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# Ovarian dynamics and hemodynamics of pubertal fat-tail ewes supplemented Moringa seed cake before and after exogenous progesterone synchronization

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**ABSTRACT**

To study the effect of supplementation of moringa seed cake on the ovarian dynamics and hemodynamics, hormonal and antioxidant capacity of yearling ewes, native ewes (n=20) were equally divided into control supplemented the basal diet and moringa seed cake (MSC) supplemented orally 7.0g daily for 45 days. After 17 days of starting supplementation, exogenous 45mg progesterone sponges were inserted for a short period (6 days) and each animal was administrated 2.5mg Dinoprost tromethamine on the day of sponge removal. Ultrasound Doppler was conducted and blood samples were collected on the day of sponge insertion (Day -6), sponge removal (Day 0), Days 2, 9, 15, and 21. Results indicated increased dominant follicle diameter and number in ewe-lambs supplemented with MSC on Day 2 (P=0.0007), 9 (P=0.009), and Day 21 (P=0.005)Subordinate follicle diameter of MSC increased on Day -6, 2 (P<0.0001), 9 (P=0.004) and 21 (P=0.012). The follicle's average circumference, area, and perimeter increased (P<0.0001) in ewe-lambs supplemented with MSC on Days 2, 9, and 21. CL diameter, perimeter, and area of MSC reached the highest (P<0.0001) on Day 15 but those of control reached the maximum diameter (P=0.032) on Day 21. Ovarian color area of MSC increased (P<0.01) on days 0 and 2. The concentrations of total antioxidants, glutathione reduced, catalase, and SOD increased in ewe lambs supplemented MSc. In conclusion, MSC improved follicle and luteal development, ovarian hemodynamics, and response to synchronization using short-term exogenous progesterone. MSC improved the antioxidant capacity and decreased lipid peroxidation during the estrus of ewes.

## Introduction

Sheep are the most abundant ruminant livestock species in Egypt. Sheep are a valuable livestock species because of their ability to convert forages and are not suitable for other farm animals into meat and milk (Elshazly and Youngs, 2019). Recently, *Moringa oleifera* leaf was included in the nutrition of buffalo calves (Abdel-Raheem and Hassan, 2021), rabbits (Bhatt *et al*., 2023), goat kids (Leitanthem *et al*., 2022), and humans (Brar *et al*., 2022). Moringa seed cake was included in the nutrition of cattle (Lins *et al*., 2019; Mahmood *et al*., 2022). Moringa seed cake modulated the rumen microbial activities of dairy cattle and this modulation was related to its content of protein and carbohydrate and their metabolism (Mahmood *et al*., 2022). The spraying of moringa leaf extract on other feedstuffs ameliorated the gas emission and improved the digestion of steers (Parra-Garcia *et al*., 2019). In rabbits, moringa leaf extract improved the number of services per conception and progesterone under heat-stress conditions (Mutwedu *et al*., 2022). During the dry season, moringa supplementation in rams compensated for the shortage in protein intake (Gebregiorgis *et al*., 2012). The inclusion of moringa leaf in the concentrate ration of the prolific Avishan ewes during heat stress improved the antioxidant status, ovulation rate, conception, and multiple birth percentages (Sarkar *et al*., 2023). Reproductive and productive performance are important factors in the success of this livestock industry. Improvement of reproductive performance is an honorable goal and is closely associated with increasing productivity. Therefore, as the reproductive efficiency improves, the herd net return and national income increase (Gafer *et al*., 2023). Nutrition is one of the main factors influencing the reproductive performance of ewes, from the onset of puberty to the total number of lambs produced during their lifetime (Rassu *et al*., 2004).

The moringa seeds are antipyretic, acrid, and bitter. MO seeds contain a significant amount of oil (up to 40%) with a high-quality fatty acid composition (oleic acid > 70%) The extracted oil is known commercially as "Ben" or "Behen oil". The fatty acid composition of Moringa oil is quite similar to olive oil in its contents of C18:1 and C18:0 (Anwar and Bhanger, 2003), which explains its potential as an edible oil. For both human consumption and commercial purposes (Anwar *et al*., 2005). Moringa leaves were used to improve the sexual libido (Cajuday and Pocsidio, 2010)

Moringa seed cake (MSC) is the byproduct of squeezing moringa oil from moringa seeds. It increased post-rumen protein availability by reducing dietary protein breakdown in an in vitro rumen study (Folkard *et al*., 2001; Makkar *et al*., 2007). Moringa seed cake was suggested to be utilized as an alternative protein source to replace cottonseed meal in the diets of male Damascus goats (Makkar *et al*., 2007; Abdel-Rahman *et al*., 2019 ) and soybean meal in ration of fattening calves (Hassan *et al*., 2023) MSC has a high natural antioxidant content like ascorbic acid and flavonoids that possess the antioxidant activity to enhances the performance of ruminants (Nadeem *et al*., 2013; Babiker *et al*., 2017). In lactating ewes, 2.5% MSC was supplemented in their diets to improve milk production performance without resulting in any adverse effects (Aboamer *et al*., 2020). Therefore, the objective of this study was to feed moringa seed cake to pubertal ewes to study its effects on the animal's ovarian dynamics and hemodynamics, antioxidants status, and ovarian hormone dynamics.

## Materials and methods

This study was approved by the Animal Care and Use Committee of the Faculty of Veterinary Medicine (Vet CU 18042024936). Current study was carried out following Guidance on the operation of the Animals (Scientific Procedures) Act 1986 and associated guidelines, EU Directive 2010/63 for the protection of animals used for scientific purposes or the NIH (National Research Council) Guide for the Care and Use of Laboratory Animals (PDF) or those of an equivalent internationally recognized body and according to ARRIVE (Animal Research: Reporting of *In Vivo* Experi-


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ments) guidelines.

Moringa seed cake was purchased from the National Research Center (Moringa Products public office), Egypt, after extracting seed oil by using the squeezing mechanical cold press method.

*Animal housing and management*

The study was conducted from November 2023 to February 2024 at the research farm of the Faculty of Agriculture (EL-Azhar's University, Cairo, Egypt). Yearling pubertal ewe lambs (n.=20) with average body weight (30.0 kg) were housed in semi-open yards under natural daylight and temperature. Ewes were fed their maintenance requirements of pelleted concentrate (NRC, 2007) containing 14% crude protein, 2% lipids, 15% crude fiber, 9% ash, and 6.39 MJ/ kg diet as an energy requirement in addition to hay and wheat straw. Fresh water and mineral licks were available *ad libitum*.

