm motility. Observation has significant (P<0.05) differences in progressive sperm motility between (M1, M2 and M3) and other treated extenders (M0 and M4) during 5th days of short-term of storage. The highest progressive sperm motility was observed among all treatments between the 1st and 2nd days of short-term storage comported with other chilling days. Therefore, treatment of semen extenders with different concentrations of maca aqueous extract had significantly (P<0.05) increased in progressive sperm motility at 5th days of chilled storage compared with 0 µg/mL in M0 extender. The results indicated that gradually significantly (P<0.05) decrease in the progressive sperm motility among different treatments through 5th days of short-term storage Table (3).

Table 3. Progressive motility of ram spermatozoa extended with aqueous extract of maca during short-term storage at 5°C from 1st to 5th days.

Semen media with aqueous extract of maca levels (µg/mL)	Days of short-term storage					
	0 day	1 day	2 days	3 days	4 days	5 days
M0, 0 µg/mL	85.27^{Aa}±0.81	75.13^{Bb}±1.22	71.12^{Bb}±1.76	60.13^{Cc}±2.11	52.12^{Dc}±1.59	44.13^{Ec}±1.96
M1, 5 µg/mL	86.22^{Aa}±0.82	77.53^{Bb}±0.94	73.52^{Bb}±1.33	65.71^{Cb}±1.75	59.17^{Db}±2.13	49.48^{Eb}±2.45
M2, 10 µg/mL	86.39^{Aa}±0.98	78.24^{Bb}±1.12	74.21^{Bb}±1.36	66.61^{Cb}±2.01	60.44^{Db}±2.42	50.41^{Eb} ±2.32
M3,20 µg/mL	86.98^{Aa}±0.77	82.45^{Aa}±1.11	79.59^{Aa}±1.55	71.55^{Ba}±2.11	65.55^{Ca}±2.42	59.55^{Da}±2.23
M4, 40 µg/mL	85.85^{Aa}±0.71	76.45^{Bb}±1.11	72.69^{Bb}±1.41	63.19^{Cbc}±2.33	57.55^{Db}±2.44	47.15^{Ebc}±2.36

The means values of the different superscripts in the same row with $^{A, B, C, D \text{ and } E}$ and column with $^{a, b \text{ and } c}$ are significantly at P<0.05.

Effect of different concentrations of maca aqueous extract on the percentage of live spermatozoa

Changing in percentages of live spermatozoa as affected by supplied different levels of maca aqueous extract to ram semen extenders during short-term storage at 5°C from 1st to 5th days were illustrated in Table (4). The results indicated that at 2nd days of short-term storage, the addition of 20 µg/mL of maca aqueous extract to semen media (M3) has a significant increase (P < 0.05) effect on percentage of live spermatozoa when compared with other concentrations during the chilling period. It was cleared that no significant (P>0.05) effect between M1, M2 and T4 extenders on livability of spermatozoa at days of storage, but less livability spermatozoa was obtained in M0 than the previous semen extender media (M1, M2 and T4). The most percentages of live spermatozoa observed among all extenders semen media between the 1st and 2nd days of short-term storage. The decreased (P<0.05) in percentages of live spermatozoa in all semen extender media were observed during short-term storage from 2nd to 5th days except M0 extender which is began to decreased at 1st day of storage.

Table 4. Live spermatozoa of ram spermatozoa extended with aqueous extract of maca during short-term storage at 5°C from 1st to 5th days.

