

**Original Paper****The influence of selenium nanoparticles and L-Carnitine on various biochemical markers and oxidative stress status in Ossimi ewes during post-partum periods**Eman H. Halawa<sup>1</sup>, Tharwat A. Imbabi<sup>1</sup>, Omar A. A. Farid<sup>2</sup>, Ahmed A. Radwan<sup>1</sup>, Abdelkarim I. M. El-Sayed<sup>1</sup><sup>1</sup> Animal Production Department, Faculty of Agriculture, Benha University, Egypt.<sup>2</sup> Physiology Department, National Organization for Drug Control and Research (NODCAR), Giza 12553, Egypt**ARTICLE INFO****Keywords**

L-Carnitine  
Nanoparticles  
Ossimi ewes  
Oxidative markers  
Selenium

**Received** 31/01/2023**Accepted** 23/03/2023**Available On-Line**  
xx/xx/2023**ABSTRACT**

A total of 27 adult Ossimi ewes were divided into three groups (9 ewes/ each). The 1<sup>st</sup> group, which served as a control, was treated with distilled water (10 mL/kg B.W. /day), the 2<sup>nd</sup> group was treated with selenium nanoparticles (SeNPs 1 mg/kg B.W./day), and the 3<sup>rd</sup> group was treated with L-Carnitine (L-Car 350 mg/Kg/day). Data revealed that triglyceride levels decreased at 45 days of postpartum due to SeNPs treatment, while total cholesterol increased at 11 days and decreased at 45 days of postpartum due to SeNPs and L-Car. HDL levels increased at 11 day and decreased at 60 days of postpartum by using SeNPs and L-Car. LDL decreased at day 11 by L-Car administration. AST decreased at 11, 45, and 60 days in the SeNPs group and ALT decreased at day 11 in the treated group and decreased at day 45 in the L-Car group. Urea levels decreased at day 11 in treated groups. On the other hand, creatinine levels decreased at days 45 and 60 of postpartum in treated groups. SeNPs increase SOD along postpartum and decreased GSSG at days 11 and 60 and increased at day 45, while L-Car decreased GSSG at days 11 and 60 and increased at day 45 of postpartum. NO concentrations decreased along postpartum in treated groups and increased in ATP at mid and late postpartum in treated groups. As a result, SeNPs and L-Car oral administration enhanced various biochemical parameters and serum antioxidant activities for Ossimi ewes during the post-partum periods.

**1. INTRODUCTION**

The postpartum is the period between parturition and the return to the normal cycling state of the ovaries and uterus before pregnancy. Different physiological statuses (e.g., pregnancy, parturition, and lactation) in the animal reproductive life are important variable which modify its metabolism (Iriadam, 2007) and affects the concentrations of blood biochemical (Roubies *et al.*, 2006). Good health of ewes during periparturient period is mandatory for the successful reproduction and/or production of viable lambs due to the changes in body metabolism occur during pregnancy and postpartum (Balikci *et al.*, 2007).

The level of some blood constituents may correspond to animal's physiological status or response to various situations. Markers may be beneficial in indicating animal health statuses and may aid in the identification of several metabolic and/or infectious distress (Russell and Roussel, 2007). Blood biochemical parameters e.g., total protein, triglycerides, cholesterol, glucose, urea, and creatinine are important indicators for the metabolic activity, energy status and growth in lactating animals (Karaphelivan *et al.*, 2007).

Selenium is essential for growth, reproduction, fertility, immune function, metabolism, and antioxidant defense mechanisms in all mammals (Pappas and Zoidis, 2012). As a result, supplementary Se in organic or inorganic forms is

required in animal diets to give a margin of safety against deficiency and to maintain productive performance (Dokoupilová *et al.*, 2007). Selenium nanoparticles (SeNPs) have recently received an extensive interest because of its high bioavailability, as well as its strong absorption capacity (Zhang *et al.*, 2008). Selenium nanoparticles have been employed as a reactive oxygen species (ROS) scavenger to protect against oxidative damage (Khalil *et al.*, 2019).

Carnitine is a transporter for free fatty acid (FFA) into the mitochondria for oxidation (Woldegiorgis *et al.*, 2000). Carnitine effectively is involved in some metabolic processes e.g., oxidation of long-chain fatty acids, regulation of ketosis, support of the immune system, enhancement of the antioxidant system, and improvement of reproduction (Citel *et al.* 2009). L-Carnitine (L-Car) also controls the lipid metabolism process (Blanchard *et al.*, 2002). In general, carnitine supplementation can decrease liver lipid accumulation and diminishing the risk of developing metabolic disorders during early lactation (Carlson *et al.* 2007). Zhang *et al.* (2010) indicated that L-Carnitine helps animals maintain their nitrogen balance, reduce body fat, and increase protein retention.

