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IMPROVEMENT OF CHILLING SPERMATOZOA CHARACTERISTICS ANDTHEIR FERTILITY BY SUPPLEMENTATION OF L-ARGININE TO RAM SEMEN EXTENDER

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SUMMARY

This study was conducted to investigate the effect of L-arginine addition to ram semen on its short-term storability. Three healthy Rahmani rams were used for semen collection. Collected semen samples were diluted and divided into five equal aliquot treatments as T0, T1, T2, T3 and T4 including 0.0, 0.5, 1.0, 2.0- and 4.0-mM of L-arginine, respectively. All treatments were kept in refrigerator up to1 to 5 days. The sperm motility, viability, abnormal sperm, acrosome integrity, lipid peroxidation (LPO) level and fertility rate were evaluated. T4 group showed decreased (P<0.05) results in sperm motility, viability and acrosome integrity and also increased (P < 0.05) results in abnormal sperm and LPO level compared with T0, T1, T2 and T3. The highest (P<0.05) sperm motility, viability and acrosome integrity and the lowest (P<0.05) abnormal sperm and LPO levels were observed in T1 compared with T0, T2 and T3. Fertility rate was better with T1 thanT0. It is assumed that 0.5 mM addition of L-arginine to ram semen may be useful, and increasing doses may be harmful to sperm characteristics during short-term storage.

Keywords: Supplementation of L-arginine, ram spermatozoa quality, chilled Tris extender.

INTRODUCTION

Brown-Woodman and White (1974) showed that amino acids levels in ram seminal plasma as alanine and lysine were similar, while there were less levels of L-arginine, glycine, aspartic acid, glutamic acid and serine. Also, L-arginine concentrations were 0.14, 0.55, 1.11 and 0.78 mM in rete testis, epididymal plasma, seminal vesicle fluids, but it was 1.17 mM in seminal plasma collected by artificial vagina. Razmi *et al.* (2004) found that arginase enzymes activity at least at 9-10 to 20-31 IU/mg protein and bulbourethral gland has to > 60 IU/mg protein. Hence, L-arginine should be added a littleto semen extenders to improve functional integrity of spermatozoa (Sharideh *et al.*, 2015). Oyeyipo *et al.* (2015) found that L-arginine prevented bilayer phospholipids membrane peroxidation and production of nitric oxide (NO) which protected spermatozoa. In semen extenders, L-arginine being the main precursor of NO which is needed in living organisms for protein synthesis and thereby reducing lipid peroxidation (Santana *et al.*, 2016). Furthermore, El-Shahat *et al.* (2016) indicated that NO granted from L-arginine increases phosphorylation of flagella proteins and activation of sperm to penetrate the zonapellucid viscose. In addition, Kaya *et al.* (2017) found that no regulates ??? sperm capacitation and associated protein tyrosine phosphorylation that mediated through a cAMP/PKA-dependent pathway. Susilowati *et al.* (2019) reported that L-arginine has antioxidant properties which effectively scavenges hydrogen peroxide (H_2O_2) and superoxide anions (O_2-). These authors reported that chilling (up to 2 days) of goat semen extender contained 0 and 4 mM of L-arginine had sperm motility of 56.17 and 76.83%, livability sperm of 68.17 and 79.83% and intactness of plasma membrane of 46.33 and 56.53%, respectively. On the other hand, Abd-Allah *et al.* (2019) concluded that adding L-arginine improved the freezability and fertility of poor-quality Holstein bull spermatozoa. Also,Badr *et al.* (2020) demonstrated that supplementation of L-arginine to buffalo semen extender had positive effects on quality, fertilizing potentials, and improved antioxidant activities.

Artificial insemination (AI) by chilling ram semen extended with addition of L-arginine has not been adopted in the farms yet, probably due to a lack of information about the use of chilling semen under field conditions. Therefore, the addition of L-arginine was investigated to evaluate motility, livability, abnormal, acrosomal integrity and lipid peroxidation in chilling ram semen extender. Besides, fertility under field conditions after short-term storagability was evaluated.

MATERIALS AND METHODS

All animals' care and use procedures were approved by El-Serw Research Station belonging to

Animal Production Research Institute *(APRI)*, Agricultural Research Center, Ministry of Agriculture, Egypt. The experimental period was carried out from August 2020 up to February 2021.

