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Age-Dependent Computer-Assisted Sperm Analysis of Buck Semen Supplemented with Different Antioxidants

Norhan Hamdy Nasr[1], Nehal A. Abu Elnaga[1], Mervat Ahmed AbdRabou[2], Nasser Ghanem [3*]

[1]Zoology and Entomology, Department, Faculty of Science (Girls), Al-Azhar University, Cairo, Egypt

[2]Biololgy Department, College of Science, Jouf University, P.O. Box: 2014, Sakaka, Saudi Arabia

[3] Animal Production Department, Faculty of Agriculture., Cairo University, Giza, Egypt.

[*]**Corresponding author**: **Nasser Ghanem**, **Email**: nassergo@agr.cu.edu.eg,

Mobile Phone: +01002986157.

ABSTRACT

Background: Sperm cryopreservation is necessary for genetic sharing on a national and international scale.

Objective: This study aimed to examine, after treatment with various antioxidants, the link between buck age and sperm quality using the computer-assisted sperm analysis (CASA) method.

Material and methods: Collected semen from 16 younger and 14 older New Zealand rabbit bucks. The obtained semen was assessed by the CASA system using ejaculates with at least 70% progressive motility. Separated semen samples into eight liquors and diluted (1:1 v/v) with rabbit semen extender supplemented with all antioxidant combinations (melatonin (M), L-carnitine (LC), cysteine (Cys), LC+M, M+Cys, LC+Cys, and LC+Cys+M) and untreated group.

Results: The casa's characteristics were higher in young than older bucks. In comparison to controls, which recorded the lowest values, the addition of antioxidants increases total and progressive sperm motility in all experimental groups significantly. All experimental group's antioxidant supplementation levels and the control group were significantly lower than fresh pooled semen. Additionally, all CASA parameters increased compared to the control group when buck semen was supplemented with different antioxidants.

Conclusion: In contrast to younger bucks, administering several antioxidants (LM, LS, MS, and LMS) has a more substantial positive effect on sperm motility and CASA parameters in older bucks. Thus, age has a substantial role in defining semen's quality. Additionally, treatment with either a single (L, M, and S) or a combination of antioxidants enhances buck semen's motility and CASA properties (LM, LS, MS, and LMS).

Keywords: Bucks; CASA; Antioxidants; Melatonin; L-carnitine; Cysteine.

INTRODUCTION

Because commercial hybrid lines account for the majority of the world's rabbit meat output, the relevance of rabbit breeds chosen for their ability to provide meat has considerably declined [1]. FAO recommendations have incorporated most rabbit breeds into national strategies to safeguard genetic resources [2]. Semen cryopreservation is a helpful tool for protecting animal species when it comes to assisting the storage of gametes in a gene bank using the ex-situ in vitro procedure [3,4].

Rabbit cryopreserved sperm has primarily been employed for experimental purposes due to reduced fertility/prolificacy outputs [5]. In contrast, rabbit artificial insemination (AI) is often performed for a short period with fresh or cooled semen [6,7]. Changes in sperm cell integrity are detected during cryopreservation and are ascribed to intracellular ice formation, and cry-protectant[8].

One of the critical structures altered by cryopreservation is the sperm plasma membrane [9], where reactive oxygen species (ROS) formation rises, and antioxidant levels decrease during sperm cryopreservation and thawing. The water volume of cells was dramatically affected by freezing and thawing. Furthermore, spermatozoa lose most of their cytoplasm at the end of development and lack significant antioxidant-rich cytoplasmic components [10]. Therefore, the cryopreservation procedure has severe sperm damage, significantly lowering its fertilization ability [11].

Deterioration of the cell membrane may be due to both short-term (liquid) and long-term (frozen) storage of semen [12]. The mature sperm cells lack a major cytoplasmic component carrying antioxidants to combat the harmful effects of lipid peroxidation (LPO) and reactive oxygen species (ROS). Their membranes include a high concentration of unsaturated fatty acids. Therefore, during the freezing and thawing processes, free radicals such as hydrogen peroxide, superoxide anion, and hydroxyl radicals can cause structural damage to sperm membranes, making sperm cells extremely vulnerable to LPO [12,13].