The main purpose of this study is to look into the effect of SeNPs and L-Car on physiological and oxidative statuses of Ossimi ewes during post-partum.

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## 2. MATERIAL AND METHODS

### 2.1. Animals, experimental design, and management:

All experimental procedures were accomplished with reference to local Experimental Animal Care Committee and approved (20208) by the Institution Committee of the Department of Animal Production, Faculty of Agriculture, Benha University, Egypt.

A total number of 27 mature Ossimi ewes 18 months old,  $42.24 \pm 1.44$  kg body weight, were divided randomly into three groups (n= 9 per each). The first group served as a control and treated with distilled water (10 ml/kg B.W./day), whereas the other two groups T1 and T2, were orally supplied with 1 mg/kg body weight/day selenium nanoparticles (SeNPs) according to Kachuee *et al.* (2019), and 350 mg/Kg/day L-Carnitine (L-Car), respectively.

Throughout the experimental period, ewes were fed the same standard iso-caloric and iso-nitrogenic diet. The basal diet composition and the calculated analysis were performed by the Nutritional Research Council according to Nutrient Requirements of sheep NRC (1985). Drinking water was offered to animals *ad libitum*. The treatments were given to the animals by dosage daily in the morning.

### 2.2. Blood samples:

Blood samples from all ewes were collected without an anticoagulant at 11-, 45-, and 60-days post-partum for serum separation used to determine biochemical parameters. The blood tubes were centrifuged at 4000 rpm for 20 min and the supernatant layer of clear serum was carefully withdrawn and kept at -20 °C until the measurements of the biochemical parameters (lipid profile, liver and kidney functions, and oxidative stress indices).

### 2.3. Biochemical parameters:

Serum total cholesterol (mg/dL) was quantified spectrophotometrically using Stanbio Cholesterol Liquid Color® Kit (Proc. No. 1010) produced by Stanbio Laboratory Inc., Boerne, Texas, USA according to Allain *et al.* (1974). Serum triglycerides concentrations (mg/dL) were quantified spectrophotometrically according to Fossati and Prencipe (1982) by using Stanbio Liquid Color®, Triglycerides Kit (Proc. No. 2100) employed from, Stanbio Laboratory Inc., Boerne, Texas, USA. The intensity of the color was measured at 505 nm by using a double beam spectrophotometer. Serum high-density lipoprotein HDL-cholesterol (mg/dL) was determined by the method of Lopez (1977), HDL-cholesterol was determined in the supernatant fluid by the same method used for total cholesterol at 500 nm by Cobas Mira, Roche Company. Biodiagnostic's enzymatic colorimetric kits were used for this assay following their manual of instruction, the kits (Proc. No. 2100) employed from, Stanbio Laboratory Inc., Boerne, Texas, USA, and the serum low-density lipoprotein LDL-cholesterol (mg/dL) was according to Fossati and Prencipe (1982). LDL-Cholesterol was determined as the difference between total cholesterol and the cholesterol content of the supernatant after precipitation of LDL fraction by polyvinyl sulfate in the presence of polyethylene glycol mono methyl ether. Concentrations of total protein (g/dL) and albumin (g/dL) were estimated using Biuret method in the presence of alkaline cupric sulfate according to Doumas *et al.* (1971). Globulin (g/dL) value was obtained by subtracting the value of albumin from the corresponding value of total protein. The intensity of the Violate color was measured at wavelength 550 nm. Transaminase enzymes (ALT, AST) (U/L) activities were performed according to Reitman and Frankel (1957), the color intensity was measured at

wavelength of 520 nm. Serum urea (mg/dL) concentration was measured using the enzymatic colorimetric method described by Patton and Crouch (1977). This determination was carried out using wavelength of 578 nm.

Test kits were provided by Diamond Diagnostics Company.

Serum creatinine (mg/dL) concentration was measured using the enzymatic colorimetric method described by Patton and Crouch (1977). This determination was carried out using spectrophotometer with wavelength of 578 nm. Test kits were provided by Diamond Diagnostics Company.

