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Dietary supplementation with thyme oil improves the reproductive characteristics of Barki adult and prepubertal ewes

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Abstract

The reproductive technology has a significant impact on the development of livestock production. The thyme oil, rich in phytoestrogen chemicals like apigenin, has been found to enhance reproductive performance by mimicking estrogen's action. This study aimed to investigate the effects of oral supplementation with thyme essential oil on the reproductive organ biometry, and reproductive performance in adult and prepubertal Barki ewes. Seventy ewes were treated with intravaginal sponges impregnated with 40 mg. medroxyprogesterone acetate for 14 days and simultaneously assigned randomly to two groups (20 adult and 15 prepubertal ewes per group), i.e., a control and a thyme oil treated (2.25 mg/kg body weight) group. The number of follicles, follicle diameter, corpora lutea diameter, and estrogen concentration were higher in the thyme oil-treated group than in the control group without comparing between adult ewes and prepubertal ewes. Additionally, the number and diameter of the large follicles were higher ($P < 0.05$) in the right-side ovary of adult ewes compared with that in prepubertal ewes at day 15. Moreover, thyme oil treatment resulted in higher conception ($P < 0.01$), lambing rates ($P < 0.05$), and fecundity ($P < 0.01$), with values 95.12, 136.73, and 130.25 compared with those in the control group, with values 63.51, 105.24, and 66.97, respectively, without comparing between adult ewes and prepubertal ewes. The adult ewes had a stronger estrus response and better fertility measurement values than prepubertal ewes. Additionally, the estrogen serum levels were positively correlated with the number ($P < 0.05$) and diameter ($P < 0.01$) of follicles, and the progesterone serum concentration was positively correlated with the corpora lutea diameter ($P < 0.01$), without treated group. In conclusion, dietary supplementation with thyme oil improved the follicular population and reproductive performance, which has a good effect on the adult and prepubertal ewes in the Barki ewes.

Keywords Thyme essential oil, Dietary supplementation, Gonads, Fertility rate, Ovarian activity

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Introduction

Mutton and other sheep products represent a large part of agricultural income in many countries worldwide [1]. Subtropical sheep, including Egyptian sheep, are non-seasonal and able to breed year-round [2]. Furthermore, the Barki sheep is one of the Egyptian sheep breeds that is characterized as adapted to harsh conditions with a higher lambing frequency and fecundity rate compared to local Egyptian sheep [3].

Farming profitability is increased by decreasing the animal age at first lambing. For instance, the entry of prepubertal ewes into breeding at 7–12 months of age increases the economic value of sheep [4, 5]. Thus, many investigations were conducted to decrease the age at first lambing, which can improve ewe efficiency [6].

Essential oils have been suggested to improve the reproductive performance of sheep [7]. These oils prevent amino acid deamination and limit methane production [8]. Thyme essential oil is the most widely used in animal nutrition because it contains various bioactive compounds with antioxidant, antimicrobial, and aroma-regulating activities. These compounds include thymol, carvacrol, monoterpene hydrocarbons, p-cymene, and γ-terpinene [9–11].

Several studies have examined the effects of thyme essential oil as a natural feed additive on ruminal fermentation, reproductive performance, antioxidant indices, and blood parameters during the estrous cycle of adult Barki ewes using in vitro batch culture technique [12, 13], or in the field [14]. Furthermore, thyme and its derivatives possess these qualities because thyme is a source of phytoestrogen chemicals, which bind to estrogen receptors and may influence the synthesis and metabolism of estrogen [15–17]. Also, the studies were treated with thymol oil at different doses ranging from 0.5 to 3 mg and in different forms, whether extract or whole herb [18, 19].

Among the flavonoid components of thyme oil is apigenin. It resembles the estradiol hormone in chemical structure, can mimic estrogen function, and may improve the estrous response, signs of estrus, acceptance of the ram for mating, and conception rate in ewes [20]. Estrogen and progesterone are the main ovarian hormones, estrogen is responsible for estrus and the progesterone is considered to maintain pregnancy and are used as an indicator of the health of the estrous cycle [21]. However, no previous studies have investigated the impact of thyme oil on the corpora lutea diameter and follicular population or correlated these factors with estrogen and progesterone levels.

The hypothesis of this study was that improving reproductive performance through dietary supplementation with thyme oil [22–24]. Therefore, the present study aimed to evaluate the effects of dietary thyme oil on the follicular population, reproductive performance, and sexual hormone profile of adult and prepubertal Barki ewes.

Table 1 Chemical composition of the experimental diet

Chemical components	Forage/concentrate ratio
Dry matter	93.62
Organic matter	92.18
Crude proteins	15.90
Neutral detergent fiber	55.45
Acid detergent fiber	16.55
Ether Extract	4.28
Ash	7.82

Materials and methods

The study was carried out at the Experimental Sheep Farm belonging to the Faculty of Agriculture, Al-Azhar University, Cairo, Egypt, located on the Nile River, 160 km inland from the Mediterranean Sea and 135 km (80 miles) west of the Red Sea, latitude 27.18∘N, and longitude 31.19∘E. The experiments were conducted during the summer season when the highest (maximum) temperatures ranged from 24 °C to 40 °C and the lowest (minimum) from 12 °C to 27 °C.

Experimental animals

The present study included 70 Barki ewes comprising 40 adult ewes (weighing 45±3.5 kg and aged 2.5–3 years) and 30 prepubertal females (weighing 34±2.5 kg and aged 10–12 months). The prepubertal are a nulliparous female who reached puberty between 6.5 and 8 months of age and weighed 31 to 35 kg, according to farm records. The animals appeared healthy and were clinically normal. Fresh water was available *ad libitum*. The animals were kept outdor with access to a shelter during the day and housed in a semi-open barn at night. They were fed a daily farm ration, adjusted according to the National Research Council (NRC; 1985). The chemical composition of the experimental feedstuff is described in Table 1.