Animals and diets:

Three mature Rahmani rams were clinically healthy and fertility-proven ranging from 3.0 and 3.5 years old and live body weight ≥ 75 kg were used. Rams kept in pen under uniformly environment conditions. All rams received 60% concentrate feed mixture (CFM) and 40% berseem hay (BH) as a source of roughage and rice straw (RS) presented as *Ad libitum*. Table (1) shows the compositions of CFM, BH and RS according to AOAC (2007). The pen was equipped with a separate feeder and drinker during experimental period.

Table 1. Chemical composition of CFM, BH and RS

Items	Chemical composition (% on dry matter basis)						
	DM	OM	CP	EE	CF	NFE	Ash
CFM*	89.91	87.77	14.40	7.09	2.41	63.87	12.23
BH	88.65	88.43	14.12	2.15	23.29	48.87	11.57
RS	92.83	80.23	3.08	1.49	36.88	38.78	19.77

DM=Dry matter, OM=Organic matter, CP=Crude protein, EE=Ether extract, CF=crude fiber NFE=Nitrogen free extract and Ash contents according to AOAC (2007) procedure, nitrogen free extract (NFE) was calculated by deference. *The CFM consisted of 26 % undecortecatedi cotton meal, 40 % yellow corn, 27 % wheat bran, 3.5 % molasses, 2 % limestone, 1 % common isalt and 0.5 % minerals imixture.

Semen collection and spermatozoa examinations:

Estrous ewe was used to stimulate rams for sexual activity. Semen samples were collected by warmed artificial vagina. Semen was collected as two ejaculates /ram /week up to 6 weeks. Immediately semen was brought to a laboratory to be examined physically (volume, color, smell and density) and microscopically (mass motility, individual motility, viability, abnormal, sperm cells concentration and acrosomal integrity). Semen contaminated with urine, water, blood or feces was discarded. Then, ejaculates that contained a volume from 0.5 to 2.0 ml, minimum semen concentration of 3 x 10^9 spermatozoa/ml, total motility higher than 80%, abnormal sperm less than15% and acrosome integrity more than 85% were used in this experiment.

Semen volume, concentration, progressive sperm motility, dead/life, abnormal spermatozoa, and acrosome integrity were described according to **Omar *et al.* (2021)**.

Lipid peroxidation (LPO):

The LPO activity was measured on 0, 1, 2, 3, 4 and 5 days of chilled extended semen in T0, T1, T2, T3 and T4. The LPO concentration wasquantified bycommercial kit LPO-586 with sensitivity at 0.5µM and 0.5 to 4.0 µM as range curve (Oxis Research, Burlingame, CA, US).

Preparation of Tris semen extenders with L-arginine levels:

Collected semen samples were distributed in clean tubes as T0, T1, T2, T3 and T4 and diluted with Tris-based extender at a rate of 1: 6. Then, semen extenders in T0, T1, T2, T3 and T4 tubes were supplied with 0.0, 0.5, 1.0, 2.0 and 4.0 mM of L-arginine, respectively. Then, all treatments were stored in chilling state for up to 5 days in refrigerator. Tris -based semen extenderingredients with L-arginine levels are presented in Table (2).

Table 2. Ingredients of Tris-based extenders and different L-arginine levels

Ingredients	Semen extender media				
	T0	T1	T2	T3	T4
Tris (g)	3.634	3.634	3.634	3.634	3.634
Citric acid(g)	1.990	1.990	1.990	1.990	1.990
Fructose (g)	0. 500	0. 500	0. 500	0. 500	0. 500
Egg yolk (ml)	15.000	15.000	15.000	15.000	15.000
L-arginine(mM) *	0.000	0.500	1.000	2.000	4.000
Penicillin (IU)	1000	1000	1000	1000	1000
Streptomycin (mg)	200.000	200.000	200.000	200.00	200.00
Distilled water added up to	100ml	100ml	100ml	100ml	100ml

* Produced by: Bst Feed Additives Co., Limited.

Chilling extended semen procedures:

The five diluted semen tubes were dropped in a 500 ml beaker containing water at room temperature with a thermometer in order to facilitate periodic checking of temperature during the chilling periods. Tubes were covered with dark plastic sheath. Then, the beaker was placed in refrigerator and gradually cooled until their temperature reached 5°C during a period of 1.5 - 2.0 hours. During each storing time (0, 1, 2, 3, 4 and 5 days) the percentages of sperm motility, livability, abnormal spermatozoa, acrosomal intact and LPO concentration were determined.

