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EFFECT OF SUPPLEMENTING EXTENDER WITH MELATONIN ON IRAQI NATIVE SEMEN QUALITY DURING COOLING STORAGE

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ABSTRACT : This study aimed to evaluate supplementation of Melatonin to Lake 1960 chicken semen extender and semen liquid storage under 5 C to semen quality. In this study, 20 fertile Iraqi native roosters used. Semen collected from all roosters (pooled sample) in a glass tube for 3 times, 3 days per another one. Semen divided into 4 tubes and diluted 1:1 by using Lake 1960 extender with 0, 0.5, 1 and 1.5 mM/L melatonin to the extender, then stored under 5C in glass tubes for 0, 12, 24, 48, 72 and 96 hours. Sperms individual motility, viability, abnormality, and acrosomes abnormality evaluated for each treatment and time storage. The results showed that decreasing (Pd≤0.0001) in sperms individual motility for all Melatonin treatments compared with control groups at 96 hours. No significant differences between Melatonin treatments and control for another storage times. Decreasing (Pd≤0.05) in sperms abnormality in 1.5 mM/L Melatonin for 96-hour storage compared with other groups. Decreasing in acrosomes abnormality (Pd≤0.05) in 1.5 mM/L Melatonin for 24- and 48-hours storage. Moreover, Melatonin supplementation to semen extender improved sperms motility and decreasing sperms and acrosomes abnormality.

Key words **:** Chicken semen, melatonin, semen quality, cooling storage.

INTRODUCTION

Artificial insemination in poultry becomes an important role in the poultry industry, but there are several factors reduce semen quality and fertility during semen *in vitro* storage. Sperms always accompanied by membrane phospholipid peroxidation, which cause deterioration in semen quality (Meamar *et al*, 2016). Sperm membrane component from Phospholipids and it's responsible for liquidity of sperm membrane bilayer (Zaniboni and Cerolini, 2009). Polyunsaturated fatty acids (PUFA) is a main component of sperm membrane structure and cause cell very sensitive to peroxidation during *in vitro* storage (Fujihara and Koga, 1984; Cerolini *et al*, 2006; Meamar *et al*, 2016). Consequently, the sperm cell membrane is sensitive to oxidative effects, especially it has limited scavenging enzymes, thus making them highly susceptible to ROS damage (Henkel, 2005). So, the level of ROS is required for sperm function (Agarwal and Prabakaran, 2005). Because of high concentrations of sperm with minimal seminal plasma in avian species (Etches, 1994). This makes oxidative stress affect negatively on semen quality. In avian species spermatozoa, antioxidant defense system Include enzymes such as glutathione peroxidase (GSH-PX) and superoxide dismutase (SOD), water and lipid soluble antioxidant like vitamin A, C and E, protease, phospholipase and transferase which repair or remove damaged molecules by cellular oxidative stresses (Breque *et al*, 2003; Mohammed et al,2018a). Melatonin is a hormone secreted mainly by the pineal gland and act powerful antioxidant by inhibiting the activity of nitric oxide synthase (NOS) (Reiter *et al*, 2001; Mohammed *et al*, 2018b). Scavenger of free radicals and counteracts the generation of free radicals (Barrenetxe *et al*, 2004), Potently scavenge ROS (Tan *et al*, 2007), Protect cells from free radicals through metabolism (Kang *et al*, 2009). Melatonin plays a role by inducing antioxidant enzymes activity by increasing SOD and GSH-Px gene expression in sperms membrane (Ramadan *et al*, 2009). Melatonin shows a powerful antioxidant than vitamin E by purifying peroxide radicals (Roo-) a consequence of lipid peroxidation (Hardeland *et al*, 2011; Yassa *et al*, 2011). Reducing oxidation of lipids due to the purifying activity of OH Radicals (Ahmed *et al*, 2011). Reducing the sensitivity of the sperms and prevent damage by oxidation (El-Raey *et al*, 2014). This study conducted to evaluate the effect of Melatonin addition to rooster extender (Lake 1960) and semen liquid storage at 5C to semen quality.

Table 1 : Chemical composition of Lake Semen extender.