Semen media with aqueous extract of maca levels (µg/mL)	Days of short-term storage					
	0 day	1 day	2 days	3 days	4 days	5 days
M0, 0 µg/mL	90.11^{Aa}±1.81	84.42^{Ba}±1.35	78.19^{Cc}±1.63	63.31^{Dc}±2.26	57.35^{Ec}±1.86	45.45^{Fc}±1.44
M1, 5 µg/mL	91.12^{Aa}±1.65	86.29^{Aa}±1.26	79.17^{Bb}±1.34	73.45^{Cb}±1.85	62.37^{Db}±2.66	53.38^{Eb}±2.53
M2, 10 µg/mL	92.29^{Aa}±1.84	86.71^{Aa}±1.22	80.26^{Bb}±1.41	74.49^{Cb}±2.15	63.66^{Db}±2.52	54.98^{Eb}±2.65
M3, 20 µg/mL	92.98^{Aa}±1.77	89.19^{Aa}±1.43	85.75^{Ba}±1.44	78.51^{Ca}±2.14	68.88^{Da}±2.68	60.55^{Ea}±2.35
M4, 40 µg/mL	91.55^{Aa}±1.75	85.93^{Aa}±1.23	78.66^{Bc}±1.56	72.69^{Cb}±2.25	61.56^{Db}±2.42	51.45^{Eb}±2.44

The means values of the different superscripts in the same row with $^{A, B, C, D, E \text{ and } F}$ and column with $^{a, b \text{ and } c}$ are significantly at P<0.05.

Effect of different concentrations of maca aqueous extract on the percentage of abnormal spermatozoa

As shown in Table (5), extender free of aqueous extract of maca 0 µg/mL (M0) had significantly (P<0.05) increased the percentages of abnormal spermatozoa during all time of short-term storage compared with other concentrations of aqueous extract of maca. However, lower significantly (P<0.05) abnormal spermatozoa in M3 media than M1, M2 and M4 media at the 2nd day of short-term storage time. Besides that, the lowest percentage of abnormal spermatozoa was noticed in sperm supplied with 20 µg/mLof aqueous extract of maca from 1st to 5th days of stored compared with M0, M1, M2 and M4 semen extender. Furthermore, the percentage of abnormal spermatozoa in M0, M1, M2, M3 and M4 semen extender were increased (P<0.05) by advanced of short-term storage time and reached the maximum values up to 44.38, 38.52, 34.35, 29.49 and 37.15%, respectively. Likewise, the percentage of abnormal spermatozoa significantly increased gradually at the 5th days of short- term storage when compared with those chilling times at 1st, 2nd, 3rd and 4th days.

Table 5. Abnormal spermatozoa of ram spermatozoa extended with aqueous extract of maca during short-term storage at 5°C from 1st to 5th days.

Semen media with aqueous extract of maca levels (µg/mL)	Days of short-term storage					
	0 day	1 day	2 days	3 days	4 days	5 days
M0, 0 µg/mL	10.65^{Ea}±0.39	14.41^{Da}±0.51	17.97^{Da}±0.44	26.59^{Ca}±2.66	36.52^{Ba}±1.21	44.38^{Aa}±1.18
M1, 5 µg/mL	9.27^{Ea}±0.22	12.15^{Ea}±0.33	15.62^{Da}±0.94	21.05^{Cb}±0.88	29.56^{Bb}±1.19	38.52^{Ab}±1.57
M2, 10 µg/mL	9.76^{Ea}±0.16	11.13^{Ea}±0.36	15.31^{Da}±0.47	20.22^{Cb}±1.41	27.95^{Bb}±1.19	34.35^{Ab}±1.39
M3, 20 µg/mL	8.98^{Da}±0.27	10.05^{CDba}±0.38	13.12^{CDb}±0.96	18.64^{Bc}±0.81	21.55^{Bc}±1.16	29.46^{Ac}±1.57
M4, 40 µg/mL	9.43^{Ea}±0.21	13.19^{EDa}±0.47	16.88^{Da}±0.47	22.12^{Cb}±1.46	30.18^{Bb}±1.14	37.15^{Ab}±1.19

The means values of the different superscripts in the same row with $^{A, B, C, D \text{ and } E}$ and column with $^{a, b \text{ and } c}$ are significantly at P<0.05.