Experimental design

All animals were treated using an intravaginal sponge (IVS) impregnated with 40 mg of medroxyprogesterone acetate (Pfizer manufactured, NV/SA, Puurs, Belgium). The IVS was withdrawn after 14 days. The animals were randomly divided into a control and a thyme oil-treated group. Each group comprised two subgroups of 20 adult ewes and 15 prepubertal ewes. The thyme oil treated group received 2.25 mg/kg body weight thyme oil orally; the dose was adjusted based on body weight modified according to Shehata et al. [17], during the IVS treatment period (14 days). Thyme oil was obtained from Menachem, Strategic Partner for Specialty Chemicals, Certified Contract Manufacturer, India (Batch No: TO/CAL/5021/21–22) (Table 2). The experimental design is shown in Fig. 1.

Ultrasonographic examination

Ultrasonography was performed using a real-time, B-mode scanner (Sonoscape, A5, vet, China) with a vet rectal linear array auto-adapted frequency transducer range of 4–9 MHz. At days 15, 16, and 17 of the estrous cycle, i.e., 24, 48, and 72 h after IVS withdrawal, respectively, 10 animals (5 adult ewes and 5 preadult ewes) from each thyme oil-treated group and the control group were examined for the follicular population follicular population (number of small and large follicles), the diameter of the largest (ovulatory) follicles, and cross-sections of the uterine horn. The transducer was equipped with a self-made connector to facilitate transrectal manipulation. The images of the ovaries and uteri were frozen on the monitor, and the ultrasound device's built-in calliper was used to measure the structures' maximum diameters. Ovulation occurs when large, expanding antral follicles were no longer visible [25]. On the 51st experimental day (representing day 35 after mating), the uterine content was scanned to determine pregnancy. To prevent individual variance, the same operator performed all inspections.

Reproductive performance and sexual behavior

Ewes in estrus were mated with six healthy fertile Barki rams (2–3 years old) at days 15, 16, and 17 of the estrous cycle. Estrus signs in ewes were checked twice a day, in the morning and evening, 24 h after IVS withdrawal. All rams were used for estrus detection and insemination for both the treated and control groups. Soliciting, Sniffing scrotum, Anogenital sniffing, and Walking were determained as an estrus sign, and the ewe was considered in estrus when it stood firmly to be mounted by the ram. The conception rate was estimated using ultrasonography 39 days after mating. Fertility, fecundity, and lambing rates were recorded after parturition. All experimental groups were video recorded using a digital video recording system to observe the presence of estrus signs and study the estrous behaviors of ewes described in Table 3. A network camera (Samsung, China) running Android was used and installed on the ceilings to cover the entire area in a way that animals could be monitored.

Table 2 Certificate of thyme oil analysis*

Items	Analysis
Batch No.	TO/CAL/5021/21–22
Country of origin	India
Appearance	Yellow to pale yellow liquid
Odor	Characteristic odor of thyme and sharp burning taste
Solubility in water	Insoluble
Specific gravity at 25 °C	0.919 (0.900–0.930)
Optical rotation	+0.5° (−5° to +5°)
Refraction index at 25 °C	1.4998 (1.4900–1.5100)
Thyme content	50.21% (50.00% minimum)

*performed by Monachem, Strategic partner for Specialty Chemicals, Certified Contract Manufacturer, India

Blood sampling

Four hundred twenty blood samples (70 animal × 2 groups × 3 times) were collected from the jugular vein at days 15, 16, and 17 of the estrous cycle. Blood samples were centrifuged at 3000 rpm for 15 min, and the resulting serum samples were harvested and stored at −20 °C until further analysis. Serum progesterone and estrogen (17ß-estradiol) concentrations were measured using an ELISA kit (Biocheck, Inc. Foster City, CCA 94404; U.S.A.). Serum triiodothyronine (T3) and thyroxine (T4) concentrations were also analyzed using ELISA kits

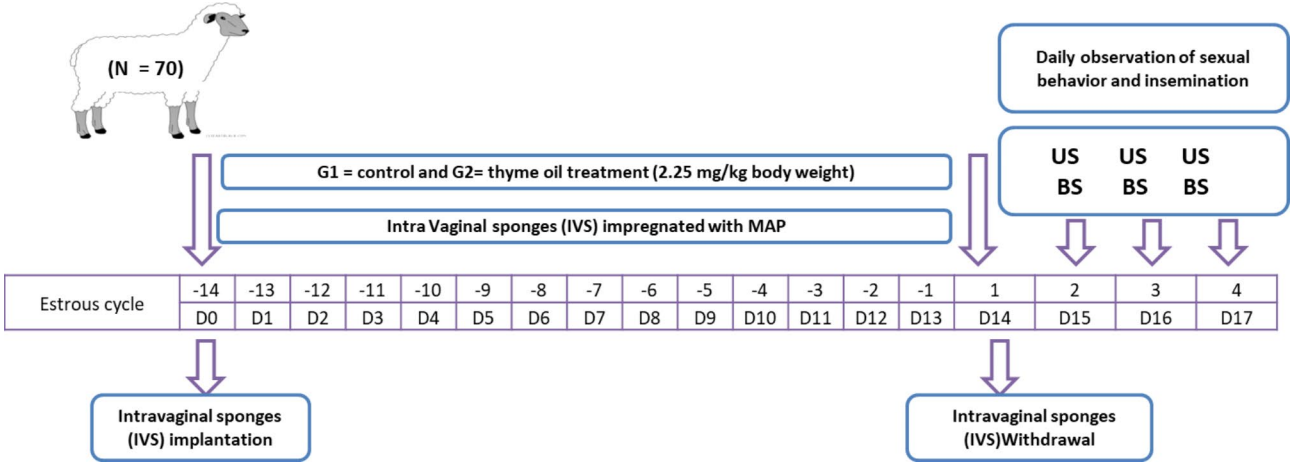

Fig. 1 Schematic diagram of the experimental protocol of dietary supplementation with thyme oil improvement the reproductive characteristics of Barki adult and prepubertal ewes. Essential thyme oil was given for 14 days from day 0 to day 14 of the estrous cycle during IVS treatment. Ultrasonography (US) and Blood samplings (BS) were performed on days 15, 16, and 17. G1: control group, G2: thyme oil-treated group, IVS: intravaginal sponge, MAP: medroxyprogesterone acetate