Increased ROS generation during cryopreservation is related to lower post-thaw viability, sperm function, motility, and fertility [10]. The term "responsibility" refers to the act of determining whether or not a person is responsible for his or her actions. They found that the quality of post-thaw spermatozoa improved when compared to controls using standard andrological testing [14, 15].

Antioxidants applied before freezing may prevent the harmful impacts of oxidative stress [16]. The scavenging of free radicals, which can result in the lipid peroxidation of sperm plasma membranes, is a crucial role of antioxidants [17]. Furthermore, antioxidants have been shown to improve the viability and motility of cryopreserved and liquid-stored sperm cells [18].

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Antioxidants are, therefore, the first line of defense against free radical-induced oxidative stress [19].

This study's objective was to assess and compare the effects of antioxidant supplementation on CASA parameters in rabbit semen at various ages using melatonin, cysteine, and L-carnitine, among other antioxidants.

MATERIAL AND METHODS

Experimental design

Using three well-known antioxidants alone or combined with recommended-dose supplements, the study had planned to investigate the effects on the sperm quality of buck semen following cryopreservation. They evaluated separately the semen sample and the afterward cryopreserved ejaculates with progressive motility of 70%. Eight aliquots of the high-quality pooled semen samples were created. They were then diluted (1:1 v/v) with rabbit semen extender and supplemented with all possible antioxidant combinations at the specified doses (melatonin 10-6M - cysteine 10-3M & L-carnitine 10-3M). Finally, the present study analyzed the pre-freezing and motion characteristics of all CASA parameters.

Experimental animals, feeding and management

This study was conducted between December 2019 and March 2020 and September 2020 and December 2020 at the Animal Physiology Laboratory, Animal Production Department, Faculty of Agriculture, Cairo University, Giza, Egypt. The Faculty of Agriculture at Cairo University's research ethics committee approved each experimental procedure.

In the present study, twenty-four New Zealand buck rabbits had been used with an average weighed of 3.5 to 4 kg at the start of the trial were kept in a semi-closed rabbit housing system for the study. They continued to operate under normal climatic conditions, with a cycle of 16 hours of light and 8 hours of darkness. Fed on commercial pellet meal (20% protein) and were given unrestricted access to water. The animals were divided into two groups, the first group (12 rabbits): aged 10 to 12 months, and the second group (12 rabbits): aged 5 to 6 months.

Semen collection

All experimental rabbits' sperm samples were taken in calibrated tubes between 8:30 and 9:30 in the morning using a sterile artificial vagina that was adjusted to have the proper internal pressure and temperature (41–44 ^{0}C).

The collecting tubes were wrapped in rubber to protect against the effects of light and heat. Collected two consecutive ejaculates twice a week from each rabbit. Ejaculates are immediately transported to the lab after collection and maintained there in a water bath at 38.5 ^{0}C until the relevant examination [20].

Semen evaluation using Computer Assisted Sperm Analysis (CASA)

CASA device used to examine rabbit sperm (Sperm vision TM software mini tube Hauptstra Be 41.84184 Tiefenbach, Germany). The semen samples were analyzed through a technique under the manufacturer's instructions. During the analysis, the extended semen samples were kept at 370C. The sample's total volume (300uL) and dilution ratio (1:20) were 20 ML of semen and 280 ML of sodium chloride (0.9%). The motion characterization was recorded using the CASA instrument, which also recorded the following motion parameters: Distance Curved Line (DCL, microns), Distance Average Path (DAP, microns), Distance Straight Line (DSL, microns), velocity curved Line (VCL, microns/sec), Velocity Average Path (VAP, microns/sec), straight velocity Line (VSL, microns/sec), linearity (LIN=VSL/VCL), Straightness (STR= VSL/ (ALH, microns). The ratios of spermatozoa with linearity and straightness were manually recalculated. Only ejaculates with progressive motility of at least 70% were pooled [20].