### 2.4. Determination of antioxidants activity:

Determined the malondialdehyde (MDA) in blood serum according to Karatepe (2004). Determination of oxidized glutathione (GSSG) and reduced glutathione (GSH) in blood serum detected by using the method of Jayatilleke and Shaw (1993). Glutathione (oxidized and reduced) reference standard purchased from Sigma Chemical Co. Dissolved in 75% methanol in stock 1 mg/mL and diluted before application to HPLC, and the determination of blood serum nitric oxide (NO) ( $\mu\text{mol}/\text{ml}$ ) detected by using the method of Papadoyannis *et al.* (1999). The activity of superoxide dismutase (SOD) was determined according to the method of Marklund and Marklund (1974) and the Adenosine Tri-phosphate (ATP) determined according to the method of Hai *et al.* (2006), reference standard purchased from Sigma Chemical Co..

### 2.5. Statistical analysis:

All data were expressed as means  $\pm$  standard error of means (SEM) and were subjected to analysis of variance (ANOVA) using SAS (2004). Duncan multiple-range test ( $P < 0.05$ ) was used to define the differences among treatment groups. The applied static model was as follows:  $X_{ijk} = \mu + T_i + e_{ijk}$ , whereas:  $\mu$  = Overall mean,  $T_i$  = Effect of the  $i^{\text{th}}$  treatment (i, 1-3),  $e_{ijk}$  = Random error associated with the individual observation.

## 3. RESULTS

### 3.1. Lipid profile

The data in table (1) showed the effect of oral administration of SeNPs and L-Car on ewes during postpartum. The triglyceride (TG) in SeNPs group showed fluctuated levels during postpartum periods characterized by significant decrease at day 45, and a significant increase at day 60. On the other hand, L-Car promoted significant increase in TG during postpartum periods, which reach to sign levels at day 11 and 60 postpartum. Cholesterol, compared to control, both treated groups promoted an increase in it at day 11 and a decrease at day 45 postpartum periods. HDL markedly increase in L-Car group at day 11, while it decreased in SeNPs group at day 60 of postpartum compared to control. LDL noticeably decreased in L-Car at day 11 of postpartum only.

### 3.2. Liver function:

Table (2) shows the liver function indices of Ossimi ewes during postpartum periods. The results showed that AST levels decreased in response to SeNPs along postpartum periods, while it increased at day 11 in L-Car compared to the control. On the other hand, both treated groups lowered ALT activity during early postpartum period (day 11). The

data showed that total protein increased insignificantly at day 11 and 45 of postpartum.

### 3.3. Kidney function

Data in table (3) reveals the effect of SeNPs and L-Car on urea and creatinine concentrations on Ossimi ewes during postpartum periods. Compared to the control group, both treatments decreased urea at early postpartum (Day 11) and creatinine at late postpartum periods (Day 60).

Table 1 Effect of selenium nanoparticles (SeNPs) and L-Carnitine (L-Car) on lipid profile in Ossimi ewes during post-partum period.

Treatment	Triglycerides (mg/dL)	Total cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
<u>Day 11 post-partum</u>				
Control	80.83±6.38b	80.78±5.63c	29.25±3.25b	39.50±4.32a
SeNPs	82.22±7.85ab	87.00±7.52a	30.33±3.62b	38.66±3.85a
L-Car	87.60±6.96a	84.60±6.35b	32.60±4.21a	35.40±3.54b
P-Value	0.0675	0.0008	0.0166	0.0054
<u>Day 45 post-partum</u>				
Control	90.25±5.96a	89.50±5.63a	31.75±3.68	37.00±4.25
SeNPs	83.33±6.38b	87.00±6.53b	30.00±3.25	37.80±4.31
L-Car	92.00±7.42a	86.00±6.85b	30.40±3.68	37.80±3.98
P-Value	<.0001	0.0130	0.1603	0.5286
<u>Day 60 post-partum</u>				
Control	88.00±5.98b	88.75±7.21	32.50±3.63a	37.00±4.25
SeNPs	91.80±6.35a	87.11±6.87	30.33±3.21b	37.77±3.56
L-Car	91.80±6.74a	86.80±6.25	31.00±3.05ab	38.40±3.38
P-Value	0.0051	0.1083	0.0896	0.3013

HDL: High density lipoprotein. LDL: Low density lipoprotein. Means with different letters (a, b, c) in the same column within the same postpartum period were significantly different.