Table 3 Reproductive performance and sexual behaviors recorded during the experimental period

Parameter	Definition	Reference
Estrus duration	Period of sexual receptivity and mating characterized by distinct behavioral symptoms of estrus and estimated from the first to last signs of estrus	[41]
Onset of estrus	Time (in hours) from the vaginal sponge withdrawal to the appearance of estrus signs	[42]
Conception rate	Percentage of ewes conceived from all inseminated ewes	[43]
Lambing rate	Number of lambs born/number of ewes inseminated expressed as a percentage	
Fecundity	Number of lambs born/number of ewes bred, expressed as a percentage	[43]
Soliciting	The ewe approaches the ram, nuzzles the ram's body, and tends to stay in the vicinity of and follow the ram	[44]
Scrotum sniffing	The ewe sniffs the scrotum and anogenital region of the ram	
Anogenital sniffing	The ewe allows the ram to sniff her tail and genitalia	
Walking	Walking around	

(Atlas Medical Co, CB4, 0WX United Kingdom; UK). To quantify the glutathione peroxidase (GPX) activity, the oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSG), which is mediated by GPX, is the basis for the approach of Paglia and Valentine [26], that was used Using the usual equation, GPX activity was calculated from the NADPH consumption in the enzyme-coupled processes.

Statistical analysis

Data regarding the reproductive organ biometry and blood levels were analyzed using a two-way ANOVA according to the following General Linear Model: $Y_{ijk}=\mu+T_i + A_j + TA+e_{ijk}$. where Y_{ijk} = experimental observation, μ=general mean, T_i = effect of treatments (Ti=control and thyme oil-treated groups), A_j = effect of age (A_j = adult and prepubertal ewes), TA=interaction between treatments and age, e_{ijk} = experimental error. Estrus signs and conception, fecundity, and lambing rates were statistically analyzed using the Chi-square test. Statistical Package for Social Sciences version 20 (SPSS Inc., Chicago, IL, USA) was used. Analyses of the correlation between ovarian follicle diameter and estrogen hormone levels or corpora lutea diameter and progesterone hormone levels were performed using Pearson's correlation coefficient with 95% confidence limits ($P<0.05$). We conducted a simple linear regression analysis using the following coefficient model: $y=b_0+b_1\times_1$. Correlation and regression were analyzed using XLSTAT software (2016). Statements of statistical significance were based on $P<0.05$. The results were expressed as means±SEMs.

Results

Ultrasonographic examination of ovarian follicles and the estrogen concentration

Ultrasonographic data of the follicular population and estrogen concentrations are presented in Table 4; Fig. 2. The number of small follicles in the right and left ovaries was higher in the thyme oil-treated group than in the control group at days 15, 16, and 17 of the estrous cycle ($P<0.01$). Moreover, the number of large follicles was higher on both sides in the thyme oil-treated group than that in the control group ($P<0.05$) on days 15 and 17, whereas no significant differences were observed at day 16 ($P>0.05$). The diameter of the large follicles was more significant in the thyme oil-treated group than in the control group on days 15, 16, and 17 ($P<0.01$).

The estrogen concentration was higher ($P<0.01$) on days 15, 16, and 17 of the estrous cycle in the thyme oil-treated group than in the control group. Additionally, at day 15, the number and diameter of the large follicles were higher in the right-side ovary of adult ewes compared with that in prepubertal ewes ($P<0.05$). Furthermore, the estrogen concentration was slightly increased ($P>0.05$) in prepubertal ewes compared with that in adult ewes at days 15 and 16 (Table 4).

Generally, the interaction between treatment with thyme oil and the effect of ewes age (adult and Prepubertal ewes) were not significantly improved in the results of ultrasonography examination of ovarian follicles (number and diameter). At the same time, the estrogen concentrations were in the prepubertal ewes with a thyme oil treatment.

Estrous response and behavior

Data regarding reproductive performance and sexual behavior patterns are provided in Table 5. The estrus duration, estrus onset, sexual behaviors, conception, fecundity, and lambing rates were assessed in control and thyme oil-treated adult and prepubertal ewes at days 15, 16, and 17 of the estrous cycle.

In the thyme oil-treated group, estrus was detected in 1, 3, 9, 12, 7, and 3 out of 35 ewes 36, 42, 48, 54, 60, and 66 h after IVS removal, respectively. In the control group, 2, 9, 11, 9, and 4 ewes were in estrus 36, 42, 48, 54, and 60 h after IVS removal, respectively. Furthermore, 6, 13, 7, and 4 out of 30 prepubertal ewes exhibited estrus 42, 48, 54, and 60 h after IVS removal, respectively, and 4, 8, 9, 12, 4, and 3 out of 40 adult ewes exhibited estrus 36, 42, 48, 54, 60, and 66 h after IVS removal, respectively (Fig. 3).

Table 4 Ultrasonographic biometric measurements of the follicular population (number and diameter of follicles in the right and left ovaries) and estrogen concentration of control and thyme oil treated adult and prepubertal ewes at days 15, 16, and 17 of the estrous cycle ($n=10$/group, data are presented as mean ± SEM)