Fertility trial:

Fertility was carried out by using T0 and T1 after two days of storage at 5°C. The fertility test used40 Rahmani ewes similar in reproductive and productive performance. Ewes were divided into two groups (*n*=20/group), the first and the second groups were inseminated with T0 and T1, respectively. In the breeding season, the oestrus symptoms of ewes were checked twice a day with a time interval of about 12 hours using a teaser ram. Ewes exhibited estrus were inseminated using1.0 ml of warm extended semen (1.0 ml for T0 and 1ml for T1). The cervical insemination was applied two times after12 and 24 hours of showing estrous by using a vaginal speculum and penlight. At insemination time, the vaginal speculum lubricated with glycerol and inserted into ewe vagina to open it. Then, the gun of the extended semen dose was slowly deposited as deep as possible into the front of Os-cervix. The ewe considered pregnant when two oestrous cycles passed without expressing heat. If any ewe returned to estrus, it was served once again. The conception rate, fertility rate, lambing rate and litter size were determined according to Khalifa *et al.* (2023).

Statistical Analysis:

Data were tabulated according to the observed variables. The results of statistical analysis were carried out using Statistical Product and Service Solutions (SPSS Statistics version 26 at 2020). The Duncan's *post hoc* test of SPSS (with less significance difference test at 95% level of significance) used to determine significant differences among all L-arginine treatments during different days of chilling. The test in a completely randomized design as the following model: -

$y_{idK} = \mu + T_i + C_d + e_{idK}$

Y_{ik} = an observation.

μ = the overall mean.

T_i= the effect of L-arginine treatments (from T0 to T4).

C_d= the effect of chilling days (from day 0 to 5 days)

e_{idk}= residual error.

RESULTS

The physical and microscopic raw semen characteristics of Rahmani rams are presented in Table (3) which showed that there were no significant differences among physical and microscopic examination of raw semen samples.

Table 3. Physical and microscopic examination of raw semen characteristics of Rahmani rams

Semen indicators	Physical characters of ram semen samples		
	Ram1	Ram2	Ram3
Volume(ml)	2.69±0.05	2.68±0.09	2.66±0.05
Color by observation	White yellowish	White yellowish	White yellowish
Odor by smelling	Idealistic	Idealistic	Idealistic
Density, scale from 0 to 3*	2.62±0.14	2.64±0.16	2.68±0.15
	Microscopic characters of ram semen samples		
Mass motility, scale from 0 to 5**	3.96±0.22	3.92±0.26	4.08±0.23
Individual motility (%)	85.42±1.44	85.85±1.89	85.55±1.85
Viability (%)	89.75±0.95	89.48±1.13	89.88±1.02
Abnormalities (%)	9.83±0.34	9.92±0.39	9.58±0.31
Sperm cells concentration(n×10^9)	3.78±0.07	3.79±0.06	3.80±0.12
Acrosome integrity (%)	90.67±0.86	90.58±1.05	90.90±0.98

* Scales 0: watery, 1:soapy, 2: milky and 3: creamy, ** scale 0; no waves, 1: slow waves, 2: moderate waves, 3: rapid waves, 4: rapid and succession waves and 5: rapid, succession and vigorous waves.

Effect of L-arginine on percentage of progressive sperm motility:

The highest sperm motility was observed between the 1st and 2nd days of chilling. The T2, T3 and T4 had decreased (P<0.05) progressive sperm motility at the 4th and 5th days compared with T1. The current data indicated that there was gradually decrease (P<0.05) in sperm motility among T0, T1, T2, T3 and T4 through the 5th days of chilling Table (4).

Table 4. Progressive motility of ram spermatozoa extended without L-arginine (T0) and with L-arginine (T1, T2, T3 and T4) during chilling for up to 5th days

Semen extenders with L-arginine levels	Days of chilling					
	0 day	1 day	2 days	3 days	4 days	5 days
To	85.17^{Aa}±0.83	78.93^{Ab}±1.12	76.52^{Ab}±1.79	68.33^{Bc}±2.16	60.92^{Bd}±1.89	45.83^{Be}±1.92
T1	85.20^{Aa}±0.85	80.50^{Ab}±0.97	78.92^{Ab}±1.30	73.75^{Ac}±1.95	65.67^{Ad}±2.49	51.58^{Ae}±2.43
T2	85.19^{Aa}±0.88	77.42^{Ab}±1.02	72.72^{Bc}±1.56	67.69^{Bd}±2.11	57.30^{Be}±2.52	46.21^{Bf}±2.25
T3	85.18^{Aa}±0.87	75.75^{ABb}±1.01	71.39^{Bc}±1.53	66.65^{Bd}±2.13	54.50^{BCe}±2.52	44.25^{Bf}±2.21
T4	85.15^{Aa}±0.81	75.55^{Bb}±1.11	70.29^{Bc}±1.43	65.59^{Bd}±2.23	54.50^{Ce}±2.32	43.25^{BCf}±2.31