Complete clinical, cardiovascular, gynecological and ultrasonographic examinations were done for each female to ensure the absence of pregnancy and any abnormalities related to the reproductive tract before beginning the research and to include healthy normal sound animals. The animals were regularly vaccinated and dewormed before beginning the experiment.

Before starting the experiment, the composition of moringa seed cake was analyzed (Ramteke *et al*., 2024) and antioxidant properties (El-Seadawy *et al*., 2022) were determined.

*Experimental design*

Ewe lambs fed the basal maintenance diet served as control. Ewe lambs fed the basal diet and supplemented daily with a Moringa seed cake of 7.0 g/animal for 45 days served as the MSC group (Ben Salema and Makkar, 2009). Ewe-lambs were allowed to adapt dietary treatments for the first 17 days before starting the experiment (Ben Salema and Makkar, 2009).

*Oestrous synchronization*

After adaptation time, animals were subjected to estrous synchronization using intravaginal sponge (Day -6, synchro vet 45 mg for 6 days (Day 0) and injected intravulvo-submucosal (Ono *et al*., 1982; Rovani *et al*., 2012) 2.5 mg of Dinoprost tromethamine (Lutalyse, Zoetis, Belgium SA) on day of sponge removal (Meira *et al*., 2006).

*Ultrasonographic and Doppler examination*

Ovarian structures of ewes were monitored using real-time ultrasonography, B-mode, diagnostic scanner equipped with 12.0 MHz linear array real-time B-mode trans-rectal (SonoVet R3, Samsung Madison, South Korea). Used to determine the number, diameter, and position of the follicles and corpora lutea on both ovaries (Ravindra *et al*., 1994). The numbers of CL were counted, and their diameters were measured (Senosy *et al*., 2017). The ovarian follicles were categorized into small (2 to ≤2.9 mm), medium (≥3–≤4.9 mm), and large-sized follicles (≥ 5 mm; Bartlewski *et al*., 1999; Al Mufarji and Mohammed, 2022). The follicle's largest diameter is considered the dominant and the second largest one is considered the subordinate. Follicles with diameters < dominant and subordinate are considered others. Ultrasonographic color Doppler mode was used to determine the vascularization within the ovaries, ovarian structures, and the uterus. Pulsed-wave Doppler was used to assess the blood flow velocity of the uterine and ovarian arteries (Meinecke-Tillmann, 2017).

The uterine artery was located transrectally, and laterally to the bladder, using the external iliac artery as a reference point. From this, we lateralized the transducer by viewing the uterine artery in a cross-section and sliding it dorsally to the branch of the iliac artery (Beltrame *et al*. 2017).

The area of the ovary, corpus luteum, and follicles in cm2 were estimated using the equation of the sphere (4 π r2) where r is the radius of the sphere.

*Blood sampling*

After each ultrasonographic examination, blood samples (5.0 ml) were collected from the jugular vein into plain as well as anticoagulant vacuum tubes. Sera and plasma were harvested after centrifuging blood samples at 3000 rpm for 15 min. Sera and plasma were separated and stored at -20°C till the assessment of circulating ovarian hormones and antioxidants.

*Hormonal and blood biochemical analysis*

Progesterone and estradiol hormones were assayed using a commercial ELISA kit (Monocent, Monocent Inc USA - California). For progesterone, the range of the assay was between 0.0 ng/ml to 40 ng/ml, the sensitivity was 0.045 ng/ml, and intra- and inter-assay variability was 6.81% and 7.25%, respectively. For estradiol, the range of the assay was between 9.7 pg/ml to 200 pg/mL, the sensitivity was 9.714 pg/ml, and intra- and inter-assay variability were 6.86% and 5.59%, respectively. Glutathione reduced (GSH), catalase, superoxide dismutase (SOD), total antioxidants, malondialdehyde (MDA), and nitric oxide (NO) were assayed using the colorimetric diagnostic kits (Biodiagnostics, Egypt).

*Image analysis*

Images of the ovarian follicles and corpora lutea were subjected to image analysis using Photoshop software. The area and perimeter (circumference) of the ovaries, follicle, and corpus luteum were estimated using the magnetic lasso tool. The color area percentages of ovaries were calculated by dividing the color area by the area in pixels.

*Statistical analysis*

The data are presented as means±SEM (standard error of the mean). Statistical analysis was carried out using the SPSS program, version 26. Split simple one-way ANOVA to study the effect of the days within each treatment. Duncan's Multiple Range test was used to differentiate between significant means at $P<0.05$. Repeated Measures A Univariate General Linear Model (2×6) was used to study the effect of treatments (controls and MSC) and time intervals from inserting the sponge (Day -6) till the second follicular phase after removing the sponge (-6, 0, 2, 9, 15, 21) and the interaction of treatment × time. An Independent Sample T-test was used to compare between parameters of the control and MSC at each time interval.

## Results

Moringa seed cake (MSC) used in the current study has 41.32±2.36% carbohydrates, 39.43±1.33% proteins, 13.85±0.78% fat, and 5.39±0.25 Ash. In control ewe lambs, only one dominant follicle was recorded throughout the experiment except on Days 2 (1.20±0.20), 15 (1.33±0.33), and 21 (1.50±0.29). In the ewes supplemented with MSC, the number of the dominant follicle was (1.00±0.00) on Day 15, (1.20±0.20) on Days 0 and 9, (1.40±0.24) on Day 2, (1.60±0.40) on the Day -6, and (1.80±0.20) on Day 21.

In the control ewes, the dominant follicle (Fig. 1) diameter reached the lowest ($P=0.032$) diameters of 3.35±0.22 and 3.65±0.21 mm on the first follicular wave after sponge removal (Day2) and 9 days after sponge removal (Day 9) compared to Day -6 (Day of sponge insertion) and Day 15 (4.20±0.17), Day 0 (4.23±0.4), and 21 (4.25±0.19 mm). The diameter of the dominant follicle (DF) of MSC (Fig. 1) reached the highest ($P=0.038$)

diameters on Days -6 (4.96±.0.24), 2 (4.77±.40), and 21 (5.20±0.22). Compared to controls, MSC had higher DF diameters on Day 2 (P=0.07), 9 (P=0.009), and 21 (P=0.005). Both control and MSC ewes have the same dominant follicle diameters on Day 0 and Day 15. Treatment (P<0.001), time (P<0.05), and treatment ×time (P>0.05) influenced the DF diameters (Fig. 1).