Semen Cryopreservation

After CASA testing, the pooled semen was evenly divided into eight groups and diluted into 1:1 v/v with rabbit semen extender enriched with antioxidants, melatonin 10-6M, cysteine 10-3M, and L-carnitine 10-3M. All groups were cooled to 5 °C for 2 hours as an equilibrium period. All studies used the CASA equipment to assess motion characteristics again after cooling. French plastic straws (0.25 ml) were filled with diluted semen and sealed with polyphenol powder using an automatic machine. After that, to prevent cold shock, straws fixed horizontally on a metal rack were placed in a freezing floating boat and exposed to liquid nitrogen (N2) vapors. After 15 minutes, the loaded straws were instantly lowered into the liquid nitrogen and held until their respective evolution [20].

Semen extender diluent: Tries hydroxyl methyl amino methyl (3.03gm) from sigma company –T1503-500G; 1.59 grams of citric acid from Riedel de Haen -27109; From Sigma - G8270-100G, D + glucose (0.9 gm). Also, 0.5 mL of antibiotic from Bio west-penicillin–streptomycin checker-dispersed in 100 mL of distilled water. Adding three moles of dimethyl sulfoxide (Riedel de Haen -27109) and 0.1 moles of sucrose (BioBAstigimgC12H2201) to 100 mL of extender will cause it to freeze (diluent) [20]. Antioxidant preparation: The molecular weight of melatonin is 232.28 g/L (Sigma, M5250-1G). Then re-dilute by taking ten microliters from the diluent and adding them to 9.990 ml of another freezing extender after adding 0.002328 gm of melatonin to 10 mL of extender (10-3 M) (10-6 M) [20]. The molecular weight of L-carnitine is 2.0 g/L. (sigma, A6706-1G). After that, add 10ml of extender and 0.0029370 g of L-carnitine (10-3 M) [21]. The molecular weight of cysteine is 121.16 gm/L. (sigma,

C7352). The extender (10-3 M) is added along with 0.0012116 gm of cysteine [22].

Statistical data analysis

Data were analyzed as a two-way analysis of variance using the SAS software by the general linear model (v. 9.3, SAS Inst. In., Cary, NC, USA, 2011). The main effects were rabbit age (young and old) and treatment (Coc, Col, Com, Cos, Llm, Lms, Ls, Ms, Pool). The traits examined were progressive percentage, DAP, DCL, DSL, VAP, VCL, VSL, STR, LIN, WOB, ALH, and BCF.

The following model was used:

$Y_{ijk} = \mu + A_i + T_j + AT_{ij} + e_{ijk}$

Where:

Y_{ijk}: The K th observation of the j th treatment within i th rabbit age

μ: The overall mean.

A_i: The effect of the i th rabbit age.

T_j: The effect of the j th treatment group.

AT_{ij}: The interaction between the i th rabbit age and the j th treatment.

e_{ijk} : Random error.

All data are reported as least square means (LSM) ± standard errors (SE). Mean values were separated, when significance existed, using Duncan's multiple range tests (Duncan;1955). The significance level was set at 5%.

Ethical consent

The research was conducted with approval from a scientific research ethics commission Cairo University (Institutional Animal Care and use committee (CU-IACUC) Cairo University Approval Number CU 11 F27 22).

RESULTS

According to the results of the current study, young males had substantially higher total and progressive sperm motility (p0.05) than older males (64.73% and 45.25%), as shown in **Table 1.** However, the results also showed that older bucks had higher levels of all CASA parameters (DAP, DCL, DSL, VAP, VCL, VSL, STR, LIN, WOB, ALH, and BCF) than younger ones did (p 0.05).

Table 1: Overall mean of computer-assisted semen analysis (CASA) parameters of old and young bucks.

Age	Mot	Prog	DAP	DCL	DSL	VAP	VCL	VSL	STR	LIN	WOB	ALH	BCF
New	77.64^{a}±2.23	64.89^{a}±2.41	19.37^{b}±0.94	29.82^{b}±2.24	14.52^{b}±0.80	46.86^{b}±1.97	72.89^{b}±4.65	35.17^{b}±1.71	0.74^{b}±0.01	0.45^{a}±0.01	0.60^{a}±0.01	4.68^{a}±0.16	18.37^{b}±0.89
Old	64.73^{b}±1.34	45.25^{b}±1.45	23.49^{a}±0.56	51.92^{a}±1.35	18.00^{a}±0.48	52.75^{a}±1.18	115.48^{a}±2.80	40.51^{a}±1.03	0.76^{a}±0.01	0.36^{b}±0.01	0.46^{b}±0.01	4.15^{b}±0.09	28.34^{a}±0.53