T0, T1, T2, T3 and T4 tubes were supplied with L-arginine of 0.0, 0.5, 1.0, 2.0 and 4.0 mM, respectively, The mean values with different superscripts in the same column with $^{A, B \text{ and } C}$ and row with $^{a, b, c, d, e \text{ and } f}$are significantly different at P<0.05.

Effect of L-arginine on the viability percentage of spermatozoa:

Data presented in Table (5) revealed that T1 increased ($P < 0.05$) their viability spermatozoa when compared with other concentrations at 1.0, 2.0 and 4mM during the chilling period. It is clear that no significant effect was observed between T0 and T1 extenders on spermatozoa livability at the 2nd days of chilling, but more livability spermatozoa was obtained in T1 (80.97 %) than that of T0 (78.59 %). The most livability spermatozoa were observed among all treatments between the 1st and 2nd days of chilling. The results recorded that degraded ($P<0.05$) livability in extended spermatozoa that stored from 1 to 5 days.

Table 5. Livability of ram spermatozoa extended without L-arginine (T0) and withe L-arginine (T1, T2, T3 and T4) during chilling up to 5th days

Semen extenders with L-arginine levels	Days of chilling					
	0 day	1 day	2 days	3 days	4 days	5 days
To	$91.44^{Aa}\pm1.84$	$85.52^{Ab}\pm1.25$	$78.59^{Ac}\pm1.74$	$69.31^{Bd}\pm2.16$	$61.95^{Be}\pm1.87$	$55.43^{Bf}\pm1.42$
T1	$92.52^{Aa}\pm1.75$	$86.59^{Ab}\pm1.37$	$80.97^{Ab}\pm1.33$	$74.55^{Ac}\pm1.95$	$66.67^{Ad}\pm2.69$	$61.48^{Ae}\pm2.33$
T2	$91.19^{Aa}\pm1.86$	$83.72^{Ab}\pm1.32$	$74.46^{Bc}\pm1.46$	$66.89^{Bd}\pm2.11$	$59.56^{Be}\pm2.42$	$54.68^{Bf}\pm2.35$
T3	$90.88^{Aa}\pm1.68$	$82.79^{ABb}\pm1.41$	$74.27^{BCc}\pm1.55$	$66.61^{BCd}\pm2.13$	$58.48^{BCe}\pm2.62$	$54.65^{BCf}\pm2.25$
T4	$89.55^{Aa}\pm1.65$	$81.63^{Bb}\pm1.21$	$73.63^{Cc}\pm1.46$	$65.59^{Cd}\pm2.23$	$56.56^{Ce}\pm2.42$	$52.55^{Cf}\pm2.34$

T0, T1, T2, T3 and T4 tubes were supplied with L-arginine of 0.0, 0.5, 1.0, 2.0 and 4.0 mM, respectively, The mean values with different superscripts in the same column with $^{A, B \text{ and } C}$ and row with $^{a, b, c, d, e \text{ and } f}$are significantly different at $P<0.05$.

Effect of L-arginine on the percentage of abnormal spermatozoa:

Table (6), showed that T4 had increased ($P < 0.05$) percentage of the abnormal spermatozoa during all times of chilling. However, abnormal spermatozoa were lower ($P < 0.05$) in T0 and T1 when compared with T2, T3 and T4 at the 2nd day of chilling. The lowest percentage of abnormal spermatozoa was detected in T1 from 1st to 5th days of storage. The abnormal spermatozoa in T0, T1, T2, T3 and T4 were increased ($P<0.05$) by increasing storing time and reached the maximum values up to 47.58, 41.92, 49.85, 51.96 and 52.55% at the 5th days of chilling, respectively. Generally, the percentage of abnormal spermatozoa significantly ($P<0.05$) increased gradually at the 5th days of chilling when compared with those chilling times at the 1st, 2nd, 3rd and 4th days.