Control ewes' subordinate follicle (SF; Fig. 1) diameters increased (P<0.0001) on Day 21 after sponge removal to reach 3.30±0.09 mm compared to the maximum diameter observed on the day of sponge removal (Day 0; 3.66±0.20 mm). On days -6, 2, 9, and 15 the lowest SF diameters (<0.3mm) were recorded. The SF diameters of MSC increased (P<0.0001) on Day -6 (3.50±0.11), Day 2 (3.65±0.18), and Day 21 (3.90±0.17). The lowest SF diameters of the MSC were recorded on Day 0 (3.08±0.09 mm) and Day 15 (3.10±0.10 mm). SF diameter of the cake group was higher than that of the control on Day -6 (P<0.0001), Day 2 (P<0.0001), Day 9 (P=0.004), and Day 21 (P=0.005) except Day 0 where the controls obtained a higher one (P=0.015). Treatment (P<0.0001), time (P<0.0001), and treatment ×time (P<0.001) influenced the SF diameters (Fig. 1).

Follicles having smaller diameters than DF and SF (others) of control (Fig. 1) reached the lowest diameter (P<0.0001; 1.65±0.15 mm) on Day of sponge removal (Day 0) and two days later (Day 2; 1.84±0.08). The other follicle diameters of MSC reached the lowest diameter (P<0.0001) on Day of sponge insertion (Day -6; 2.58±0.14 mm) and the maximum one on Day 2 (3.85±1.39 mm) and Day 21 (3.07±0.16mm). Only, treatment (P<0.01) influenced other follicles' diameters (Fig.1).

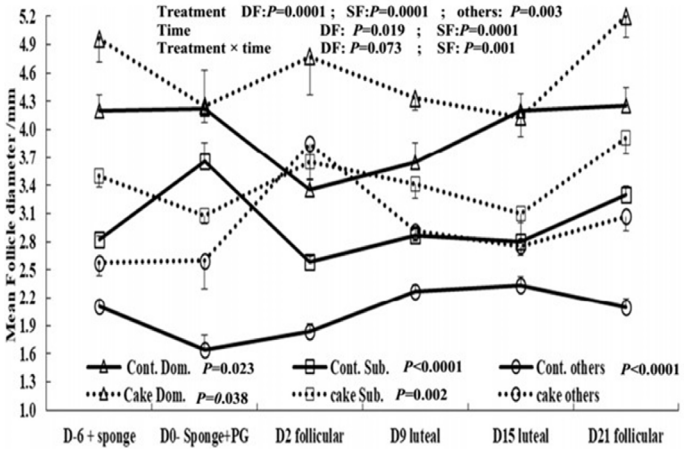


Figure 1. Mean diameter/mm of dominant, subordinate, and other smaller follicles of the control and moringa seed cake from the day of sponge insertion (Day -6) till Day 21 after the day of sponge removal (Day 0) with standard error bars, Dominant follicle (DF, Dom.), subordinate follicles (SF, Sub.), follicles smaller than dominant and subordinates (others)

DF area/pixel (Fig. 2) and DF perimeter/ pixel (Fig. 3) of in the control ewes reached high values on Day -6 (2760±447; 200±17.16 / pixel) and Day 9 (2928±444; 205±15.78 /pixel) and Day 21 (3031±489; 198±16.52), but DF of MSC ewes reached their highest values on Day 2 (3654±358; 229±13.40) and 21 (3636±199; 231±8.73). SF and others smaller than dominant and subordinate of the control ewes reached their maximum area on Days -6, 0, 9, and 21 of subordinate and Days -6 and 21 for others (Fig. 2) and perimeters Days (0, 9, 21 and Days -6, 9, 21) for perimeters of SF and others, respectively (Fig. 3). SF and others smaller than dominant and subordinate of the MSC ewes reached their maximum area (Fig. 2) and perimeters (Fig. 3) on Days 2 and 21. Treatment (P<0.051; P=0.024), time (P<0.0001; P<0.0001), and treatment ×time (P<0.0001; P<0.0001) influenced the DF area (Fig. 2) and DF perimeter (Fig.3). Treatment (P>0.05; P=0.027), time (P<0.0001; P<0.0001), and treatment ×time (P=0.007; P<0.001) influenced the SF area (Fig. 2) and perimeter (Fig. 3). Treatment (P=0.004; P<0.0001), time (P<0.0001; P<0.0001), and treatment × time (P<0.0001; P<0.0001) influenced the others F area (Fig. 2) and perimeter (Fig. 3).

In control ewes, the maximum (P<0.0001) diameter (3.14±0.16mm), circumference (9.86±0.51mm), area (2146±304 pixel), and perimeter (166.11±10.91 pixel) of the average total follicular populations were recorded on Day 21 (Table 1). Ewes of MSC had a large diameter of total follicle population (P=0.0001; 4.18±0.16 mm), and circumference (P=0.001; 13.12±0.49 mm) on Day 21 with maximum follicle area (P<0.0001; 2261±211) and perimeter (P<0.0001; 174.5±8.07 pixel) on Days 2 and 21 (2370±150; 182.5±6.3 pixel). The total follicle population average diameter and circumference of the MSC are higher than controls on Day -6 (P<0.0001), Day 2 (P<.0001), Day 9 (P<0.0001), and Day 21 (P<0.0001). A significant increase in the follicle area/pixel and follicle perimeter/pixel (P<0.0001) are observed on Day 2 in the MSC group compared to the control one. Treatment influenced the average total follicles diameters/mm (P=0.003), perimeter/mm (P<0.0001), area/pixel (P=0.0001), and perimeter /pixels (P<0.0001). Time impacted the average total follicles perimeter/mm (P<0.0001), area/pixel (P=0.0001), and perimeter / pixels (P<0.0001). Treatment × time affected the average total follicles perimeter/mm (P<0.05), area/pixel (P=0.0001), and perimeter /pixels (P<0.0001). The circulatory % (Table 1) of MSC average follicular populations (P=0.001) reached the highest value on Days 9 (84.52±0.39%) and Day 2 (85.53±0.82%) but those of controls reached high (P<0.0001) values on Day 21 (84.31±0.51%) and Day 15 (85.05±0.51%). The circulatory % of MSC is lower than control on Day -6 (P=0.029) and Day 15 (P=0.071) but is higher than controls (P=0.002) on Day 0 and Day 2 (P=0.033). Either time (P=0.0001) or treatment × time (P=0.0001) affected the average total follicles circulatory % (Table 1).