In the same row, means with various superscripts (a, b, c, d, e, and f) differ significantly (p 0.05). DAP:Distance Average Path (microns); DCL:Distance Curved Line (microns); DSL:Distance Straight Line (microns); VAP:Velocity Average Path (microns/sec); VCL:Velocity Curved Line(microns/sec); LIN:Linearity (VSL/VCL); WOB:Wobble (VAP/VCL); ALH:Ampltude of Lateral Head Displacement (microns (Hz).

The addition of antioxidants to various experimental groups increased total and progressive sperm motility significantly (p 0.05) compared to the control, which recorded the lowest values (65.70% and 45.20%), as shown in Table 2. However, compared to fresh-pooled semen (89.58% and 80.17%), the antioxidant supplementation levels in all experimental groups (L(56.97% and 49.61), M(69.56 % and 54.23%), S(69.18% and 52.19%), LM(71.96% and 55.40%), LS(68.42% and 51.87%), MS(68.90%and53.34), and LMS(71.35% and53.61%)) and the control group were considerably lower (p0.05). Additionally, as shown in **Table 2**, buck semen treated with various antioxidants (L, M, S, LM, LS, MS, and LMS) performed better than the control group in all CASA parameters (DAP, DCL, DSL, VAP, VCL, VSL, STR, LIN, WOB, ALH, and BCF).

Table 2: Overall mean of computer-assisted semen analysis (CASA) parameters of rabbit bucks cryopreserved with different antioxidants

Treat	Mot	Prog	DAP	DCL	DSL	VAP	VCL	VSL	STR	LIN	WOB	ALH	BCF
C	65.70^{b}± 2.91	45.20^{c}± 3.14	18.98^{c}± 1.22	38.08^{a}± 2.92	14.24^{c}± 1.04	44.18^{c}± 2.57	87.66^{b}± 6.06	33.19^{c}± 2.22	0.75^{ab}± 0.01	0.38^{a} ± 0.02	0.51^{b} ± 0.01	4.26^{b}± 0.20	22.52^{a}± 1.16
L	65.97^{b}± 2.79	49.61^{bc}± 3.01	22.78^{ab}± 1.17	44.68^{a}± 2.80	17.57^{ab}± 1.00	52.05^{b}± 2.47	101.1^{ab} ± 5.82	40.18^{ab}± 2.14	0.76^{a}± 0.01	0.40^{a}± 0.02	0.52^{ab}± 0.01	4.26^{b}± 0.20	24.57^{a}± 1.11
M	69.65^{b}± 2.99	54.23^{b}± 3.22	21.68^{bc}± 1.25	41.68^{a}± 3.00	16.3^{abc}± 1.07	50.21^{bc}± 2.64	95.82^{ab}± 6.23	37.7^{abc} ± 2.29	0.74^{ab}± 0.01	0.39a± 0.02	0.52^{ab}± 0.02	4.57^{b}± 0.21	23.23^{a}± 1.19
S	69.18^{b}± 2.99	52.19^{bc}± 3.22	20.71^{bc}± 1.25	38.71^{a}± 3.00	15.88^{abc}± 1.07	47.87^{bc}± 2.64	88.73^{b}± 6.23	36.75^{bc}± 2.29	0.76^{a}± 0.01	0.42a± 0.02	0.55^{a}± 0.02	4.13^{b}± 0.21	22.94^{a}± 1.19
LM	71.96^{b}± 3.12	55.40^{b}± 3.37	20.41^{bc}± 1.31	39.21^{a}± 3.13	15.45^{bc}± 1.12	48.52^{bc}± 2.76	92.01^{ab}± 6.51	36.84^{bc}± 2.39	0.75^{ab}± 0.02	0.40^{a}± 0.02	0.53^{ab}± 0.02	4.48^{b}± 0.22	22.57^{a}± 1.24
LMS	71.35^{b}± 3.05	53.61^{b}± 3.29	21.42^{bc}± 1.28	42.05^{a}± 3.07	16.5^{abc}± 1.10	49.14^{bc}± 2.70	95.69^{ab}± 6.36	37.8^{abc}± 2.34	0.77^{a}± 0.02	0.40^{a}± 0.02	0.52^{ab}± 0.02	4.18^{b}± 0.21	23.92^{a}± 1.21
LS	68.42^{b}± 3.12	51.87^{bc}± 3.37	20.04^{bc}± 1.31	38.30^{a}± 3.13	15.26^{bc}± 1.12	46.72^{bc}± 2.76	88.44^{b}± 6.51	35.64^{bc}± 2.39	0.75^{ab}± 0.02	0.41^{a}± 0.02	0.53^{ab}± 0.02	4.21^{b}± 0.22	23.19^{a}± 1.24
MS	68.90^{b}± 3.05	53.34^{bc}± 3.29	21.64^{bc}± 1.28	39.40^{a}± 3.07	16.9^{abc}± 1.10	50.16^{bc}± 2.70	90.59^{b}± 6.36	39.1^{abc}± 2.34	0.75^{ab}± 0.02	0.42^{a}± 0.02	0.55^{a}± 0.02	4.20^{b}± 0.21	23.04^{a}± 1
Pool	89.58^{a}± 2.99	80.17^{a}± 3.22	25.22^{a}± 1.25	45.72^{a}± 3.00	18.35^{a}± 1.07	59.38^{a}± 2.64	107.63^{a}± 6.23	43.32^{a}± 2.29	0.72^{b}± 0.01	0.40^{a}± 0.02	0.55^{a}± 0.02	5.42^{a}± 0.21	24.21^{a}± 1.19