Table 2 Effect of selenium nanoparticles (SeNPs) and L-Carnitine (L-Car) on concentrations of total protein, albumin, globulin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of Ossimi ewes during post-partum periods

Treatment	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	AST (U/L)	ALT (U/L)
<u>Day 11 post-partum</u>					
Control	9.00±0.93	4.70±0.56	4.30±0.52	72.00±6.93b	34.25±3.32a
SeNPs	9.61±0.91	5.38±0.71	4.23±0.62	69.55±6.52c	28.11±2.89b
L-Car	9.06±0.85	5.20±0.65	3.77±0.66	79.40±7.12a	28.80±3.10b
P-Value	0.7252	0.7040	0.8055	<.0001	0.0005
<u>Day 45 post-partum</u>					
Control	9.75±0.88	4.90±0.58	4.85±0.56	71.25±7.52a	27.50±2.52ab
SeNPs	9.78±0.92	5.58±0.67	4.20±0.68	68.88±6.95b	28.44±2.37a
L-Car	9.36±0.87	4.58±0.59	4.78±0.67	70.60±5.89ab	26.20±2.50b
P-Value	0.8519	0.4990	0.6980	0.0640	0.0860
<u>Day 60 post-partum</u>					
Control	9.85±0.82	5.00±0.61	4.85±0.71	70.25±6.85a	27.25±3.21
SeNPs	9.46±0.93	5.30±0.63	4.16±0.62	67.66±5.66b	27.11±3.56
L-Car	8.18±0.87	4.48±0.51	4.70±0.70	70.00±6.28a	26.80±2.78
P-Value	0.7248	0.6210	0.6899	0.0356	0.8564

AST: Aspartate aminotransferase and ALT: Alanine aminotransferase. (a, b, c) in the same column within the same period of postpartum are significantly different.

Table 3 Effect of selenium nanoparticles (SeNPs) and L-Carnitine (L-Car) on urea and creatinine activities of Ossimi ewes during post-partum period.

Treatment	Urea (mg/dL)	Creatinine (mg/dL)
<u>Day 11 post-partum</u>		
Control	1.10±0.02	66.75±6.63a
SeNPs	0.85±0.02	55.00±5.87c
L-Car	0.98±0.03	62.40±5.85b
P value	0.8716	<.0001
<u>Day 45 post-partum</u>		
Control	0.87±0.06b	56.50±6.34
SeNPs	0.90±0.06a	55.88±5.87
L-Car	0.88±0.05b	57.20±6.36
P value	0.027	0.3376
<u>Day 60 post-partum</u>		
Control	0.90±0.07a	57.00±4.63
SeNPs	0.88±0.06b	58.96±5.85
L-Car	0.82±0.06c	55.60±5.96
P value	0.0002	0.4851

<sup>a, b, c</sup> Means with different superscript in the same column within the same periods of postpartum are significantly different at P<0.05.

Table 4 Effect of selenium nanoparticles (SeNPs) and L-Carnitine (L-Car) on blood serum oxidative stress markers of Ossimi ewes during post-partum period

Treatment	MDA (nmol/mL)	SOD (U/mL)	GSH (µmol/ml)	GSSG (µmol/ml)	NO (µmol/ml)	ATP (µg/ml)
<u>Day 11 of post-partum</u>						
Control	4.06±0.42	49.94±5.63b	3.25±0.39	0.71±0.06a	0.35±0.04a	80.08±8.25
SeNPs	3.38±0.44	58.02±6.37a	3.69±0.41	0.55±0.05c	0.30±0.05c	62.57±6.52
L-Car	3.71±0.39	49.37±5.10b	3.51±0.44	0.58±0.05b	0.33±0.04b	82.68±8.36
P-Value	0.7202	<.0001	0.8665	<.0001	0.0025	0.7006
<u>Day 45 of post-partum</u>						
Control	2.80±0.43	60.46±6.35b	3.77±0.47	0.35±0.05c	0.28±0.04a	89.22±7.63c
SeNPs	2.70±0.38	68.24±6.10a	4.38±0.46	0.44±0.06b	0.25±0.05b	111.60±5.63a
L-Car	3.01±0.47	56.83±5.43c	4.11±0.51	0.51±0.07a	0.25±0.05b	97.35±6.53b
P-Value	0.9097	<.0001	0.7649	<.0001	0.0156	<.0001
<u>Day 60 of post-partum</u>						
Control	2.54±0.32	66.30±6.25b	4.46±0.45	0.49±0.05a	0.22±0.03a	96.88±6.23c
SeNPs	2.07±0.35	75.43±7.25a	4.99±0.41	0.33±0.04b	0.18±0.04c	125.07±7.52a
L-Car	2.46±0.38	66.43±6.53b	4.71±0.48	0.40±0.04ab	0.20±0.03b	109.25±5.68b
P-Value	0.8320	<.0001	0.8156	0.0413	0.0080	<.0001

MDA: malondialdehyde, SOD: superoxide dismutase, GSH: reduced glutathione, GSSG: oxidized glutathione NO: nitric oxide and ATP: adenosine tri phosphate. (a, b, c) in the same column within the same period of postpartum are significantly different at P<0.05.