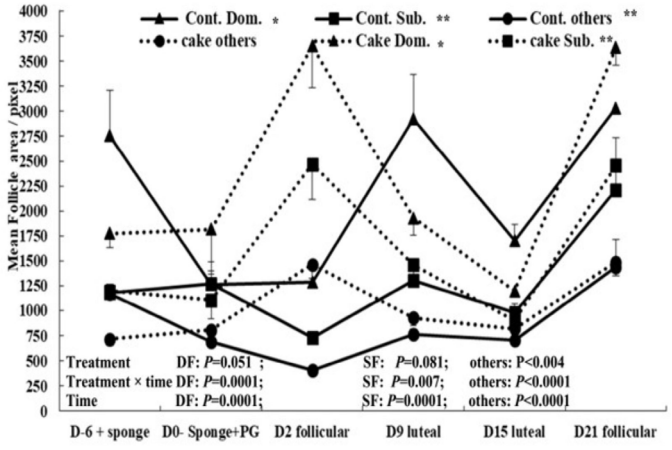


Figure 2. The mean perimeter /pixel of dominant, subordinate, and other follicles of the control and moringa seed cake from the day of sponge insertion (Day -6) till Day 21 after the day of sponge removal (Day 0) with standard error bars, * means significant at P<.05, ** means significant at P<0.001, Dominant follicle (DF, Dom.), subordinate follicles (SF, Sub.), follicles smaller than dominant and subordinates (others)

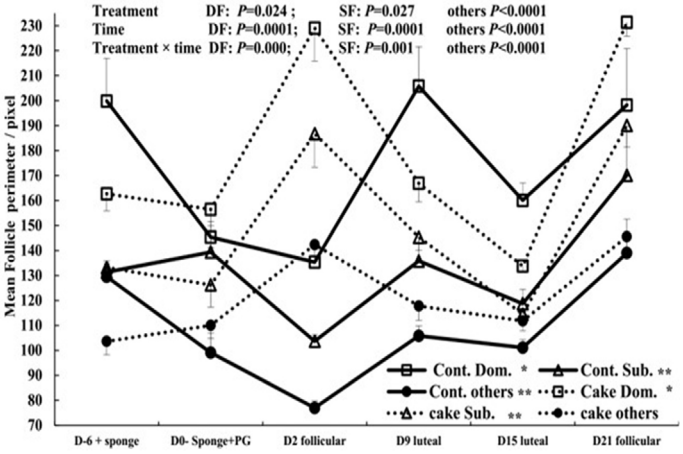


Figure 3. The mean area /pixel of dominant, subordinate, and other follicles of the control and moringa seed cake from the day of sponge insertion (Day -6) till Day 21 after the day of sponge removal (Day 0) with standard error bars, * means significant at P<.05, ** means significant at P<0.001, Dominant follicle (DF, Dom.), subordinate follicles (SF, Sub.), follicles smaller than dominant and subordinates (others).

The ovulation rate was 1.0 throughout the experiment in both controls and MSC except on Day 0 of MSC, when it reached the highest (P=0.016) value (1.50±0.019). The ovulation rate of the cake group tended to be higher (P=0.098) than control on Day 0. The control ewes' corpus luteum diameter /mm (Fig. 4; P=0.023) and perimeter/mm (Table 2; P=0.023) were low on Day 9 (8.63±0.38 mm; 27.11±1.18mm) and Day 15 (8.76±0.23; 27.49±0.73 mm) compared to the maximum diameter and perimeter on day 0 (10.73±0.27; 33.68±.86 mm) and Day 21 (10.90±; 0.03; 34.23±0.07mm). The CL diameter (P<0.001) and perimeter (P<0.001) of MSC animals showed low values on Day 21 (7.25±0.56 and 22.75±1.75 mm). MSC showed maximum CL diameter/mm (Fig. 4) and perimeter (Table 2) on Day 15 (10.06±0.2 and 31.58±0.06) and Day -6 (10.18±0.13 and 31.98±0.42mm). The CL diameter of the control ewes is lower than MSC ewes on Day 15 (P=0.002) but is higher than MSC on Day 21 (P=0.012) and the CL perimeter/mm of control is lower than MSC (P=0.0001) on Day 15.

Treatment influenced CL diameter (P=0.013) and tended to impact color area (P>0.083). Time intervals influenced (P<0.001) CL diameters and color areas. Treatment × time influenced the CL diameters (P=0.001; Fig. 4), and color area (P=0.047; Fig. 5).

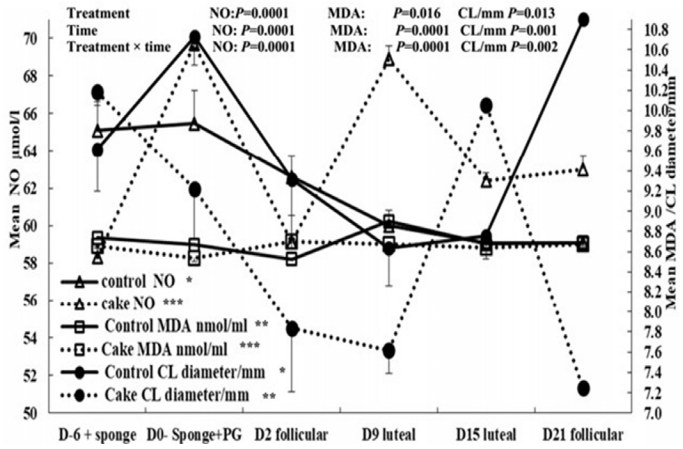


Figure 4. The mean nitric oxide (NO), malondialdehyde (MDA), and CL diameter of the control and moringa seed cake from the day of sponge insertion (Day -6) till Day 21 after the day of sponge removal (Day 0) with standard error bars, * means significant at P<.05, ** means significant at P<0.001, *** means significant at P<0.0001

The Perimeter/cm of control ewes CL is higher than ewes supplemented MSC on Day 0 (P=0.047), Day 9 (P=0.089), and Day 21 (P=0.001) but got a lower circumference on Day 15 (P=0.006).