The means of the same row that have different superscripts (a, b, c, d, e, or f) differ significantly (p 0.05). DAP stands for "Distance Average Path" in microns; DCL for "Distance Curved Line" in microns; DSL for "Distance Straight Line" in microns; VAP for "Velocity Average Path" in microns/sec; LIN for "Linearity" in microns/sec; WOB for "Wobble" in microns/sec; ALH for "Amplitude of Lateral Head Displacement" in (Hz).

According to **Table 3,** whether in the control or antioxidant-supplemented groups, the total and progressive sperm mobility was substantially (p0.05) increased in young males compared to old males. However, all experimental groups (L, M, S, LM, LS, MS, and LMS) who received antioxidant supplements had higher (p0.05) values for CASA parameters (DAP, DCL, DSL, VAP, VCL, VSL, STR, LIN, WOB, ALH, and BCF).

Table 3 shows that adding more than one antioxidant (LM, LS, MS, and LMS) had a more positive effect on sperm motility and CASA parameters in older males than in younger males.

Table 3: Computer-assisted semen analysis (CASA) of old and young bucks' semen cryopreserved with different antioxidants.

Age	Treat	Mot	Prog	DAP	DCL	DSL	VAP	VCL	VSL	STR	LIN	WOB	ALH	BCF
New	**C**	77.36± 4.90	58.80±5.29	16.30±2.06	26.08±4.92	11.63±1.76	40.60±4.33	65.85±10.22	29.14±3.75	0.72±0.02	0.42±0.03	0.58±0.03	4.76±0.34	17.01±1.95
	L	69.89±4.90	56.88±5.29	20.74±2.06	32.58±4.92	16.21±1.76	48.97±4.33	77.08±10.22	38.27±3.75	0.77±0.02	0.46±0.03	0.59±0.03	4.42±0.34	18.99±1.95
	M	79.97±4.90	67.73±5.29	19.95±2.06	29.60±4.92	14.83±1.76	48.46±4.33	73.37±10.22	35.99±3.75	0.73±0.02	0.45±0.03	0.61±0.03	5.10±0.34	17.70±1.95
	S	78.69±4.90	67.34±5.29	17.72±2.06	26.99±4.92	12.95±1.76	42.89±4.33	66.21±10.22	31.44±3.75	0.73±0.02	0.44±0.03	0.59±0.03	4.45±0.34	17.51±1.95
	LM	79.62±4.90	66.08±5.29	19.03±2.06	30.12±4.92	13.96±1.76	47.40±4.33	75.22±10.22	35.02±3.75	0.73±0.02	0.44±0.03	0.59±0.03	4.92±0.34	17.76±1.95
	LMS	75.69±4.90	62.71±5.29	21.03±2.06	36.38±4.92	16.10±1.76	49.20±4.33	85.09±10.22	37.66±3.75	0.76±0.02	0.43±0.03	0.56±0.03	4.46±0.34	20.62±1.95