Table 6. Abnormal (%) of ram spermatozoa extended without L-arginine (T0) and with L-arginine (T1, T2, T3 and T4) during chilling up to 5th days

Semen extenders with L-arginine levels	Days of chilling					
	0 day	1 day	2 days	3 days	4 days	5 days
To	$9.58^{Af}\pm0.47$	$12.83^{Ce}\pm0.53$	$16.77^{Bd}\pm0.46$	$27.83^{Bc}\pm2.76$	$37.92^{Bb}\pm1.13$	$47.58^{ba}\pm1.08$
T1	$9.18^{Af}\pm0.29$	$12.25^{Ce}\pm0.31$	$15.42^{Bd}\pm0.95$	$22.00^{Cc}\pm0.87$	$32.50^{Cb}\pm1.16$	$41.92^{Ca}\pm1.56$
T2	$9.78^{Af}\pm0.15$	$13.43^{Ce}\pm0.34$	$18.80^{Ad}\pm0.46$	$29.32^{ABc}\pm1.41$	$39.75^{ABb}\pm1.15$	$49.85^{Aa}\pm1.19$
T3	$9.98^{Af}\pm0.26$	$14.25^{Abe}\pm0.35$	$19.92^{Ad}\pm0.94$	$31.54^{ABc}\pm0.85$	$41.53^{ABb}\pm1.14$	$51.96^{ABa}\pm1.54$
T4	$10.44^{Af}\pm0.22$	$15.79^{Ae}\pm0.37$	$21.80^{Ad}\pm0.48$	$32.22^{Ac}\pm1.44$	$42.78^{Ab}\pm1.11$	$52.55^{Aa}\pm1.17$

T0, T1, T2, T3 and T4 tubes were supplied with L-arginine of 0.0, 0.5, 1.0, 2.0 and 4.0 mM, respectively, The mean values with different superscripts in the same column with $^{A, B \text{ and } C}$ and row with $^{a, b, c, d, e \text{ and } f}$ are significantly different at $P<0.05$.

Effect of L-arginine on the percentage of acrosome integrity:

Table (7) showed that percentage of acrosome integrity between T0 and T1 at the 2nd day of chilling that the values ranged from 85.25to 86.50%, respectively. Significant differences ($P<0.05$) and were higher in acrosome integrity in T1 than T0, T2, T3 and T4 from the 3rd to 5th days of chilling. The highest percentage of acrosome integrity was observed among all treatments between the 1st and 2nd days of chilling. Treatments had ($P<0.05$) decreased the percentages acrosome integrity at the 4th and 5thdays compared with (T1). The current data indicated that there was a gradual decrease ($P<0.05$) in acrosome integrity among the different treatments through the five days of chilling.

Table 7. Acrosome integrity (%) of ram spermatozoa extended without L-arginine (T0) and with L-arginine (T1, T2, T3 and T4) during chilling up to 5th days

Semen extenders with L-arginine levels	Days of chilling					
	0 day	1 day	2 days	3 days	4 days	5 days
To	$90.25^{Aa}\pm0.51$	$88.50^{Aa}\pm0.72$	$85.25^{Ab}\pm1.29$	$78.11^{Bc}\pm1.47$	$69.17^{BCd}\pm1.57$	$66.42^{Be}\pm1.38$
T1	$91.42^{Aa}\pm0.58$	$89.73^{Aa}\pm0.90$	$86.50^{Ab}\pm1.09$	$82.33^{Ac}\pm1.50$	$75.00^{Ad}\pm1.97$	$72.67^{Ae}\pm1.96$
T2	$88.94^{Aa}\pm0.48$	$85.67^{Aa}\pm0.62$	$83.58^{ABb}\pm1.16$	$77.17^{Bc}\pm1.35$	$68.59^{BCd}\pm1.66$	$64.55^{BCe}\pm1.35$
T3	$87.88^{Aa}\pm0.68$	$85.34^{ABa}\pm0.44$	$82.25^{BCb}\pm1.65$	$75.91^{BCc}\pm2.33$	$68.48^{BCd}\pm2.65$	$64.65^{BCe}\pm2.26$
T4	$87.55^{Aa}\pm0.65$	$84.63^{Bb}\pm0.23$	$81.33^{Cc}\pm1.56$	$74.39^{Cd}\pm2.25$	$66.56^{Ce}\pm2.32$	$62.55^{Cf}\pm2.33$

T0, T1, T2, T3 and T4 tubes were supplied with L-arginine of 0.0, 0.5, 1.0, 2.0 and 4.0 mM, respectively, The mean values with different superscripts in the same column with $^{A, B \text{ and } C}$ and row with $^{a, b, c, d, e \text{ and } f}$ are significantly different at P<0.05.