Effect of different concentrations of maca aqueous extract on the percentage of integrity acrosome

It can be noticed from Table (6) has (P>0.05) insignificant differences in percentage of percentage of integrity acrosome among all treated media as M0, M1, M2, M3 and M4 at the 1st days of short-term storage then, the integrity acrosome values were 86.55, 86.78, 87.67, 89.54 and 87.93, respectively. A significant (P<0.05) differences were observed in percentage of integrity acrosome between M3 and M0, M1, M2 and M4 from 2nd to 5th days of short-term storage. The highest percentage of integrity acrosome was observed among all media treatments between the 1st and 2nd days of short-term storage. Treatment of ram semen extenders with different concentrations of aqueous extract of maca (5, 10, 20 and 40 µg/mL) had significantly (P<0.05) higher in the percentage of integrity acrosome at 4th and 5th days than ram semen extenders free of aqueous extract of maca (M0). The present data observed that significantly (P<0.05) lower gradually in the percentage of integrity acrosome among all ram semen extender (M0, M1, M2, M3 and M4) through short-term storage periods from 2nd to 5th days.

Table 6. Integrity acrosome of ram spermatozoa extended aqueous extract of maca during short-term storage at 5°C from 1st to 5th days.

Semen media with aqueous extract of maca levels (µg/mL)	Days of short-term storage					
	0 day	1 day	2 days	3 days	4 days	5 days
M0, 0 µg/mL	91.15^{Aa}±0.55	86.55^{Aa}±0.726	82.15^{Bab}±1.39	72.21^{Cc}±1.48	62.27^{Dc}±1.67	56.52^{Ec}±1.35
M1, 5 µg/mL	91.52^{Aa}±0.68	86.78^{Aa}±0.92	82.35^{Bab}±1.19	77.43^{Ca}±1.51	70.10^{Db}±1.99	62.17^{Eb}±1.93
M2, 10 µg/mL	91.94^{Aa}±0.46	87.67^{Aa}±0.61	83.51^{Bab}±1.19	78.17^{Ca}±1.28	71.49^{Db}±1.68	63.65^{Eb}±1.37
M3, 20 µg/mL	92.58^{Aa}±0.66	89.54^{Aa}±0.43	86.15^{Aa}±1.68	80.11^{Ba}±2.36	75.98^{Ca}±2.68	69.15^{Da}±2.96
M4, 40 µg/mL	92.55^{Aa}±0.63	87.93^{Aa}±0.25	82.53^{Bab}±1.58	77.49^{Cb}±2.27	71.66^{Db}±2.33	64.35^{Eb}±2.35

The means values of the different superscripts in the same row with $^{A, B, C, D \text{ and } E}$ and column with $^{a, b \text{ and } c}$ are significantly at P<0.05.

Lipid peroxidation (LPO) concentration in semen extenders supplemented with different levels of maca aqueous extract during short-term storage at 5°C from 1st to 5th days.

Figure (1) is summarized the concentration of malondialdehyde (MDA) as a lipid peroxidation marker in M0, M1, M2, M3 and M4 of ram semen extenders during short-term storage up to 5th days. The maximum less (P<0.05) concentration of LPO was obtained with ram semen extenders containing 20 and 40 µg/mL in M3 and M4 media compared with M0, M1 and M2 during times of short-term storage. Interestingly, the LPO concentration has no significant values among M3 and M4 media during short-term storage. Also, the current results indicated that addition different levels of maca aqueous extract to the ram semen media had a safety effect on reduce LPO concentration through the short-term storage of ram semen extender up to 5th days.