	LS	73.99±4.90	60.83±5.29	17.84±2.06	25.17±4.92	13.54±1.76	43.45±4.33	62.52±10.22	33.01±3.75	0.75±0.02	0.48±0.03	0.63±0.03	4.43±0.34	17.96±1.95
	MS	74.85±4.90	63.52±5.29	19.95±2.06	27.61±4.92	15.65±1.76	48.00±4.33	67.50±10.22	37.63±3.75	0.75±0.02	0.48±0.03	0.63±0.03	4.25±0.34	17.65±1.95
	Pool	88.73±4.90	80.09±5.29	21.78±2.06	33.88±4.92	15.78±1.76	52.75±4.33	83.21±10.22	38.35±3.75	0.72±0.02	0.44±0.03	0.60±0.03	5.32±0.34	20.13±1.95
Old	**C**	54.04±2.91	31.60±3.14	21.66±1.22	50.08±2.92	16.85±1.04	47.76±2.57	109.46±6.06	37.24±2.22	0.77±0.01	0.34±0.02	0.43±0.01	3.77±0.20	28.04±1.16
	L	62.04±2.67	42.33±2.88	24.82±1.12	56.79±2.69	18.93±0.96	55.13±2.36	125.19±5.58	42.08±2.05	0.76±0.01	0.34±0.02	0.44±0.01	4.11±0.19	30.15±1.06
	M	59.33±3.10	40.72±3.35	23.40±1.30	53.75±3.11	17.72±1.11	51.95±2.74	118.27±6.47	39.51±2.37	0.75±0.02	0.33±0.02	0.44±0.02	4.05±0.22	28.75±1.23
	S	59.68±3.10	37.03±3.35	23.70±1.30	50.42±3.11	18.80±1.11	52.85±2.74	111.25±6.47	42.07±2.37	0.79±0.02	0.40±0.02	0.50±0.02	3.81±0.22	28.36±1.23
	LM	64.30±3.48	44.72±3.76	21.78±1.46	48.31±3.50	16.93±1.25	49.63±3.08	108.81±7.26	38.65±2.66	0.77±0.02	0.37±0.02	0.47±0.02	4.04±0.24	27.38±1.38
	LMS	67.01±3.28	44.51±3.54	21.82±1.38	47.72±3.29	16.81±1.18	49.08±2.90	106.28±6.84	37.88±2.51	0.77±0.02	0.37±0.02	0.48±0.02	3.90±0.23	27.22±1.30
	LS	62.84±3.48	42.92±3.76	22.24±1.46	51.43±3.50	16.99±1.25	49.98±3.08	114.36±7.26	38.27±2.66	0.76±0.02	0.34±0.02	0.44±0.02	3.99±0.24	28.41±1.38
	MS	62.94±3.28	43.16±3.54	23.33±1.38	51.18±3.29	18.06±1.18	52.31±2.90	113.68±6.84	40.56±2.51	0.77±0.02	0.37±0.02	0.47±0.02	4.14±0.23	28.44±1.30
	Pool	90.42±3.10	80.25±3.35	28.66±1.30	57.57±3.11	20.91±1.11	66.01±2.74	132.04±6.47	48.28±2.37	0.72±0.02	0.36±0.02	0.50±0.02	5.51±0.22	28.28±1.23

Means differ significantly (p 0.05). DCL: Distance Curved Line (microns); DSL: Distance Straight Line (microns); VAP: Velocity Average Path (microns/sec); VCL: Velocity Curved Line (microns/sec); LIN: Linearity (VSL/VCL); WOB: Wobble (VAP/VCL); ALH: Amplitude of Lateral Head Displacement (microns); BCF: Beat C Frequency.