The ovarian area (Fig. 6) of the control ewes increased (P=0.004) on Day 21 (17367±199) and Day 9 (17942±266) compared to the lowest area on Day 2 (1071±893). However, the ovarian area of MSC was high on Day 21 (23954±4653 pixels). The ovarian area of MSC is higher than controls on Day 0 (P=0.021), and Day 2 (P=0.004) but is lower than control on Day 9 (P=0.02). The ovarian color area/pixel (Fig. 6) and color area % (P=0.09) of controls showed minimal variation throughout the study but those of MSC showed minimum (P<0.0001; 0.009) values on Day 9 (1133.4±74.55 pixels; 9.57±0.89%) and maximum value on Day 2 (2871.5±304.90 pixel; 16.81±1.59%). The ovarian color area % (Table 2) was also low on Day 21 (P<0.009; 10.78±1.35). The ovarian color area of cake group MSC is

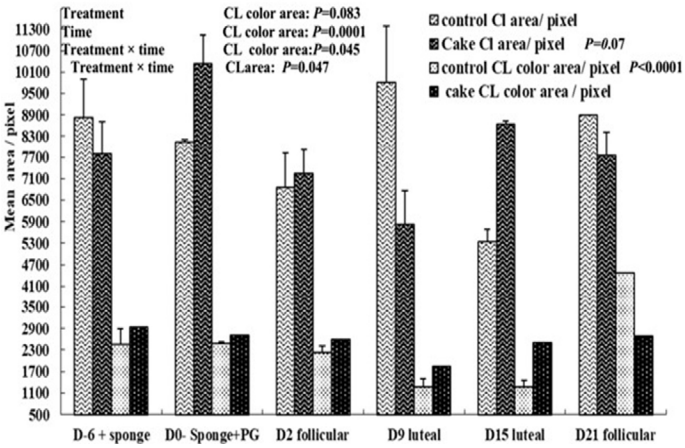


Figure 5. The mean corpus luteum (CL) area /pixel and color area /pixel of the control and moringa seed cake from the day of sponge insertion (Day -6) till Day 21 after the day of sponge removal (Day 0) with standard error bars.

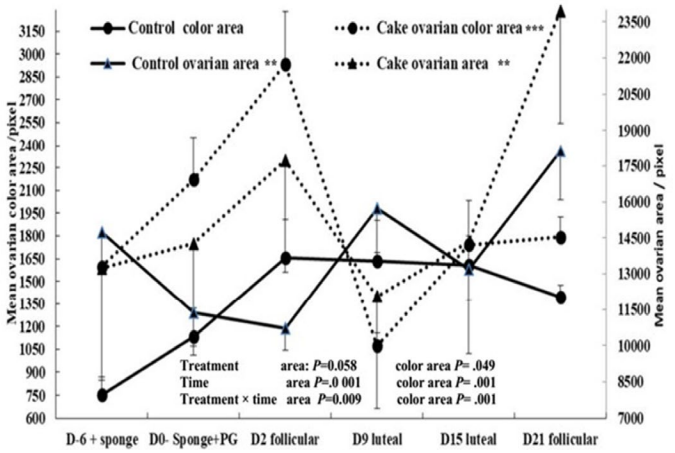


Figure 6. The mean ovarian area /pixel and ovarian color area /pixel of the control and moringa seed cake from the day of sponge insertion (Day -6) till Day 21 after the day of sponge removal (Day 0) with standard error bars.

higher than controls on Day 0 (P=0.016), and Day 2 (P=0.010) but is lower than controls on Day 9 (P=0.011). Treatment, time, and Treatment × time influenced (P<0.05) ovarian area and color area (Fig. 6).

The concentrations of nitric oxide (NO; P=0.031; Fig. 4) reached the lowest values on Day 21 (59.20±0.73µmol/l) and the highest values can

Table 1. Follicular populations (all classes) dynamics in yearling ewe-lambs showing the effect of time within treatment and between treatments.

| Days | Follicles diameter/mm | | Follicles perimeter/mm | | Follicle area/pixel | | Follicles perimeter /pixel | | Follicles circulatory% | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | cake | Control | cake | Control | cake | Control | cake | Control | Cake |
| -6 | 2.58±0.09 | 3.55±0.180** | 8.9±0.30 | 11.16±0.56** | 1305±92.9$^{b}$ | 1169±85.19 | 136.13±4.47$^{b}$ | 129.63±4.88 | 83.95±0.41$^{b*}$ | 82.17±0.68 |
| 0 | 3.10±0.25 | 3.32±0.15 | 9.75±0.79 | 10.44±0.49 | 1063.±104$^{ab}$ | 1287±177 | 126.69±6.52$^{b}$ | 133.09±7.16 | 78.92±1.19$^{a}$ | 82.65±2.48** |
| 2 | 2.37±.11 | 3.34±.22** | 7.45±.35 | 10.49±.68** | 694±61$^{a}$ | 2261.39±210.8** | 97.42±4.07$^{a}$ | 174.53±8.07** | 84.14±.49$^{b}$ | 85.53±.39* |
| 9 | 2.81±.09 | 3.63±.12** | 8.82±.28 | 11.39±.38** | 1341±161$^{b}$ | 1373.1±93.69 | 134.13±7.35$^{b}$ | 140.12±4.85 | 83.79±.43$^{b}$ | 84.53±.82 |
| 15 | 2.91±.16 | 3.15±.13 | 9.15±.5 | 9.90±.41 | 1022±88$^{ab}$ | 925.15±47.04 | 120.22±5.09$^{b}$ | 117.51±2.85 | 85.05±.64$^{b}$ | 82.98±.97# |
| 21 | 3.14±.16 | 4.18±.16** | 9.86±.51 | 13.12±.49** | 2146±304$^{c}$ | 2370.4±150.19 | 166.11±10.91$^{c}$ | 182.49±6.33 | 84.31±.51$^{b}$ | 83.6±.46 |
| P-value | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Treatment | 0.00 | | 0.00 | | 0.00 | | 0.00 | | NS | |
| Time | NS | | 0.00 | | 0.00 | | 0.00 | | 0.00 | |
| Treatment × time | NS | | 0.04 | | 0.00 | | 0.00 | | 0.00 | |