Components	Gm/100 ml water
Sodium glutamate	1.92
Potassium citrate	0.128
Sodium Acetate	0.5132
Magnesium chloride, $6H_2O$	0.0676
Fructose	1.000
p^H	7.0

(Lake, 1960)

Semen collected from all roosters (pooled sample) in the glass tube (10 ml) for 3 times, 3 days per another one then divided into four tubes. Semen diluted by using lake (1960) extender (table1) about 1:1 volume with 0, 0.5, 1 and 1.5 mMol/L (mM/L) Melatonin to extender then stored under 5 C in 5 ml glass tubes for 0, 12, 24, 48, 72 and 96 hours. Individual motility evaluated using an Olympus light microscope (Bakst, 1980). Sperms viability tested by using Eosin-Nigrosine stain (Bajpai, 1963). Sperms and acrosomes abnormality tested by using Fast-green-fast Eosin stain (Al-Daraji, 2001).

Table 2 : Effect of different levels concentration of melatonin and storage time at 5C to semen diluent on Sperms individual motility (%).

Melatonin concentration M Mol/L	Storage time/hour						Mean	SEM	Significant level
	0	12	24	48	72	96			
0	89.16 Aa	86.00 ABb	81.66 Bb	72.5 Cb	60.00 Db	28.33 Eb	69.14	3.67	0.0001
0.5	92.66 Aa	90.00 ABab	85.00 ABab	80.00 Ca	67.5 Dab	55.00 Ea	78.36	2.36	0.0001
1	94.66 Aa	91.66 ABa	87.33 ABa	85.00 Ba	72.5 Ca	50.00 Da	80.19	2.73	0.0001
1.5	90.00 Aa	92.16 Aa	82.50 Bab	82.50 Ba	67.5 Cab	35.00 Db	74.94	3.42	0.0001
Mean	91.62	90.13	84.12	80	66.87	42.08			
SEM*	0.98	0.96	0.93	1.25	1.52	2.75			
Significant level	N.S	0.05	0.05	0.0003	0.05	0.0001			

Letters in capital refer to differences between columns
Letters in small refer to differences between rows
*SEM: standard error of means

Table 3 : Effect of different levels concentration of melatonin and storage time at 5C to semen diluent on the percentage (%) of dead sperms.

Melatonin concentration M Mol/L	Storage time/hour						Mean	SEM	Significant level
	0	12	24	48	72	96			
0	1.42 Aa	18.76 ABa	27.65 ABa	30.52 BCa	47.17 Ca	62.33 Da	31.06	2.46	0.0001
0.5	12.33 Da	16.25 Dab	25.69 Ca	30.45 Ca	52.58 Ba	71.09 Aa	33.56	2.69	0.0001
1	10.63 Da	11.75 Db	21.97 Da	35.05 Ca	50.58 Ba	71.71 Aa	32.56	3/11	0.0001
1.5	13.35 Ea	19.25 EDa	30.81 CDa	37.85 CBa	48.10 Ba	70.90 Aa	37.13	2.78	0.0001
Mean	12.84	16.72	26.20	33.58	49.58	69.62			
SEM*	1.32	0.92	1.72	2.07	2.60	1.56			
Significant level	N.S	0.05	N.S	N.S	N.S	N.S			

Letters in capital refer to differences between columns
Letters in small refer to differences between rows
*SEM: standard error of means

MATERIALS AND METHODS

This study carried out in the poultry farm, Dept. of animal production, Agriculture College, University of Anbar (Iraq). Twenty fertile native roosters (30 weeks old) used and trained for semen collection by abdominal massage method as (Burrows and Quinns, 1937) and feed a balance diet for roosters (NRC, 1994).

Statistical analysis

Complete random design (CRD) within four treatments, 3 replicates used in this experiment. Data analyzed by using GLM model procedure of SAS (Statistical analysis system) (SAS, 2001). Including concentrations and times. Means for treatments compared