DISCUSSION

For the effectiveness of artificial insemination by semen collected from superior males, improving semen quality during cryopreservation is essential. According to the results of the current study, as shown in Table 1, total and progressive motility was higher in young (77.64% and 64.89%) than in old bucks (64.73% and 45.25%). Similarly, semen taken from Holstein bulls of various ages showed that the production of semen, motility and mass movement of spermatozoa had increased in bulls 1 to 2 years old. In comparison, it recorded the same value on bulls 3 – 9 years old [23]. [however, the present study also showed that all casa parameters (DAP, DCL, DSL, VAP, VCL, VSL, STR, LIN, WOB, ALH, and BCF) were improved in the aged compared to young bucks.

In fact, among five mature Black Bengal bucks, there were distinct differences in the semen parameters for initial sperm motility (ranging from 77.071.06 to 81.471.84%), on dilution sperm motility (ranging from 61.711.03 to 70.301.54%), and post-thawing sperm motility (ranging from 48.151.99 to 55.882.97% [24]. The New Zealand White bucks also had total motile spermatozoa than Chinchilla rabbit bucks [25] the British Spot, Fauve de Bourgogne rabbit breeds, and the New Zealand White also have similar motility percentages. As a result, the present work findings and those of other research teams pointed to the possibility that age, breed, and individual differences affect the motility of rabbit spermatozoa [25].

It is well-recognized that oxidative stress is one of the significant causes of reducing sperm viability and semen quality [26]. According to research, the four rabbit breeds—Chinchilla, British Spot, and Fauve de Bourgogne—New Zealand White- had the highest oxidative stress resistance levels [25].

To maintain semen quality and viability following cryopreservation, antioxidant addition (L, M, and S) is necessary [27, 28, 29]. The present study showed an increase in total and progressive motility in semen groups supplemented with various antioxidants, which is consistent with earlier investigations. Additionally, when buck semen was supplemented with various antioxidants (L, M, S, LM, LS, MS, and LMS), all CASA parameters (DAP, DCL, DSL, VAP, VCL, VSL, STR, LIN, WOB, ALH, and BCF) improved in comparison to the control group. L-c unit sup lamentation has been found to improve the chromatin integrity and motility of mouse sperm [27].

However, Motility and fertilization potential have enhanced due to the cysteine addition of semen extender in buck semen [29]. Additionally, it was shown that adding melatonin to buck semen increased sperm motility and vitality after freezing [28].

The present findings showed that young males in the control or antioxidant-supplemented groups had improved total and progressive sperm motility when age and antioxidant supplementation were considered. However, all the main groups (M, S, LM, LS, MS, and LMS) who obtained antioxidant supplements had higher (p0.05) values for CASA parameters (DAP, DCL, DSL, VAP, VCL, VSL, STR, LIN, WOB, ALH, and BCF). In line with the previous findings, melatonin-supplemented male goat semen showed enhanced total and progressive sperm motility values for CASA parameters [30]. It should be noted that the inclusion of antioxidants (L, M, and S) is necessary to maintain the viability and quality of semen following cryopreservation [27,28,29].

According to the current study findings, adding more than one antioxidant (LM, LS, MS, and LMS) essentially benefits sperm motility and CASA parameters in older males than younger males. Similarly, adding antioxidants such as zinc selenium, vitamin C, and cystine to human sperm extender increased quality and reduced sperm DNA fragmentation [31]. A combination of numerous antioxidants, including 60 mg of vitamin C, 1500 mg of l-carnitine, 10 mg of vitamin E, 20 mg of CoQ10, 200 mg of folic acid, 10 mg of zinc, 1 mg of vitamin B12, and 50 mg of selenium, has been shown to dramatically improve sperm motility and viability in humans [21]. Finally, age plays a significant role in enhancing the quality of buck semen. Additionally, adding either one (L, M, or S) or a combination of antioxidants improves the motility and CASA characteristics of buck semen.

CONCLUSIONS

As a result, age likely has a significant role in determining the quality of the semen. However, adding either a single (L, M, and S) or a combination of antioxidants improves the motility and CASA characteristics of buck semen (LM, LS, MS, and LMS).

Author Contributions:

All authors have contributed to the design of the manuscript, laboratory work of the
The study, statistical analysis, writing, and revision of the manuscript.

Conflicts of Interest:

All authors who contributed to the current study declare no conflict of interest.

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