Item		Treatment (T)			Ewe age (A)			*P*. Value		
		Control	Thyme oil	SEM	Adult	Prepubertal ewes	SEM	T	A	T*A
Number of small follicles										
D15	Right	1.63	2.88**	0.24	0.25	0.26	0.05	0.01	0.59	0.510
	Left	1.00	2.88**	0.15	0.24	0.30	0.02	0.01	0.80	0.384
D16	Right	1.50	3.00**	0.23	0.26	0.26	0.02	0.01	0.89	0.908
	Left	2.00	3.25**	0.29	0.15	0.30	0.02	0.03	0.01	0.039
D17	Right	1.38	2.25**	0.17	0.17	0.19	0.01	0.01	0.69	0.640
	Left	1.50	2.38*	0.22	0.23	0.21	0.02	0.02	0.07	0.256
Number of large follicles										
D15	Right	1.00	1.63*	0.09	1.33*	1.25	0.13	0.03	0.06	0.525
	Left	1.50	1.63*	0.22	0.15	0.13	0.14	0.04	0.37	0.525
D16	Right	1.38	2.25	0.17	0.18	0.17	0.17	0.73	0.69	0.640
	Left	1.25	2.25	0.20	0.18	0.23	0.20	0.21	0.50	0.981
D17	Right	1.63	1.75**	0.24	0.22	0.15	0.18	0.01	0.34	0.083
	Left	1.38	2.00**	0.28	0.28	0.13	0.20	0.01	0.75	0.781
Diameter of large follicles (mm)										
D15	Right	0.32	0.41**	0.01	0.38*	0.35	0.01	0.01	0.06	0.234
	Left	0.32	0.44**	0.01	0.02	0.02	0.02	0.01	0.95	0.366
D16	Right	0.35	0.48**	0.02	0.02	0.09	0.05	0.01	0.30	0.920
	Left	0.40	0.51**	0.01	0.02	0.02	0.02	0.01	0.81	0.492
D17	Right	0.46	0.61**	0.01	0.02	0.02	0.02	0.01	0.96	0.580
	Left	0.32	0.61**	0.01	0.02	0.02	0.02	0.01	0.60	0.674
Estrogen concentration (pg/mL)										
D15		12.63	14.65**	0.42	0.29	0.51	0.04	0.01	0.19	0.098
D16		16.78	19.54**	0.31	0.55	0.64	0.05	0.01	0.39	0.238
D17		21.09	24.48**	0.37	0.72	0.61	0.06	0.01	0.27	0.203

* and ** indicate significantly different means between the control and thyme oil-treated-group or between the adult and prepubertal ewes with * $P \leq 0.05$ or ** $P \leq 0.01$

Moreover, ewes receiving thyme oil supplementation exhibited estrus 60.15 h after IVS withdrawal and remained in estrus longer than in the control group. There was no difference in the estrus duration ($P>0.05$) between adult and prepubertal ewes; however, prepubertal ewes exhibited estrus after a shorter period ($P<0.05$), i.e., 53.40 h after IVS withdrawal (Table 5).

Regarding the sexual behaviors observed at days 15, 16, and 17 of the estrous cycle, soliciting, scrotum and anogenital sniffing were detected more often in the thyme oil-treated group than in the control group.

All reproductive traits, including sexual behaviors and conception, fecundity, and lambing rates, were more pronounced in the thyme oil-treated than in the control group ($P<0.01$). Also, there was a significant ($P<0.05$) effect in the estrus duration for the adult ewes treated with thyme oil than prepubertal ewes, whereas those two types of age took a long time to estrus onset. However, the sexual behaviors and fertility rate have a highly significant ($p<0.01$) effect in the interaction between treatment with thyme oil and effect of ewes age, and the result showed the high estrus intensity in the prepubertal ewes treated with thyme oil than adult ewes. However, prepubertal ewes were a higher conception rate ($P<0.05$), lambing rate ($P<0.05$), and fecundity rate ($P<0.01$) than adult ewes.

Corpora lutea diameter and progesterone concentration

Ultrasonography data on the corpora lutea diameter and progesterone concentration are provided in Table 6; Fig. 4. The corpora lutea diameter measured in the right and left ovaries were more significant in the thyme oil-treated group than in the control group at days 15, 16, and 17 of the estrous cycle ($P<0.01$). Additionally, the progesterone concentration was higher in the thyme oil-treated group than in the control group ($P<0.01$) at days 15, 16, and 17. However, there were no differences in the corpora lutea diameter and progesterone concentration ($P>0.05$) between adult and prepubertal ewes at days 15, 16, and 17 of the estrous cycle. For the interaction between treatment with thyme oil and effect of ewes age, there were not significantly improved in the ultrasonographic biometric measurements of corpora lutea diameter (in the right and left ovaries) and progesterone