Data are presented as Mean±SEM. Means with different superscript letters ($^{a, b, C}$) within columns are significant at P<0.05, non-significant (NS)

be observed on Day 0 (64.46±0.85 µmol/l). Ewes supplemented MSC showed minimum (P<0.0001) NO values on Days -6 and 2, and high values on Days 0 and 9. NO of the cake group is higher (P<0.0001) than control on Days 0, 9, 15 and 21 but lower than it on Days -6 (P=0.031) and Day 2 (P=0.086). On the day of sponge insertion (Day-6) of control ewes, MDA (8.86±0.01 nmol/ml) achieved the maximum (P<0.0001) concentrations on Day 9 but the lowest MDA (Fig. 4) values can be observed on Day 2 (8.55±0.07 nmol/ml). MSC ewes had the lowest MDA (P<0.0001) on Day 0 (8.55±0.2). MDA of the control ewes showed higher values compared to MSC on Days 0 (P<0.0001) and Day 9 (P=0.047) but low values on Days 2 (0.071) and Day 15 (P=0.002). Treatment, time, and Treatment × time affected the concentrations of NO(P<0.0001) and MDA (P<0.05; Fig. 4).

High estradiol (E2 pg/ml, P<0.0001; Fig. 7) concentrations can be observed on Day 2 (34.02±3.77 pg /ml), Day -6 of sponge insertion (43.41±0.99 pg/ml), and Day 0 (48.03±1.80) by removing sponge and injecting prostaglandin where the lowest values were recorded on Day 21 (9.67±1.43 pg/ml). E2 of the MSC group showed low (P<0.0001) concentrations on Day 9 (28.56±1.11) and Day 21 (31.51±6.52) but high values on Day 15 (59.26±6.52) and Day -6 (72.44±3.13pg/ml). E2 concentrations of the ewes-supplemented MSC are higher than control on Days -6 (P<0.0001), 2 (0.015), 9 (P<0.001), 15 (P<0.0001) and 21 (P=0.001). Treatment, time, and Treatment × time affected (P=0.001) the concentrations of E2 (Fig. 7).

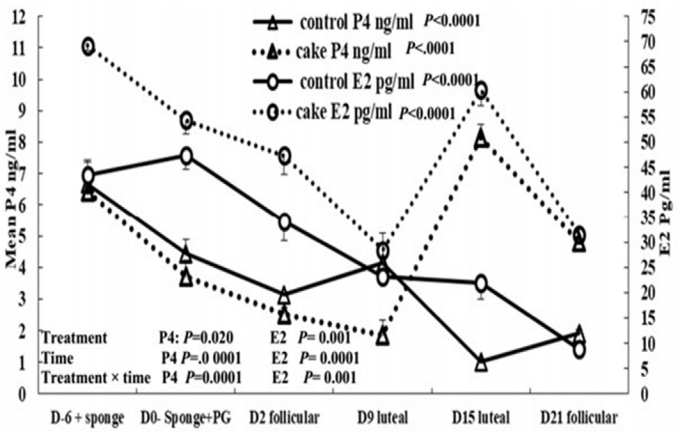


Figure 7. The mean progesterone a (P4 ng/ml) and estradiol (E2 pg/ml) concentrations in the control and moringa seed cake groups from the day of sponge insertion (Day -6) till Day 21 after the day of sponge removal (Day 0) with standard error bars.

Progesterone of control ewes (P4, P<0.0001; Fig. 7) showed minimum concentrations on Days 15 (0.99±0.08 ng/ml) and 21 (1.88±0.12ng/ml) but high concentrations on Day 2 (3.13±0.06), 9 (3.81±0.80ng/ml), Day 0 (4.60±0.08), and Day±6 (6.67±0.12ng/ml). MSC ewes had low P4 (P<0.0001) on Day 9 (2.06±0.21 ng/ml) and Day 2 (2.55±0.10) and high concentrations on Day 21 (4.79±0.89), Day±6 (6.74±0.50), and Day 15 (8.77±2.35). P4 of the control ewes are higher than cake on Days 0 (P=0.019), Day 2 (P<0.0001) and Day 9 (P=0.018) but lower than cake on Days 15 (P=0.0001) and Day 21 (P=0.03). Treatment, time, and Treatment × time affected (P<0.05) the concentrations of P4 (Fig. 7).

The maximum (P<0.0001) GSH of control ewes (78.62±1.09 mg/dl) is observed on Day 2 (Fig. 8) but the lowest GSH can be noticed on Day 21 (53.25±0.60mg/dl). MSC ewes had the lowest MDA (P<0.0001) on Day 0 (8.55±0.2) associated the lowest (P<0.0001) GSH on Day 21 (59.56±1.48) and high GSH values on Day 15 (73.52±1.43), Day 9 (76.17±1.32), and Day 2 (78.44±0.89mg/dl). GSH of the cake group showed higher concentrations on Days 9 (P=0.027) and Day 2 (P=0.0001). The highest (P<0.0001) catalase concentration (Fig. 8) of control animals is observed on days 9 (263.89±3.40 U/L), Day 15 (266.45±6.75), and Day 21 (273.71±4.86 U/L). Whereas the minimum catalase was recorded on Day 0 (231.57±1.50U/L). Catalase of MSC ewes showed minimum values on Day 0 (261.49±4.19 U/L) and high activity on Day 15 (274.67±2.72), Day 9 (275.41±1.12), and Day±6 (282.46±3.09). Catalase concentrations of the cake group showed increased activity (P<.001) from Day -6 to Day 15 compared to controls. The highest (P<0.0001) SOD (295.82±9.11U/ml) and total antioxidants (1.14±0.03 mM/L) concentrations can be observed on Day 0 but the lowest ones were recorded on Day 15 (256.52±6.07 U/ml), and (0.56±0.02 mM/L). The concentrations of total antioxidants (Table 2) of the cake groups are higher than controls on Day -6 (P<0.0001), Day 2 (P=0.32), and Day 9 (P=0.006). SOD (Fig. 8) of MSC showed the minimum activity (P<0.001) on Day 2 and high activity on Day -6. The total antioxidant (Table 2) of control and MSC-supplemented ewes was high (P<0.0001) on Day -6 and Day 0 compared to Days 2 to Day 21.MSC ewes obtained

