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Effect of Obesity on Some Reproductive Hormones and Pregnancy Outcomes in Goats

Sawsan M. Ahmed[1], Amal M. Abo El-Maaty[2], Hamed A.A. Omer[1] and Hazem A. El-Debaky[2]

[1]Animal Production Department, Agricultural & Biological Research Institute, National Research Centre, 33 El-Buhouth Street, P.O: 12622, Dokki, Giza, Egypt.
[2]Animal Reproduction and AI Dept., Veterinary Research Institute, National Research Centre, 33 El-Buhouth Street, P.O: 12622, Dokki, Giza, Egypt.

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ABSTRACT

This study aimed to point out the effect of increased back fat (BF) on leptin, insulin, progesterone (P4), and estrogen (E2) hormones, as well as pregnancy rates in goats. We hypothesized that BF thickness influences hormonal profiles and reproductive performance. Goats (N=45) of mixed breeds were classified into obese (N=15), moderate (N=18), and lean goats (N=12). Obese females were fed ration-free-concentrate for one month (green clover) *ad libitum*. After the dietary regime, all females were kept with fertilizable males until pregnancy was detected 30 days later. Ovarian follicles diameters and numbers were also compared. Our results revealed that obese goats before restricting diets had the highest BF (2.69±0.39 mm; $P \leq 0.0001$), corpus luteum diameter (CL, $P<0.01$; 1.05±0.12); leptin concentration ($P<0.01$; 1.79±0.13); total follicles (5.89±0.27; $P<0.0001$), number of large follicles ($P<0.05$; 1.45±1.24), medium follicles ($P<0.01$; 1.55±0.74). After diet restriction, obese and moderate BF goats had nearly the same BF, P4, and leptin concentration. Compared to goats with moderate BF, replacing concentrated ration with green clover for 30 days increased CL diameter, area, total ovarian activity, number of large and medium follicles, and the diameter of large follicles. Goats of moderate BF, obese dietary restricted, and lean ones had 46%, 31%, and 23% pregnancy rates respectively. In conclusion, obesity can be detected by determining BF and leptin hormone. Obesity is associated with disturbed leptin, insulin and ovarian hormones, ovarian follicle growth, luteal development as well as reduced pregnancy rates. Diet free from concentrated reduced back fat and improved pregnancy outcomes.

Keywords: leptin, insulin, ovarian hormones, pregnancy, obesity, goats

1. Introduction

Goats were the first animal domesticated by humans (FAOSTAT, 2020) for milk and meat production. Goat milk has health promotion: lower allergenicity, easier digestibility, and higher content of short and medium-chain fatty acids (dos Santos *et al.,* 2023). Fermented goat milk is suitable to cure cardiovascular diseases implicated in obesity physiopathology with anti-atherogenic, antihypertensive, lipid-lowering, hypoglycemic, antioxidant, and gut microbiota regulation properties (Andrada *et al.,* 2024). For successful goat breeding, a healthy reproductive system and sufficient nutrition with essential elements are crucial (Niyigena *et al.,* 2022). Nutrition is one of the reproductive success parameters that control fertility and litter size in ruminants especially those kept indoors (Cristodoro *et al.,* 2024; Ding *et al.,* 2024; Šavorić *et al.,* 2024). Both undernutrition (Estrada-Cortés *et al.,* 2009) and or short-term overnutrition (Nogueira *et al.,* 2016) badly impacted goat fertility leading to extended anestrus, irregular ovarian cycles and reduced conception (Chaves *et al.,* 2024).