### 3.4. Oxidative stress markers:

Table (4) shows that SeNPs and L-Car administration improved stress marker concentrations. SeNPs supplementation, compared to the control, significantly increased SOD activity (along the postpartum period), and decreased GSSG (at day 11 and 60) and NO levels (along the experiment). Both SeNPs and L-Car supplementation increased ATP considerably during the mid- and late postpartum periods.

#### 4. DISCUSSION

Lower cholesterol levels during early breastfeeding are consistent with an increased energy requirement and a negative energy balance (Antunovic *et al.*, 2011). Supplement with SeNPs increased the HDL concentration while decreased LDL according to Çicek *et al.* (2021). Mohamed *et al.* (2015) observed a drop in blood triglycerides as milk production increased, which required triglyceride removal by the mammary gland for milk fat synthesis (Mantovani *et al.*, 2010). Huang *et al.* (2013) found no effect of L-Car treatment was observed on blood cholesterol or triglycerides, while ours results showed an increase in triglycerides and total cholesterol concentrations. On the other hand, Mohamed *et al.* (2015) noticed a decrease in blood triglycerides with increasing the amount of milk production which required triglycerides withdrawal by the mammary gland for synthesis the milk fat (Mantovani *et al.*, 2010). This finding may indicate the positive role of SeNPs and L-Car on lipid profile and energy metabolism.

Serum total protein concentrations were quantitatively lower after parturition and 60 day later. This, though, was insignificant. It was possible to draw the conclusion that fluctuations in the levels of total proteins were caused by globulins. According to Taghipour *et al.* (2010), the decline in serum total protein was mostly caused by a drop in the alpha 1 and gamma fractions of globulin. This was attributed to the creation of colostrum that was high in globulins. Three to four weeks prior to parturition, sheep begin to have the ability to manufacture the components of milk. One major contributing reason to the decline in blood total protein may be the drainage of globulins to the mammary glands for the production of colostrum (Taghipour *et al.*, 2010). The use of SeNPs in this study had no significant effect on total protein, albumin and globulin concentrations, these results disagreed with El-Shahat and Abd El-Monem (2011), who showed an increasing in serum total protein and globulin, but not albumin, on Baladi sheep supplementing with SeNPs. Mahmoud *et al.* (2013) also attributed the significant increases in blood TP and its fractions to the improvement in protein anabolism and decrease of protein catabolism. According to Qin *et al.* (2016) SeNPs supplementation raises ALT and decreases AST activities compared with the control group. These results disagreed with ours which indicated that AST and ALT levels decreases by using SeNPs.

The renal function principally indicated by urea and creatinine concentrations during lactation period was significantly affected by L-Car. The concentration of urea decreased as results of L-Car and SeNPs administration. The considerable decrease in plasma urea concentration seen at the end of lactation is consistent with the findings of Karapehlivan *et al.* (2007) and Yokus *et al.* (2006) in ewes. These data support the theory that variations in blood urea levels during lactation may be affected by the pace of milk synthesis (El-Sherif and Assad 2001). It is most likely related to the usage of urea for protein synthesis via the ruminal hepatic route to compensate for the poor protein intake during the dry season (Yokus *et al.* 2006). When significant amounts of body reserves are used up, it could potentially be the result of muscle protein being catabolized (Antunovic *et al.*, 2011).

Using SeNPs and L-Carnitine during post-partum periods improves oxidative stress markers and SeNPs is more effective than L-Car compared with control group. When SeNPs was applied, the cell's oxidative status was improved, which was implied by lower levels of

glutathione and superoxide dismutase (Hassanin *et al.*, 2013). Antioxidant enzymes such as SOD and glutathione peroxidase form a natural defensive system against oxidant activity. The ability of an organism to withstand the harmful effects of ROS may be critical for its survival (El-Ramady *et al.*, 2014). L-Car administration reduced cytotoxicity by enhancing blood serum parameters, lowering oxidative stress, sustaining cell energy, and increasing ATP in blood serum (Abd-Elrazek and Farid, 2018). L-Car supply boosted total antioxidant enzyme status as a function of treatment duration, lowering free radical levels accessible for lipid peroxidation (Basini and Grasselli, 2015).

#### 5. CONCLUSION

Presented results indicated the beneficial effects of oral administration of SeNPs and L-Car during post-partum through improving lipid profile, AST, ALT, GSH and ATP, and decreasing urea and creatinine as well as serum oxidative markers (MDA, GSSG, NO).

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