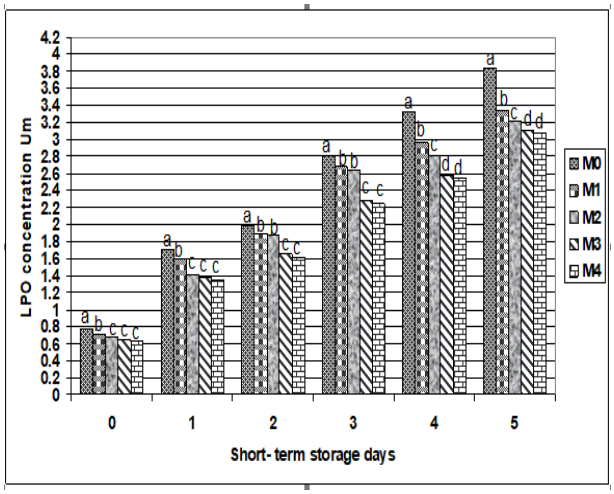

Fig.1. Lipid peroxidation (LPO) concentration in semen extenders supplemented with different levels of maca aqueous extract during short-term storage at 5°C from 1st to 5th days.

Fertility trial

Table (7) is discussed the fertility trial of ram semen extender (M0 and M3) after short-term storage at 5°C up to 2nd days. Interestingly, the concentration of 20 µg/mL of maca aqueous extract in M3 media was used in a fertility trial which has the best sperm characteristics after dilution and storage up to 2nd days (Tables 3, 4, 5 and 6) compared with other media. Then, the fertility trial was carried out between M0 and M3 to decide which the best treatments that can be used in diluents futurity. The results revealed that the use of M3 (80.00%) gave slightly higher pregnancy rate (conception rate) than of M0 media (70.00%). Also, the conception rate was 50.00 and 75.00% for ewes inseminated twice (which did not conceive from the first insemination) with semen storage in M0 and M3 extenders, respectively. Then, the results calculated that higher pregnancy rate during the 1st and 2nd services in ewes inseminated with M3 semen extender compared to those ewes in M0 after short-term storage up to 2 days. The improvement in pregnancy rate through 1st and 2nd services was 65.38 and 79.17 % for M0 and M3 media, respectively. Therefore, artificial insemination of ewes by ram semen extender supplemented with 20 µg/mL of maca aqueous extract which storage at 5°C up to 2 days could be achieved more pregnancy rate percentage than ram semen extender free aqueous extract of maca.

Table 7. Fertility trial of ram semen extender media (M0 and M3) after short-term storage at 5˚C up to 2nd days.

Items	Ram semen extender media	
	M0	M3
No. of ewes inseminated at 1st service	20.00	20.00
No. of ewes conceived at 1st service	14.00	16.00
*Pregnancy rate at 1st service,%	70.00	80.00
No. of ewes inseminated at 2nd services	6.00	4.00
No. of ewes conceived at 2nd services	3.00	3.00
Pregnancy rate at 2nd services	50.00	75.00
The total insemination services:-		
Ewes inseminated at 1st and 2nd services	26.00	24.00
Ewes conceived at 1st and 2nd services	17.00	19.00
Total Pregnancy rate through 1st and 2nd services	65.38	79.17

All the differences were not significant.

*** Pregnancy rate (conception rate) = No. of ewes conceived ×100 / No. of ewes inseminated**

Discussion

It is known; maca has traditionally been used as folk medicine and is considered a food supplement. It was first cultivated at least 2000 years ago in the Andes Mountains of Peru at an altitude of 4000-4500 meters above sea level, has been used as both a food and a traditional medicine in the region for over 2000 years (Tung *et al.,* 2015). There are numerous substances in the tubers of maca as amino acids, alkaloids, fatty acids (linoleic, palmitic, oleic acid, etc), tannins, saponins, and several microelements as Cu, Su, Mn and Al (Lee *et al.,* 2016) which improved sexual dysfunction by increased serum testosterone concentration by enhancing the steroidogenesis ability of Leydig cells. Also, Ohta *et al.* (2016) indicates that maca might be enhanced bioavailability of testosterone or testosterone receptors and an improved response of Sertoli cells to follicle stimulate hormone (FSH) which effect positively on sperm functions.