Lipid peroxidation (LPO) concentration on semen extenders as affected without L-arginine (T0) and with L-arginine (T1, T2, T3 and T4) during chilling days up to 5th days:

Figure (1) summarizes the concentration of LPO in T0, T1, T2, T3 and T4 semen extenders during short-storage time up to 5th days. In the 1st day of chilling, the T1 tube has lower (P<0.05) LPO concentration up to 1.39 µM than 1.61, 1.51, 1.58 and 1.59 µM in T0, T2, T3 and T4 tubes, respectively. The results observed that the lowest (P<0.05) of LPO concentration was in T1 (1.69 µM) compared with T0 (1.99 µM), T2 (1.87 µM), T3 (1.92 µM) and T4 (1.95 µM) at 2nd days of chilling. However, at 5th days of chilling, the LPO concentration reached to 3.73, 2.94, 3.25, 3.61 and 3.79 µM in T0, T1, T2, T3 and T4 tubes, respectively.

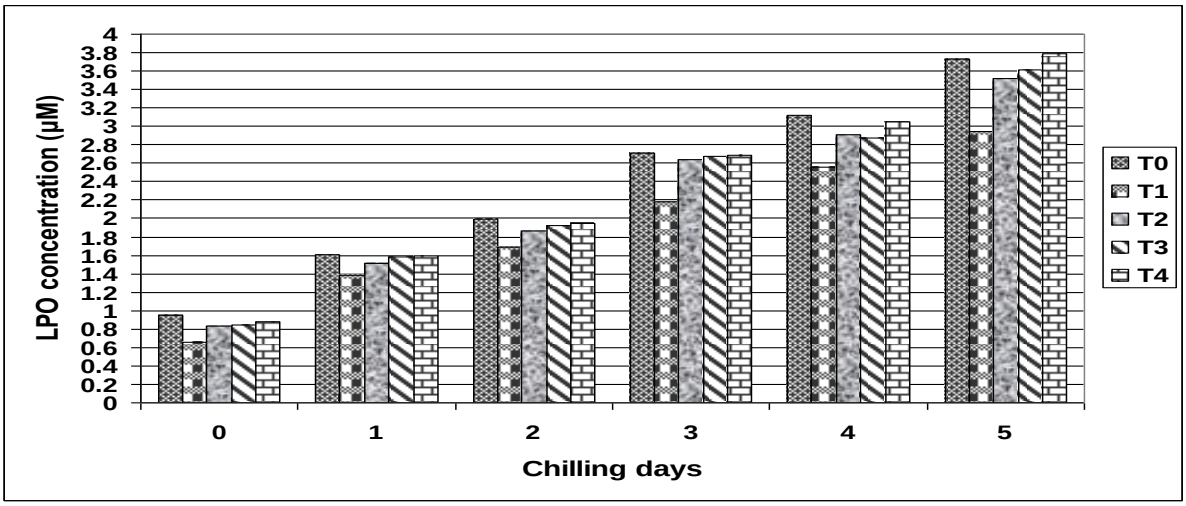

Fig.1. Lipid peroxidation (LPO) concentration of semen extenders as affected by addition of L-arginine (T0, T1, T2, T3 and T4) during chilling up to 5th days.

***Fertility trail**:*

The fertility trail was carried out to compare between T0 and T1 after short- storage up to 2nd days. The results indicated that calculation of the best conception rate, number of lambing, fertility rate and litter size were obtained in T1 ewes compared with those ewes in T0 after two services of AI processing. The improvement in lambing rate included single, twins and triplets were 10.00, 85.00 and 5.00% in T1. While as 42.11, 57.89, 0.00% in T0 ewes through the 1st and 2nd services of AI processing. Therefore, AI processing of the ewes with T1 of chilling up to 2 days showed slightly higher fertility percentage than chilling semen without L-arginine Table (8).