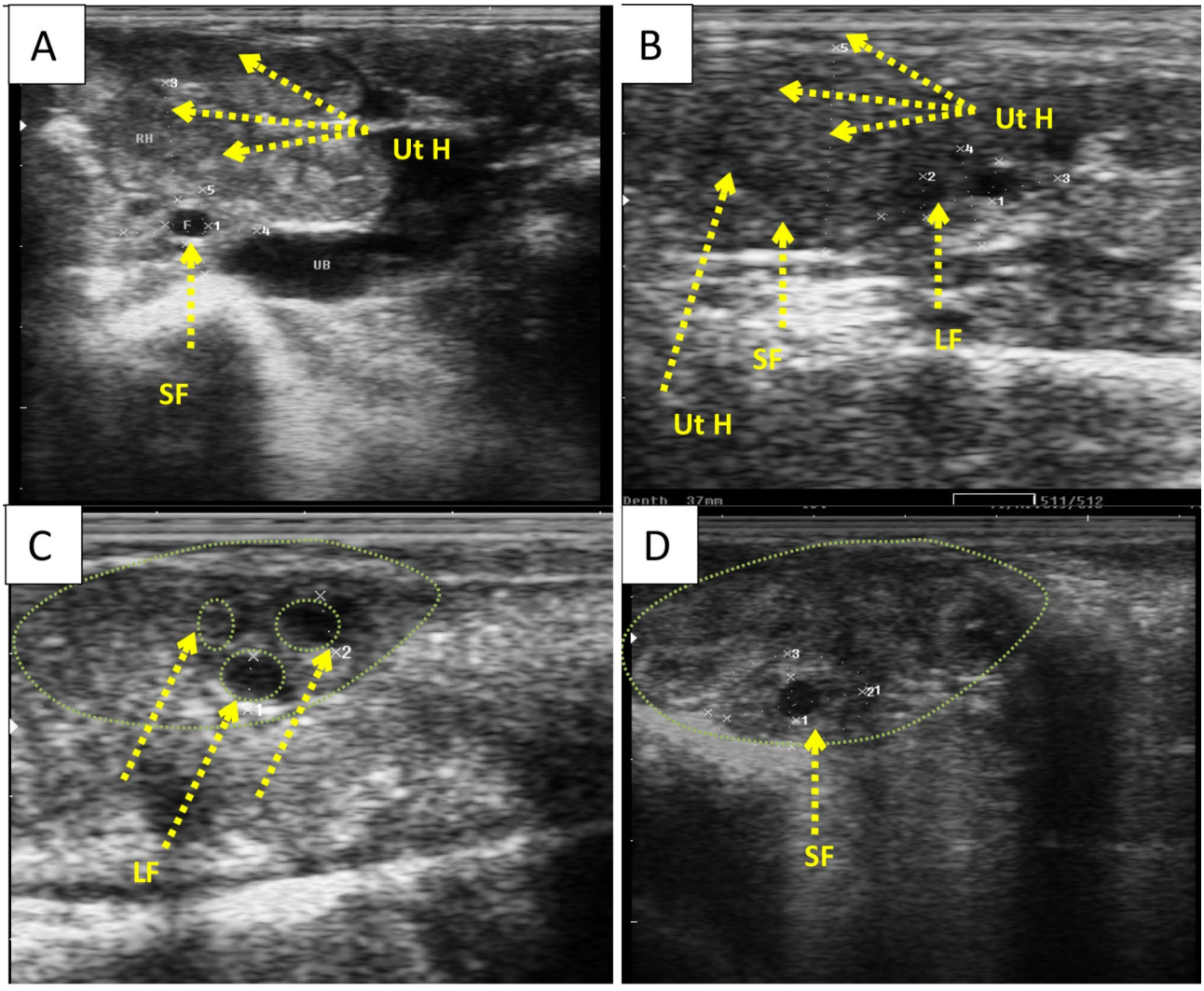

Fig. 2 Representative ultrasonograms of genital tracts of non-pregnant ewes treated with thyme essential oil. (**A**) Empty cross-sections of the uterus during proestrus showing small sized follicles (SF). (**B**) Empty cross-sections of the uterus during the follicular phase at day 15 by the estrous cycle showing large sized follicles (LF) and SF. (**C**) Empty cross-sections in the uterus during the follicular phase at day 16 of the estrous cycle showing LF. (**D**) Empty cross-sections of the uterus during the follicular phase at day 17 of the estrous cycle showing SF

Table 5 Estrus duration, estrus onset, sexual behaviors, and reproductive performance of control and thyme oil treated adult and prepubertal ewes at days 15, 16, and 17 of the estrous cycle ($n = 70$/group, data are presented as mean ± SEM)

Item	Treatment (T)			Ewe age (A)			*P*. Value		
	Control	Thyme oil	SEM	Adult	Prepubertal	SEM	T	A	T*A
Estrus duration (h)	27.90	35.50**	0.22	34.82	28.98	1.14	0.01	0.18	0.031
Estrus onset (h)	62.20	60.15	0.49	68.95*	53.40	2.80	0.06	0.03	0.410
Soliciting	15.97	26.76**	0.15	14.80	21.10**	0.09	0.01	0.01	0.001
Sniffing scrotum	9.82	14.12**	0.06	8.94	12.08**	0.06	0.01	0.01	0.001
Anogenital sniffing	6.36	10.10**	0.06	5.87	8.34**	0.06	0.01	0.01	0.001
Walking	25.55	25.67	0.07	23.23	27.43**	0.07	0.06	0.01	0.001
Conception rate (%)	63.51	95.12*	0.54	81.56	87.95*	0.52	0.05	0.05	0.001
Lambing rate (%)	105.24	136.73*	0.40	120.12	125.03*	0.48	0.05	0.05	0.001
Fecundity rate (%)	66.97	130.25**	0.15	99.69	111.52*	0.99	0.01	0.05	0.001

* and ** indicate significantly different means between the control and thyme oil-treated-group or between the adult and prepubertal ewes with * $P \leq 0.05$ or ** $P \leq 0.01$

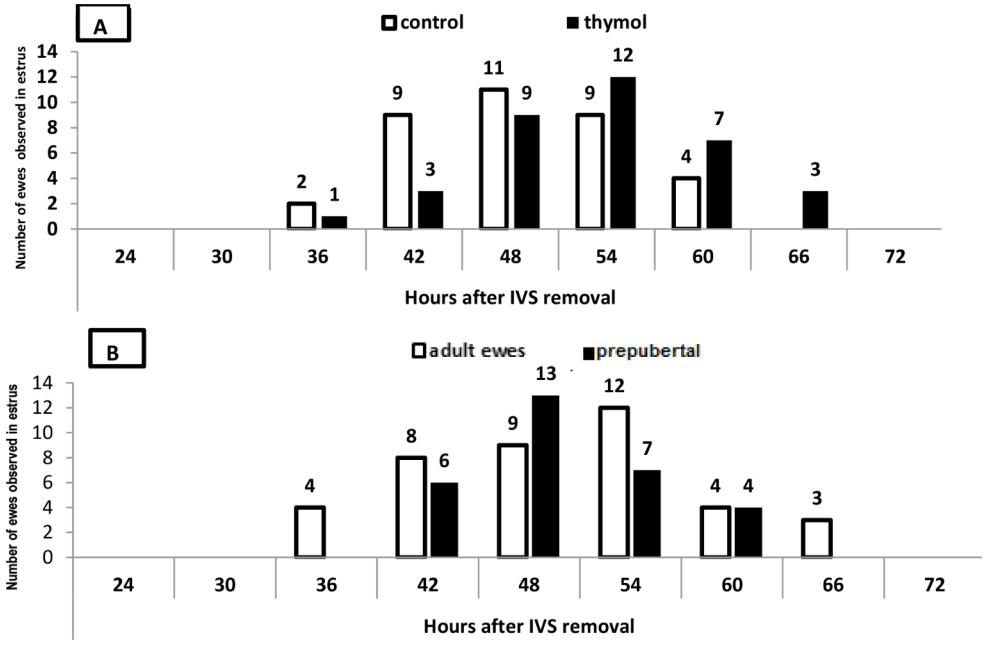

Fig. 3 Distribution of the number of ewes in estrus over 72 h. The effects of oral administration of thyme essential oil (**A**) and ewe sexual maturity (**B**) were investigated IVS, intravaginal sponge