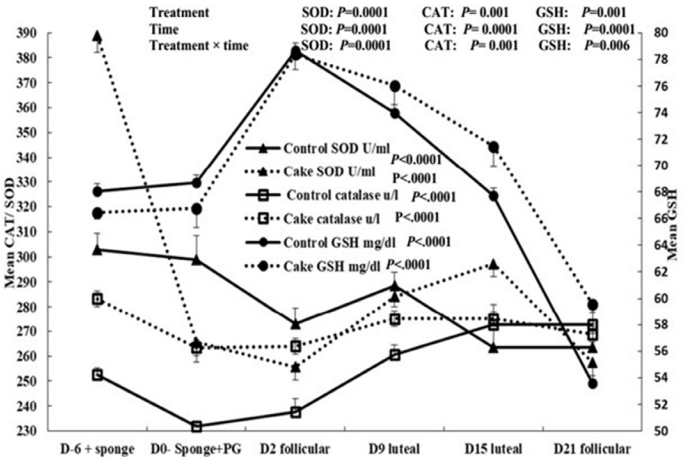


Figure 8. The mean catalase (CAT), superoxide dismutase (SOD), and glutathione reduced (GSH) of the control and moringa seed cake from the day of sponge insertion (Day -6) till Day 21 after the day of sponge removal (Day 0) with standard error bars.

Table 2. Luteal and ovarian dynamics in yearling ewe-lambs showing the effect of time within treatment and between treatments.

| Days | CL perimeter /mm | | CL area /cm$^2$ | | Ovarian color area% | | Total antioxidants | |
|---|---|---|---|---|---|---|---|---|
| | Control | cake | Control | cake | Control | cake | Control | cake |
| -6 | 30.20±1.31$^{ab}$ | 31.98±0.42$^{c}$ | 2.69±.30$^{ab}$ | 3.24±0.09$^{a}$ | 11.48±1.75 | 11.53±1.09$^{a}$ | 1.05±0.02 | 1.12±0.01** |
| 0 | 33.68±.86$^{b}$ | 28.95±1.57$^{bc*}$ | 3.59±.17$^{bc}$ | 2.49±0.28$^{bc}$ | 12.96±1.43 | 12.87±1.32$^{ab}$ | 1.14±.03 | 1.09±0.01 |
| 2 | 29.26±2.17$^{ab}$ | 24.60±1.96$^{ab}$ | 2.36±.40$^{a}$ | 1.94±0.19$^{ab}$ | 13.27±1.97 | 16.81±1.59$^{b}$ | 0.70±.03 | 0.77±0.02* |
| 9 | 27.11±1.18$^{a}$ | 23.91±.71$^{ab\#}$ | 2.34±.22$^{a}$ | 1.47±0.11$^{a}$ | 9.57±1.01 | 9.57±.89$^{a}$ | 0.79±.02 | 0.85±0.01** |
| 15 | 27.49±.73$^{a}$ | 31.58±0.06c$^{**}$ | 2.09±.22$^{a}$ | 3.15±0.08$^{c}$ | 12.77±1.19 | 12.81±2.16$^{ab}$ | 0.56±.02 | 0.59±0.02 |
| 21 | 34.23±.07$^{b}$ | 22.75±1.75$^{a**}$ | 4.13±.08$^{c}$ | 1.38±0.23$^{a}$ | 12.63±2.21 | 10.78±1.35$^{a}$ | 0.69±.04 | 0.66±0.02 |
| P-value | 0.02 | 0.00 | 0.01 | 0.00 | NS | 0.01 | 0.00 | 0.00 |
| Treatment | 0.01 | | 0.01 | | NS | | 0.02 | |
| Days | 0.00 | | 0.00 | | 0.01 | | 0.00 | |
| Treatment × Days | 0.00 | | 0.00 | | NS | | 0.00 | |

Data are presented as Mean±SEM. Means with different superscript letters (a, b, C) within column are significant at P<0.05, non- significant (NS)

higher total antioxidant capacity on Day -6 (P<0.0001), Day 2 (P=0.032), and Day 9 (P=0.006) than the control. Treatment, time, and Treatment × time affected the concentrations of total antioxidants (P<0.01), CAT (P<0.001), GSH (P<0.01), and SOD (P<0.0001; Fig. 8).

## Discussion

Moringa seed cake (MSC) is a by-product of oil extraction that has a high protein content (61.4% to 70.3% CP on a DM basis) and was recommended to be used as a protein supplement (Hansen *et al*., 2020; Makkar *et al*., 2007). Moringa seed cake protein content (25%-60%) differs according to the methods of extraction (Fuglie, 2001; El-Naggar *et al*., 2017). Hassan *et al*. (2023) suggested that MSC can efficiently be used as an alternative protein source to ration at levels up to 50% to improve growth performance and net profit without any adverse effects on nutrient digestibility and blood metabolites. Defatted seed (cake) is free of most plant secondary metabolites such as tannins, saponins, alkaloids, inhibitors of trypsin and amylase, lectin and cyanogenic glucosides, but contains glucosinolates which give it the bitter taste (Makkar and Becker, 1997).

In the investigated ewe lambs, the number and diameters of the dominant follicle improved when MSC was supplemented. The increase in the diameter of the dominant and subordinate follicles 2 and 21 days after removing the sponge compared to Day 0 (Sponge removal) and Day 15 in control non-supplemented ones, the comparison between Day 2 of MSC and Day 0 of control confirms the improvement in the follicle diameter, circumference, perimeter, ovarian area, and color area. Moringa extract increased the gene expression of oocyte maturation in sheep (Barakat *et al*., 2015). The authors added that this improvement in the ovarian blood flow, ovarian area, color area, and dominant and subordinate follicle diameters of ewe-lambs supplemented MSC is comparable to the improvement in the oocyte maturation and embryo developments recorded when moringa extract was supplemented in the maturation media of sheep oocytes and this improvement was referred to its action as a promotor in the induction of mRNA expression and synthesis of essential proteins for oocyte maturation of ewes in vitro.