Table 8. Effect of addition of L-arginine as T0 or T1 on fertility assessments of cooling ram semen dilution up to 2 days

Items	Insemination ewe groups	
	T0	**T1**
No. of ewes inseminated at 1st service	20.00	20.00
No. of ewes conceived at 1st service	16.00	18.00
Conception rate at 1st service (%)	80.00	90.00
No. of ewes lambed at 1st service	16.00	18.00
No. of ewes lambed single at 1st service	5.00	2.00
No. of ewes lambed twins at 1st service	11.00	16.00
No. of lambs born at1st service	21.00	34.00
Fertility rate at 1st service (%)	105.00	170.00
Litter size at 1st service (%)	131.25	188.88
No. of ewes inseminated at 2nd service	4.00	2.00
No. of ewes conceived at 2nd service	3.00	2.00
Conception rate at 2nd service	75.00	100.00
No. of ewes lambed at 2nd service	3.00	2.00
No. of ewes lambed single at 2nd service	3.00	0.00
No. of ewes lambed single at 2nd service	0.00	1.00
No. of ewes lambed triplet at 2nd service	0.00	1.00
No. of lambs born at2nd service	3.00	5.00
Fertility rate at 2nd service (%)	100.00	250.00
Litter size (%)	100.00	250.00
Lambing rate		
Total ewes lambing through 1st and 2nd service	19.00	20.00
Total ewes lambing single through 1st and 2nd service	8.00	2.00
Single rate (%)	42.11	10.00
Total ewes lambing twins through 1st and 2nd service	11.00	17.00
Twinning rate (%)	57.89	85.00
Total ewes lambing triplet through 1st and 2nd service	0.00	1.00
Triplet rate (%)	0.00	5.00

DISCUSSION

L-arginine plays an important role in improving ram spermatozoa characters (Hassanpour *et al.,* 2010) by enhancing the rate of glycolysis, resulting in higher rates of ATP and lactate generation in spermatozoa. Sangeeta *et al.* (2015) reported that L-arginine has a protective effect against lipid peroxidation on ram spermatozoa. Preservation processes can cause loss of motility and viability of spermatozoa, consequently fertilization deteriorate (Sarıozkan *et al.,* 2013). The lowest levels of reactive oxygen species (ROS) are factors essential for normal functions of spermatozoa (Sarıozkan *et al.,* 2014). Excessive ROS production caused impaired spermatogenesis, sperm motility count decreased, membrane damage and fertilization deterioration (Turk, 2015). L-arginine protects spermatozoa against motility losses and membrane disorders, inhibits lipid peroxidation and supports the antioxidant capacity (Shan, *et al.,* 2013). In some studies, to determine the effects of L-arginine on sperm characteristics, El-Shahat *et al.* (2016) concluded that 0.04 mM of L-arginine for 90 min was the best concentration to be used for *in vitro* induction of acrosome reaction. Also, Özer Kaya *et al.* (2018) 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 motility at 74.00, 77.00, 79.00, 77.00, 75.00 and 55.00% and integrity membrane at 65.00, 65.00, 67.00, 68.00, 68.00and 63.00%, respectively. Besides, Omar *et al.* (2021) noticed that 0.001, 0.100 and 1.000 µmol of L-arginine in ram semen extender indicated sperm motility at 30.00, 19.16 and 13.33%, dead sperm at 42.00, 54.33 and 65.00% and abnormal sperm at 22.33, 24.00 and 34.50% after storage in 4°c up to 72 hours, respectively. On the other hand, Shikh *et al.* (2014) found that sperm goat motility in Tris-citric egg yolk extender contain 0.00, 0.01 and 0.10 mM of L-arginine was 76.13, 79.00 and 77.00% after 6 hours of incubation, respectively. Furthermore, AL-Ebady *et al.* (2012) suggested that 0.005 mM of L-arginine into cooling bull semen extender can improve sperm motility up to 59.41% compared with 40.29% in control. Also, Susilowati *et al.* (2019) found that high L-arginine up to 6 mM did not improve goat sperm motility (41.17%), viability (42.17%), number of motile sperm (4111.85), intact plasma membrane (18.83%) and LPO concentration (3245.25 nmol/ml) compared with low level up to 4 mM can achieve sperm motility at 46.53%, viability at 47.83%, number of motile sperm at 4647.18, intact plasma membrane at %22.67 and LPO concentration at 2850.25 nmol/ml after 5thdays of cooling.

In our study, L-arginine in T1 has the highest (P<0.05) sperm characteristics after storage at 5°C up to five days. Similarly, Özer Kaya *et al,* (2018) and Omar *et al.* (2021) confirmed that positive result could be obtained from cooling sperm motility, viability and integrity acrosome at 0.5 mM of L-arginine. The lowest sperm motility, viability and integrity acrosome in extenders that supplemented with the most of L-arginine after cooling may be an indicative of the toxicity of these doses (Omar *et al.*, 2021). High level of L-arginine has a drastic effect on semen quality (El-Shahat *et al.*, 2016, Kaya *et al.*, 2017, and Özer Kaya *et al.*, 2018).