Table 6 Ultrasonographic biometric measurements of corpora lutea diameter (in the right and left ovaries) and progesterone serum concentration of control and thyme oil treated adult and prepubertal ewes at days 15, 16, and 17 of the estrous cycle ($n=10$/group, data are presented as mean ± SEM)

Item		Treatment (T)			Ewe age (A)			*P*. Value		
		Control	Thyme oil	SEM	Adult	Prepubertal ewes	SEM	T	A	T*A
Corpora lutea diameter (mm)										
D15	Right	0.34	0.41**	0.01	0.41	0.34	0.02	0.01	0.52	0.432
	Left	0.44	0.42**	0.01	0.40	0.39	0.01	0.01	0.33	0.286
D16	Right	0.46	0.63**	0.02	0.61	0.57	0.02	0.01	0.69	0.238
	Left	0.53	0.63**	0.01	0.60	0.57	0.02	0.01	0.78	0.685
D17	Right	0.54	0.77**	0.01	0.69	0.68	0.01	0.01	0.95	0.040
	Left	0.59	0.84**	0.04	0.75	0.75	0.04	0.01	0.98	0.364
Progesterone concentration (ng/ml)										
D15		0.08	0.31**	0.03	0.23*	0.13	0.04	0.02	0.41	0.185
D16		0.23	0.64**	0.07	0.36	0.54	0.07	0.01	0.18	0.328
D17		0.47	1.40**	0.01	0.92	0.94	0.01	0.01	0.20	0.928

* and ** indicate significantly different means between the control and thyme oil-treated-group or between the adult and prepubertal ewes with * $P \leq 0.05$ or ** $P \leq 0.01$

serum concentration of control and thyme oil-treated adult and prepubertal ewes at days 15, 16, and 17 of the estrous cycle.

Correlation and regression analyses

Correlation and regression analyses of the ovarian follicle biometric measurements and estrogen hormone levels are provided in Table 7. First, the correlation coefficient (r) was calculated. The estrogen concentration was positively correlated with the number of follicles ($P<0.05$) and the large follicular diameter ($P<0.01$). Linear regressions were obtained using the correlation coefficients, and the determination coefficient R^2 of all the regression equations was greater than 0.78.

The correlation coefficient r and regression analysis of the corpora lutea size and progesterone levels in the control and thyme oil-treated Barki adult and prepubertal ewes are shown in Table 8. The concentration of progesterone was positively correlated with the number and diameter of the corpora lutea ($P<0.01$). The determination coefficients R^2 of all the regression equations were greater than 0.65.