The presented study is showed; the addition of maca aqueous extract to short- storage semen extenders had cytoprotective effects on increasing sperm characteristics by the direct free radical scavenging and by its ingredients as proteins, carbohydrates, amino acids, minerals, saturated fatty acids, unsaturated fatty acids and antioxidant substances (Dutta *et al.,* 2019). Our results indicated that supplementation aqueous extract of maca to ram semen extenders at 20 µg/mL was more suitable for amelioration ram sperm parameters (motility, livability, abnormality and integrity acrosome) than 5, 10 and 40 µg/mL during short term-storage at 5°C for 5th days. Furthermore, the current study was evaluated the influence of chilling extended ram sperm storage up to 2nd days at 5°C on conception rate as another direct effect between extenders contain aqueous extract of maca at 20 µg/mL and 0 µg/mL. On the basis of our findings, results speculate that adding maca aqueous extract to extender during short term-storage may increase its conception rate by improving sperm characteristics and extended semen antioxidant activity.

Then, supplementation aqueous extract of maca to ram semen extenders could cause a progressive and significant higher in ram semen quality compared with control semen extender. In this context, Del Prete *et al.* (2018) found that the best effects of maca supplementation on total motility, progressive motility and integrity acrosome of stallion sperm than in the control group during cooling storage. Furthermore, Aoki *et al.* (2019) indicated that the fertilization rate and sperm motility were 33.4% and 82.1% in medium containing maca extract than 14.3% and 53.9% in medium without maca extract, respectively. Also, the previous authors defined that rate of acrosome-reacted sperm in medium containing maca extract at concentrations of 4.0%, 8.0% and 16.0% (w/v) were significantly higher (68, 71, and 71%, respectively) than 44% in medium without maca (0% w/v). Also, several studies reported the effectiveness of maca on improving semen quality and quantity in stallions (Del Prete *et al.,* 2018), humans (Tafuri *et al.,* 2021) and canine (Del Prete *et al.,* 2022). Besides that, Leiva-Revilla *et al.* (2022) could be reported that in bovine semen the four dilutions of maca extract at 0, 1, 10, and 100 mg/mL post-thawing incubation for 24 hours indicated rectilinear motility up to 49, 60, 53 and 48 %, normal 88, 90, 87 and 87 %, intact live acrosome 21, 25, 40 and 30%, livability 22, 25, 41 and 30% and normal DNA 90.60, 92.29, 91.35 and 91.44%, respectively.

Then, the best of aqueous extract of maca on ram semen extenders during the short-term storage at 5˚C for 5 days may be related to antioxidant activity. In this context, Buyanbadrakh *et al.* (2020) who revealed that macaridine, macaene, macamides and alkaloids in maca has free-radical scavenging capacity and increase the levels of superoxide dismutase and glutathione. Also, amino acid in maca like arginine showed an improvement in sperm quality. A study presented by Özer Kaya *et al.* (2018) who found that equilibrated semen extender (up to 2 hours) including 0.0, 0.1, 0.5, 1.0, 5.0 and 10.0 mM of L-arginine had sperm motility 74.00, 77.00, 79.00, 77.00, 75.00 and 55.00% and integrity membrane 65.00, 65.00, 67.00, 68.00, 68.00 and 63.00%, respectively. Likewise, Mohammed *et al.* (2020) found that arginine acts as antioxidant substance through its ability to remove