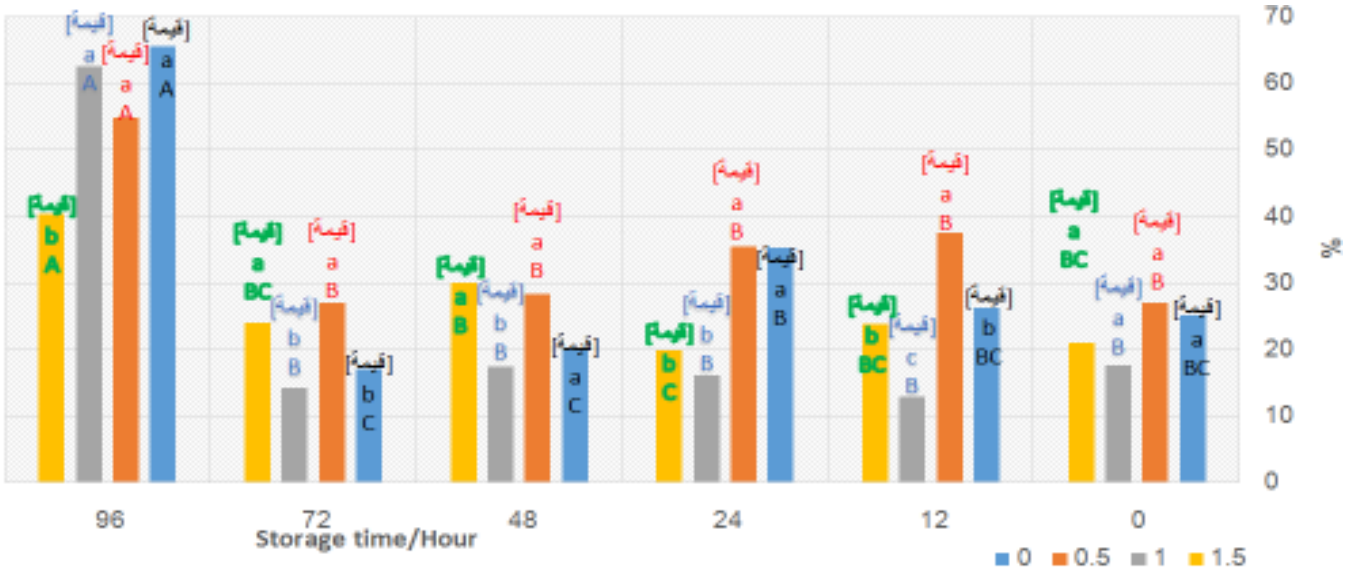
by using Duncan's polynomial using different significance levels to determine significant differences between the averages (Duncan, 1955).

RESULTS AND DISCUSSION

Results in table 2 show significantly improved in sperms individual motility for melatonin groups 0.5 mM/L (Pd≤0.003) and 1 mM/L (Pd≤0.0001) compared with control groups. Moreover, sperms individual motility significantly (Pd≤0.0001) highly for groups 0.5 and 1 at 96 hours storage at 5c compared with control. Sperms individual motility decreasing significantly (Pd≤0.0001) with time storage progress.

Results in the table (3) show no significant differences between Melatonin and control groups in dead sperms except for melatonin addition 1 mM/L at 12 hours storage at 5C compared with the control group. Dead sperms increasing significantly (Pd≤0.0001) with time storage progression.

The results in a figure 1 showed that treatment with 1 mM/L of Melatonin decreased significantly (Pd≤0.0001) sperms abnormality for 12, 24, and 72 hours at 5C and decreasing significantly (Pd≤0.002) at 96 hours storage. Therefore, treatments with 1.5 mM/L Melatonin was affected significantly (Pd≤0.0001) by decreasing sperms abnormality for 12- and 24-hours storage and significantly (Pd≤0.01) at 96 hours storage at 5C . In 96 hours storage at 5C semen abnormality decreasing significantly (Pd≤0.0001) for 0.5 and 1 mM/L of Melatonin