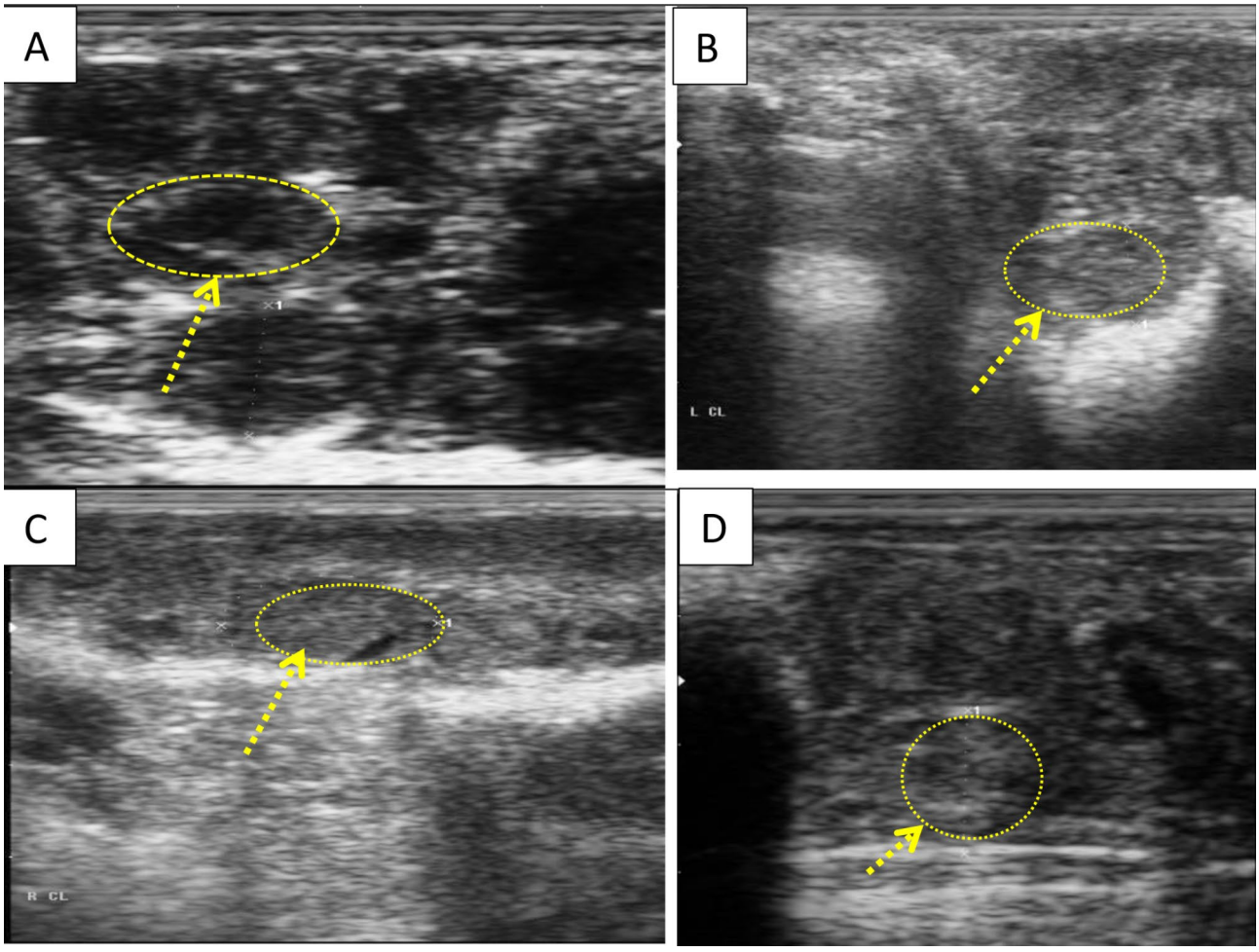

Fig. 4 Representative ultrasonograms of genital tracts of pregnant ewes of control and thyme oil treated adult and prepubertal ewes at days 15, 16, and 17 of the estrous cycle ($n = 10$/group). (**A** and **C**) Assessment of the corpora lutea diameter (yellow circle) in empty cross-sections of the left ovary. (**B** and **D**) Assessment of the corpura lutia diameter (yellow circle) in empty cross-sections of the right ovary

Table 7 Correlation and regression analyses of biometric measurements of the ovarian follicles (number and diameter of follicles in the right and left ovaries) (X_i) and estrogen serum concentration (Y) in control and thyme essential oil-treated Barki adult and prepubertal ewes

Item	Number of follicles	Follicular diameter (mm)
Correlation		
Estrogen concentration (pg/mL)		
r	0.402**	0.885**
P	< 0.005	< 0.001
Regression		
Estrogen concentration (pg/mL)		
Regression equation	$Y = 0.085 + 41.84 \times_1$	$Y = 12.84 + 0.77 \times_2$
R^2	0.16	0.78
Constant	12.84	−0.085
Reg. coefficient	0.77**	41.84**
P	< 0.004	< 0.001

X_1 = number of follicles and X_2 = follicular diameter

Table 8 Correlation and regression analyses of corpora lutea diameter (X) (in the left and right ovaries) and progesterone serum concentration (Y) in control and thyme essential oil-treated Barki adult and prepubertal ewes

Item	Corpora lutea
Correlation	
Progesterone concentration (ng/mL)	
r	0.805**
P	< 0.001
Regression	
Progesterone concentration (ng/mL)	
Regression equation	$Y = -1.14 + 2.79 \times_1$
R^2	0.65
Constant	−1.14
Reg. coefficient	2.79**
P	< 0.001

X_1 = corpora lutea diameter in mm

The thyroid hormone concentrations and GPX activities

The thyroid hormone concentrations and GPX activities are presented in Fig. 5. Concentrations of T3 and T4 and GPX activities were higher at days 15, 16, and 17 of the estrous cycle in the thyme oil-treated group than that in the control group ($P<0.05$).

Discussion

The obtained data confirms the possibility of using thyme essential oil as dietary supplements to influence reproductive ability in both the Barki adult and prepubertal ewes groups. Furthermore, the background obtained from the impact of the studied thyme oil on the corpora lutea diameter, follicular population, sexual hormone profile, and reproductive performance makes the task topical. The results obtained from the study allow for the development of an optimal regimen of thyme essential oil use with dietary supplements to improve reproductive organ biometry and reproductive performance in the ewes.

The supplementation with thyme oil enhanced the number of small and large follicles and the diameter of large follicles and corpora lutea. According to Hasan et al. [27] the thyme improves the blood flow and stimulates sexual activity in the ovarian female rabbits. The greater corpora lutea diameter observed in the thyme essential oil-treated group might be due to the antioxidant properties of thyme [28]. This result can be supported by improved progesterone concentrations in animals treated with thymol in this study. Indeed, antioxidants have important functions in corpora lutea as they play significant roles in the corpus luteum physiology during the estrous cycle [29, 30].

The present study evidenced a positive correlation between the number of follicles and estrogen concentration. Additionally, thyme oil enhanced estrogen concentration. Similarly, El-Zaher et al. [13] investigated ovarian activity and antioxidant indices during estrous cycle of Barki ewes under effect of a variety of herbs, including thyme, and reported that thyme has a relatively high binding activity to the estradiol receptor and estrogenic effects. However, the rise in estrogen concentration is related to an increase in the diameter and number of ovarian follicles [31].

Our study showed a positive correlation between the corpora lutea diameter and progesterone concentration.