The improvement in the concentration of estradiol in ewe lambs supplemented MSC compared to control could be referred to the regulating effects of some plant's compounds on the reproductive functions by acting directly or indirectly on the hypothalamic-pituitary-ovarian axis by induction or inhibition of ovulation and steroidogenesis disrupting hormonal functioning of the hypothalamus and pituitary gland. Moringa leaves improved the ovine oocyte maturation rate (Barakat *et al*., 2015). Similar to the increase in estrogen in ewe lambs supplemented with MSC, moringa increased estrogen in women (Otitoju *et al*., 2019) and rabbit dams (El-kashef, 2022).

After removing sponges, the decrease in progesterone in the MSC was parallel to the decrease in corpus luteum diameter, area, and color area to reach minimum values on Day 9 and their increase was maximum on Day 15 but those of control were low on Day 15 and high on Day 21 which coordinates with the dominant and subordinate follicles development. This difference in the response of control and MSC ewe lambs indicates a delayed response to exogenous progesterone. Similar to the response of ewes supplemented MSC of this study, 47.4% of cows injected with prostaglandin F2α via intravulvo-submucosal injection came in estrus within 5 days after treatment compared to 54.7 % administrated the same dose intramuscularly (Rovani *et al*., 2012). The response of MSC ewes to sponge removal and prostaglandin F2α administration was confirmed by the decrease in progesterone to reach low values 2 days later is similar to cows administrated PGF2α via IM or intravulvo-submucosal routes and had low progesterone within 4 days later (Rovani *et al*., 2012). Ewes supplemented with MSC and administrated 50% of PGF2 α via intravulvo-submucosal showed luteolysis 2 days post administration and decreased CL diameter, area, color area and progesterone, non-lactating Nelore cows administrated 50% of PGF2 α via IM or IVSM was Sufficient to induce efficiently and effectively a complete luteal regression and hasten the onset of estrus (Meira *et al*., 2006)

Similar to the increase of the number of dominant follicles in ewe lambs supplemented with MSC of this study, Naimi ewes supplemented with 50.0 and 100.0 g /kg diet moringa had a large number of small, medium, and large follicles 18 days postpartum (Al Mufarji and Mohammed, 2022) and 21 days postpartum (Al-Mufarji *et al*., 2023). Usually follicles area /mm2 start to increase from day 18 postpartum (Elmetwally and Bollwein, 2017). The increase in the number and diameter of the dominant follicles in ewe-lambs synchronized with short-term exogenous progesterone and PGF2α and supplemented with MSC, L carnitine increased the number of follicles, number of dominant follicles, and the diameter of the dominant follicles in ewes synchronized by Ovsynch protocol during the follicular phase (Samir *et al*., 2023). In agreement with the increase in the diameter of dominant and subordinate follicles of control ewe lambs of this study, Ossimi ewes showed high dominant and subordinate follicle diameters on Day 2 and maximum ones on Day 6 after PGF2α administration (El-Sherry *et al*., 2013). As well as the ovarian color area and color area % increased two days and reached maximum values 6 days after PGF2α administration (El-Sherry *et al*., 2013). However, the difference between our study and those of Ossimi ewes, is our ewes are still yearling with smaller ovarian volumes, and they were synchronized with exogenous progesterone and single PGF2α in addition to the difference in the breed.

In agreement with the increase of ovulation rate in ewes-supplemented MSC, moringa leaves in doses of 50.0 and 100.0 g /kg diet improved the CL number and the CL diameters 18 days (Al Mufarji and Mohammed, 2022) and 21 days postpartum (Al-Mufarji *et al*., 2023). Comparable with the increase in the CL diameter, area, and color area during the luteal phase in ewe lambs fed MSC, ewes supplemented with l carnitine and synchronized with Ovsynch protocol had more CL numbers but of low diameters and lower number of follicles with the dominant having a high diameter on Day 8 (Samir *et al*., 2023). The increase in the CL area and color area, follicle area, ovarian area and color area in ewe-lambs supplemented MSC simulate the increase in the dominant follicle color area and the CL color area after L-carnitine supplementation (Samir *et al*., 2023).

Our results showed improvement in the activity of antioxidants and a decrease in MDA when ewe-lambs were supplemented with MSC. In agreement with the decreased MDA in ewe-lambs supplemented MSC of this study on Days -6, 0, and 9, Najdi ewes fed 25% *Moringa oleifera* leaves in their diet for six weeks, presented decreased concentration of malondialdehyde (MDA) in the ewes' milk and serum (Babiker *et al*., 2016). The increase in the total antioxidant capacity and catalase activity in ewe-lambs supplemented MSC of this study was also recorded in Najdi ewes-supplemented moringa leaves (Babiker *et al*., 2016). Similar improvement in the antioxidant capacity and concentrations was reported when lactating dairy cows were supplemented with moringa leaf meal (Kekana *et al*., 2019). The improvement in the antioxidant capacity of our ewe-lambs supplemented MSC could be referred to the antioxidant properties of moringa leaves (Verma *et al*., 2009). Moringa leaves increased the activity of glutathione reduced (GSH), catalase and superoxide dismutase and reduced lipid peroxidation in a dose-dependent manner in the liver and kidney of rats intoxicated with carbon tetrachloride (Verma *et al*., 2009). Similar to our ewe lambs, supplementing moringa leaves in goats' diet increased the activity of glutathione reduced, catalase, and superoxide dismutase and reduced the production of MDA in their livers (Moyo *et al*., 2012). Not only the leaves, but all parts of the *Moringa oleifera* plant have high-quality nutritional and antioxidant content (Saxena *et al*., 2013; Sivasankari *et al*., 2014; Su and Chen, 2020). Similar to the increase in the total antioxidant capacity of ewe-lambs supplemented MSC during the follicular and luteal phases of the estrous cycle, ewes treated with L- carnitine showed increased levels during the two phases after synchronization with Ovsynch protocol (Samir *et al*., 2023).

## Conclusion

Moringa seed cake (MSC) improves the response to estrous using short-term exogenous progesterone, follicular, luteal diameters, areas, and color areas in ewe lambs. MSC elevates estradiol and increase dominant follicle diameters. MSC improves the antioxidant capacity and reduces MDA production. MSC is recommended to be used in yearling sheep to improve the hormonal changes, dynamics and hemodynamics of the ovaries.

## Conflict of interest

The authors have no conflict of interest to declare.

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