Letters in capital refer to differences between columns in different time storage
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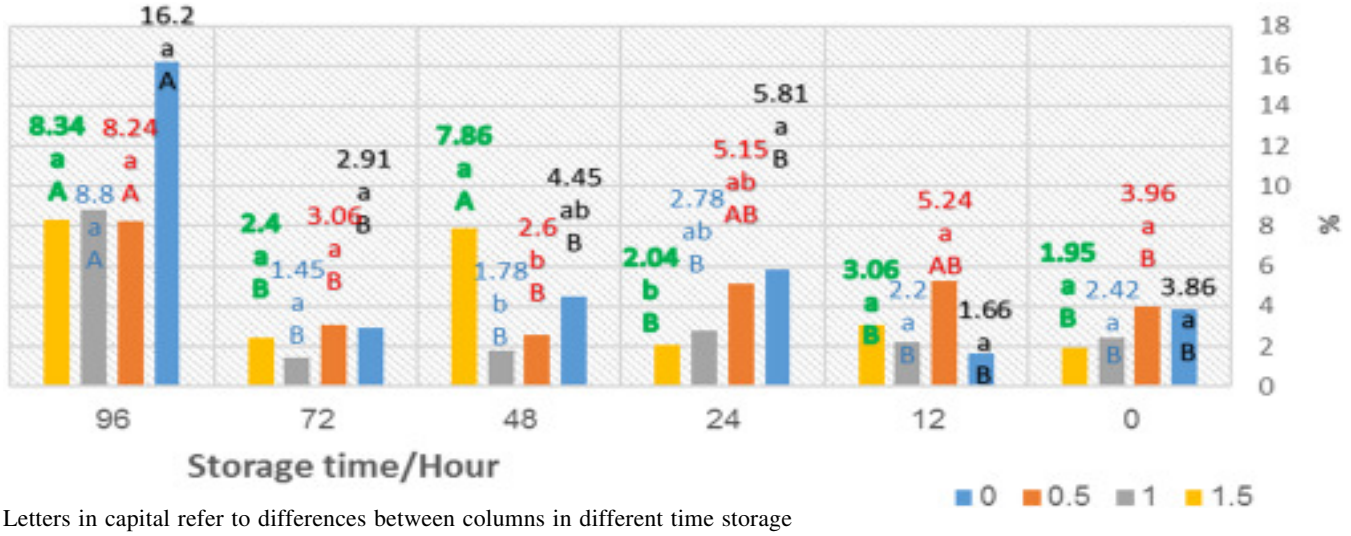
Effect of different levels concentration of melatonin with semen diluent and storage time at 5C on sperms abnormality percentage (%)



Letters in capital refer to differences between columns in different time storage
Letters in small refer to differences between columns in same time storage
Effect of different levels concentration of melatonin with semen diluent and storage time at 5C⁻ on acrosomes abnormality percentage

concentration and significantly decreasing (Pd≤0.0003) in 24 and 48 hours at 5C storage in 1.5 mM/L of Melatonin concentration

Moreover, the results in figure 1 showed that treated semen extender with 0.5 and 1 mM/L of Melatonin and storage for 24 hours, and treated semen extender with 1.5 mM/L storage for 24 hours at 5C significantly (Pd≤0.05) minimal ratio in acrosomes abnormality compared with control groups. Acrosomal abnormality significantly increasing in 96 hours storage at 5C compared with another storage periods for all melatonin concentrations.

Storage avian semen for a long time makes deterioration in quality and fertilization ability because of high concentration of avian semen compared with mammalian species. This makes seriously damaging of reactive oxygen species (Surai *et al*, 1998; Zaed et *al*, 2018). These oxygen species and free radicals make damage all major cellular components (Droge, 2002). Oxidative stresses make an increase in intracellular ROS that makes the loss in membrane integrity (Johnson *et al*, 2016).

The results showed that Melatonin addition to Lake 1960 extender during semen cooling storage affected positively in semen characteristics such as maintaining Individual motility for 24 hours of storage at 5C with 1 mM/L of Melatonin concentration. This improvement due to the ability of Melatonin in scavenging free radicals (Reiter *et al*, 2016; Malm *et al*, 2017). Then decreasing lipid peroxidation during storage by reducing (PUFAs) in sperms membrane (Meamar *et al*, 2016). This result compatible with Najafi *et al*, 2016 who reported that supplementation Melatonin to human sperms freezing medium make a protective affection against ROS production and membrane damage. The improvements in sperms quality by decreasing percentages of dead sperms, sperms abnormal and acrosomes abnormality with time storage progression due to the high ability of Melatonin to scavenge oxygen-derived free radicals (Bejarano *et al*, 2014). Then it will make a reduction in oxidative stresses (Garica *et al*, 2014, Manchester *et al*, 2015). These causes make a better decreasing in sperms organelles damage and prevent mitochondria activity (Suzen, 2018). Because of preventing oxidative stresses and ROS-induced abnormalities and hence mitochondrial dysfunction (Paradies *et al*, 2017). This improvement back to Melatonin role as mitochondria targets antioxidant (Reiter *et al*, 2014; Reiter *et al*, 2017).

In conclusion, supplementation of Melatonin in Native rooster's semen extenders in 1 mM/L and storage semen for 24 hours in 5C prevent deterioration of sperms motility and decreasing sperms and acrosomes abnormality and dead sperms. Furthermore, reinforce cooling semen storage by decreasing oxidative stresses that formed during cooling storage.

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