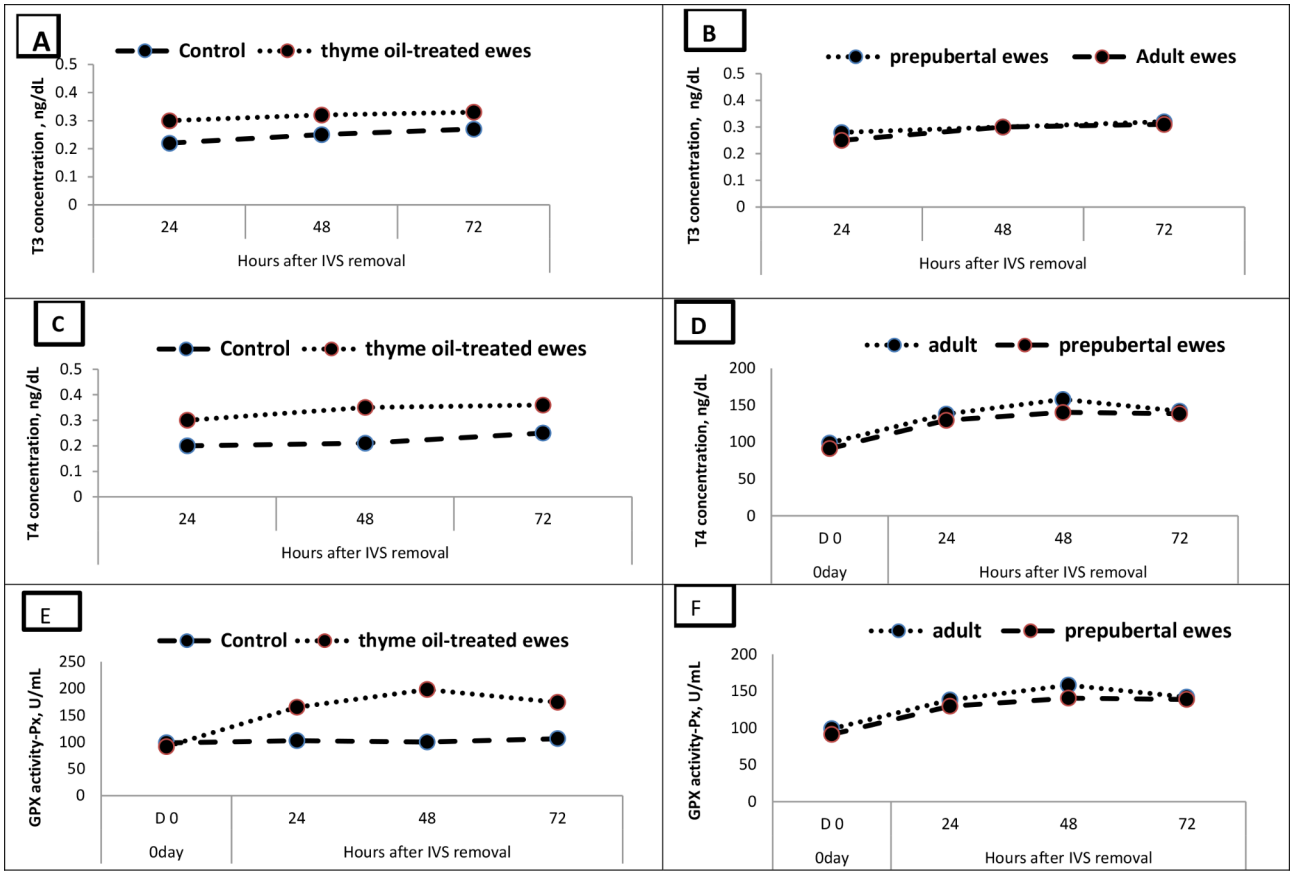

Fig. 5 (**A**–**D**) Effects of thyme oil treatment (**A** and **C**) and sexual maturity (**B** and **D**) on the serum levels of thyroid hormones. The concentration of triiodothyronine (T3, A and B) and thyroxine (T4, C and D) were measured 24, 48, and 72 h after intravaginal sponge (IVS) removal. (**E** and **F**) Effects of thyme oil treatment (**E**) and sexual maturity (**F**) on the activity of the glutathione peroxidase (GPX) at 0, 24, 48, and 72 h after IVS removal

Additionally, thyme oil enhanced progesterone concentration. We previously found that the progesterone levels are regulated by apigenin, which interferes with normal progestin signalling. Furthermore, apigenin activates a progesterone response element/luciferase construct in T47D cells, indicating that apigenin may interact directly with progestin receptors [32]. Moreover, previous studies suggested that apigenin alters progestin signalling, potentially by interacting directly with progesterone receptors.

Here, the ewes receiving thyme oil supplementation had a more extended estrus period and more pronounced sexual behaviors (soliciting and scrotum and anogenital sniffing) and other reproductive traits (conception, fecundity, and lambing rates). Increasing estradiol levels causes estrous behavior, and estrus occurrence requires an appropriate balance among estradiol, progesterone, and androgen levels [33]. Because apigenin has a chemical structure similar to estrogens [20], it enhances estrus signs and fertilization.

Serum levels of T3 and indicators of animal T4 are reliable thyroid function [34]. The thyroid gland regulates energy usage, RNA synthesis, cell oxygen consumption, overall body metabolism, growth processes, and neurological development [35]. In the present study, thyme oil treatment induced higher T3 and T4 concentrations than controls. Placha et al. [36] reported that thyme oil improves dietary value, increases the intestinal availability of essential nutrients for absorption, and improves growth. In addition, the significantly higher blood levels of GPX in the thyme oil-treated group suggested a protective effect of thyme oil in ewes, as glutathione has prooxidant and antioxidant activities [37].

Compared to adult ewes, lower rates of estrus response, ovulation, and fertility were observed in prepubertal ewes. Further investigations are needed to improve these rates because farm animals should be include in an annual reproductive programme [38]. Here, there was no difference in the estrus response, fertility rate, and pregnancy rate between adult and prepubertal ewes, although adult ewes spent more time in estrus than prepubertal ewes. We also found that, compared with prepubertal ewes, adult ewes exhibited estrus more clearly, had higher fertility rates, and had more successful pregnancies. Although these differences were not statistically significant, higher rates of pregnancy failure and fetal loss have been reported in prepubertal ewes compared with those in mature ewes [39].

Additionally, the overall ovulation rate and duration were lower in prepubertal ewes. Mulvaney et al. [40] reported a lower number of ewes breeding or ovulating, a lower ovulation rate, and a higher number of bred ewes returning to estrus activity, and a higher reproductive loss in early pregnancy for prepubertal ewes. This all culminates in reduced reproductive performance of prepubertal ewes.

Conclusions

In conclusion, oral supplementation with thyme oil is advantageous in improving the corpora lutea diameter, follicular population, and reproductive performance, which has a good effect on the adult and prepubertal ewes. Also, the thyme oil treatment improves reproductive ability in both the Barki adult and prepubertal ewes groups.

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Author contributions

M.E Ali: conceptualization, methodology, investigation, visualization, formal analysis, writing – original draft. M.Y. Zainhom: investigation. A.A. Awad, A.E. Ahmed: investigation, formal analysis. S.S. Abd-Elghfar, M Abdelrahman: formal analysis. M.E Ali, and M. H. Farouk: formal analysis, visualization, writing – original draft. F. A. Al-Saeed: interpretation of data, and editing the manuscript. All authors have read and approved the final manuscript.

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Data availability

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Declarations

Ethics approval and consent to participate

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. This study was conducted in accordance with Institutional and National Guidelines for the care and use of animals were followed according to the OIE standards, the Ethical Committee of the Faculty of Veterinary Medicine, with the permission number (no: AAA; 2022/05/1161).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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