different types of free radicals such as reactive oxygen species (ROS) included superoxide anion (O_2^-), peroxide radical (H_2O_2), hydroxyl radical (OH^-) and activate the production and catalyze of enzymatic antioxidant like superoxide dismutase (SOD), glutathione peroxidase (GOX) and catalase (CAT) which improved sperm activity. Besides that, Omar *et al.* (2021) noticed that 0.001, 0.100 and 1.000 µmol of L-arginine in ram semen extender indicated sperm motility was 30.00, 19.16 and 13.33%, dead sperm was 42.00, 54.33 and 65.00% and abnormal sperm was 22.33, 24.00 and 34.50% after storage at 4°C up to 72 hours, respectively. In addition, polyunsaturated fatty acids are presented in maca which played beneficial role in protected cell membrane of spermatozoa. In this assay, Leiva-Revilla *et al.* (2022) reported that normal head was 3, 3, 2 and 4% and fragmented DNA was 9.40, 7.71, 8.65 and 8.56 % post-thawing in bovine semen extender incubation for 24 hours contained 0, 1, 10, and 100 µg/mL of maca extract, respectively. On the other hands, elements in maca can be able to activate sperm function (Kerns *et al.*, 2018) who noticed that elements are known to play an important role for reproduction performance, specially zinc (Zn) are essential for reproduction and that their deficiency can lead to degenerative changes of sperm activity. Also, Zakošek *et al.* (2021) shown that enrichment of a sperm extender with calcium (Ca) could be improved sperm quality after thawing and consequently increase fertility rates by reducing cryopreservation-induced sperm damage.

Finally, sperm parameters were significantly higher after 2nd days of short- term storage at 5ºC with the maca dilutions contained 20 µg/mL which effects on fertility rate positively. According to Kumaresan *et al.* (2017) reported that these are the only parameters (motility, livability, normality and intact acrosome) of importance in determining fertility, reliance on only these parameters will not intuitively provide enough information regarding potential fertility to be a useful predictor. As well as, Espina-Ávila *et al.* (2021) the principal factor for artificial insemination (AI) was the best semen extenders which refluxed the most sperm characteristics led to the greatest fertility rate and productive performance.

CONCLUSION

The results showed that maca aqueous extract has an improvement in all sperm parameters (motility, livability, normality and intact acrosome), fertility rate and detraction of LPO level during short-term storage ram spermatozoa at 5ºC for up to five days. Then, the best results of sperm characteristics and pregnancy rate obtained at 20 µg /mL of maca aqueous extract at 2nd days of storage compared with all media. However, studies are required to elucidate the effects of maca aqueous extract levels on frozen sperm characteristics.

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إستخدام المستخلص المائي لمسحوق الماكا لدراسة فعاليته في التخزين القصير المدى للسائل المنوي للكباش وخصوبته

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الملخص

هدفت الدراسة إلى تقييم خصائص الحيوانات المنوية للكباش بعد إضافة المستخلص المائي للماكا إلى مخفف السائل المنوي. احتوت خمس وسائط من مخففات السائل المنوي للكبش مثل M0 و M1 و M2 و M3 و M4 على مستويات مختلفة من المستخلص المائي لماكا عند 0 و 5 و 10 و 20 و 40 ميكروغرام / مل تم تخزينها عند 5 درجات مئوية لمدة 5 أيام على التوالي. بعد ذلك ، تم تقييم خصائص الحيوانات المنوية مثل الحركة التقدمية للحيوانات المنوية ، الحيوية ، الشواذ ، سلامة الأكروسوم وتركيز بيروكسيد الدهون (LPO). كما تم تلقيح أربعين نعجة (ن = 20 في كل معامله) بواسطة M0 (كمجموعة كنترول) و M3 (كأفضل معاملة للحيوانات المنوية). أدت إضاقة المستخلص المائي للماكا بتركيز 20 ميكروغرام / مل إلي تحسين خصائص الحيوانات المنوية، وأيضاً قللت من شواذ الحيوانات المنوي و بيروكسيد الدهون (LPO) بـ مقارنة بالمخففات الأخرى أثناء التخزين عند 5 درجات مئوية لمدة 5 أيام. كذلك لوحظ تحسن في معدل الحمل الكلي للنعاج باستخدام M3 (79.17%) مقارنة مع M0 (65.38%) بعد التبريد علي 5 درجات مئوية لمدة يومين. تشير النتائج إلى أن المستخلص المائي للماكا بتركيز 20 ميكروغرام / مل يحافظ على خصائص الحيوانات المنوية عند درجة حرارة 5 درجات مئوية لمدة 5 أيام ويحافظ أيضاًعلى قدرتها على الخصوبة بعد اليوم الثاني من التبريد.