The current results showed that increasing of L-arginine level in semen extender reflected on the highest(P<0.05) LPO concentration obtained in T2, T3 and T4 tubes compared with T1 tube during chilling days. In this context, Susilowati *et al.* (2019) noticed that after cooling goat semen extender the lowest LPO concentration reached to 2850.25 nmol/ml in extender contains 4.0 mM of L-arginine however, it 3245.25 nmol/ml in extender contains 6.0 mM of L-arginine.

In the current study, semen in T1 up to two chilling days may enhance fertility rate compared with the T0 extender. The mechanism of the increments was observed in fertility rate in T1 extender could be interpreted by arginase activity of cooling semen. L-arginine can improve the rate of ATP and lactate generation (Hassanpour *et al.*, 2010, Sarıozkan *et al.*, 2014 and Turk, 2015). L-arginine could reduce toxic effects of ROS and lipid peroxidation (Sarıozkan *et al.*, 2014, Özer Kaya *et al.*, 2018, Susilowati *et al.*, 2019 and Omar *et al.*, 2021). Supplied extender by limited NO which majors to protein synthesis and thereby reducing lipid peroxidation, (Santana *et al.*, 2016). The NO is a source of reactive nitrogen species (RNS) that can act in inter and intracellular signaling and as an antioxidant (Chen *et al.*, 2018). Also, NO can activate ram sperm to penetrate the zona pellucid viscose (El-Shahat *et al.*, 2016). NO regulates sperm capacitation (Kaya *et al.*, 2017). L-arginine can scavenge effectively for H_2O_2 and O_2- which is known to deteriorate sperm motility (Susilowati *et al.*, 2019).

CONCLUSION

The concentration of0.5 mM of L-arginine is beneficial to ram spermatozoa, but either 1.0- or 4.0-mMware not effective on sperm quality during short-term storage. Also, this level (0 .5mM)in ram semen extender can improve field fertility rate of ewes compared to control semen extender.

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الملخص العربي

تحسين خصائص وخصوبة الحيوانات المنوية المبردة عن طريق إضافة الحمض الأميني إل-أرجينين إلى مخففات السائل المنوي للكباش

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أجريت هذه الدراسة لتقييم تأثير إضافة الحمض الأميني إل-أرجينين على قابلية تخزين السائل المنوي للكباش. تم استخدام ثلاثة كباش رحماني وتم جمع عينات السائل المنوي وبعد تخفيفها قسمت إلى خمس مجموعات متساوية. مجموعة كنترول (T0) وتمت إضافة إل-أرجينين في T_1وT_2وT_3وT_4بمعدل ٠,٥ - ١,٠ - ٢,٠ - ٤,٠ ملي مول إلى هذه التخفيفات. تم حفظ جميع التخفيفات في الثلاجة (لمدة٥ أيام). تم تقييم حركة الحيوانات المنوية، والحيوية، والحيوانات المنوية الشاذة وسلامة الأكروسوم ومستوى بيروكسيد الدهون ومعدل الخصوبة. وجد أن المجموعة T٤(٤,٠ ملي ال - أرجينين) قلت فيها حركة الحيوانات المنوية المبردة، وحيويتها وسلامة الأكروسوم، وأيضًا زادت بشكل ملحوظ من مستوى الحيوانات المنوية الشاذة ومستوى بيروكسيد الدهون مقارنة مع المجاميع الأخرى. وكانت أعلى حركة للحيوانات المنوية المبردة وحيويتها وسلامتها وأقل مستوى للحيوانات المنوية الشاذة ومستوى بيروكسيد الدهون في المجموعة (T1) ٠,٥ ملي مول – أرجنيين مقارنة بتركيزات إل-أرجينين في T2و T3و..T0 كان معدل الخصوبة أفضل عندما حدث التلقيح باستخدام السائل المنوي المخفف مع T1 مقارنة بالمخففT0. نستنتج من ذلك أن إضافة ٠,٥ ملي مول من الأرجينين في مخفف السائل المنوي قد يكون مفيدًا. لكن زيادة ال – أرجينين من ١,٠ الي٤,٠ ملي مول قد تكون ضارة بجودة الحيوانات المنوية أثناء التخزين على المدي القصير يمكن بمزيد من الدراسات الكشف عن مقدرة ال – أرجينين عند اضافته الي مخففات السائل المنوي المجمد.