Body condition score (BCS) is a relevant visual indicator of body fat stores that can be used during adverse environmental and physiological conditions such as heat stress, pregnancy, underfeeding, adiposity, nutritional status, and post-nutritional status (Caldeira and Portugal, 2007) that facilitate

Corresponding Author: Hazem A. El-Debaky, 2Animal Reproduction and AI Dept., Veterinary Research Institute, National Research Centre, 33 El-Buhouth Street, P.O: 12622, Dokki, Giza, Egypt. E-mail: - haldebaky@gmail.com

establishing the animal status during routine inspection (Maurya *et al.,* 2008).
Obesity attracted increasing global welfare concerns in different species (Beyene *et al.,* 2024), companions such as mares (Fresa *et al.,* 2024), and farm animals such as goats (Yao *et al.,* 2024). Obesity is a complex and progressive disease that results in a wide spectrum of reproductive disorders (Zhang *et al.,* 2024). Obesity disturbs mitochondrial function, biogenesis, and dynamics with the cells (Jun *et al.,* 2024), as well as it exhibits undesirable effects on the reproductive processes and causes infertility (Dağ and Dilbaz, 2015; Hallman *et al.,* 2023). Infertility has become a prominent co-morbidity factor of obesity (Sharma and Galvão 2023).

Leptin is one of the adipokines hormones that was impacted in the course of obesity and is a key bioactive protein mainly secreted from the adipocytes (Friedman and Halaas, 1998). It has an obvious role in the course of obesity and reproduction (Tong and Xu, 2012). Leptin hormone was produced by the obesity (*ob*) gene as 16 kDa protein consists of 167 amino acid (Zhang *et al.,* 1994). It relays information to the brain concerning the amount of energy stored and activates the hypothalamic centers that regulate energy uptake and expenditure (De la Hoya *et al.,* 2015). Leptin receptors are expressed in many reproductive organs of ruminants (Bartha *et al.,* 2005, Smolinska *et al.,* 2013), and mice (Pennington *et al.,* 2022). Leptin concentration in the blood affects body weight by controlling food intake and energy metabolism (Hafeezullah, 2006) and correlated BCS (Leòn *et al.,* 2004). In goat, serum leptin increased proportionally with the increase in BCS or body weight (Gamez-Vazquez *et al.,* 2008). In cyclic mares, obesity altered ovarian hormones, insulin and leptin concentrations (Abo El-Maaty *et al.,* 2020). It has direct effects on steroidogenesis in the ovary in cell types such as theca and granulosa cells (Kendall *et al.,* 2004).

Insulin is the hormone that regulates glucose metabolism in mammalian cells. Increased lipids are associated with insulin resistance (Itani *et al.,* 2002). Increased insulin is encountered when body condition increases in mature cyclic goats (Nogueira *et al.,* 2016). Maternal hyperinsulinemia caused intrauterine fetal growth restriction (Song *et al.,* 2024).

Thus, this study hypothesized that obesity interferes with conception and reduces fertility in non-pregnant cycling goats. For postulating this hypothesis, BF was measured to evaluate the subcutaneous fat deposition and categorize animals to compare their circulating serum leptin, insulin, ovarian hormones, ovarian follicle and corpus luteum development, ovulation rate, and pregnancy rate in goats with high, moderate, and low BF in addition to goats with high BF and subjected to concentrated diet deprivation for 30 days before starting their breeding.

2. Materials and Method

2.1. Animals and management

Animals were handled following the Institutional Animal Care and Use Committee of NRC (NRC-074101230). Forty-five goats of mixed breeds (Damashkus, Zarabi, and native) of 2-4 years and 25-30 kg body weight belonging to the research farm of NRC (Nobaria) were divided into obese goats (N=15), moderate BF (N=18), and lean group (N=12). Goats with moderate and lean BF were kept with fertilizable males until pregnancy was confirmed 30 days later. Obese goats were subjected to a nutritional regime for one month and fed their diet free from concentrate by allowing *ad libitum* access to the green clover (*Trifolium alexandrinum*) then a fertilizable buck was introduced. Goats had free access to clean water and were housed in shaded barns under the natural daylight and temperature. The study was conducted from March to May 2022. Estrous was synchronized by using the male effect (Perkins and Fitzgerald, 1994) where all females were isolated in pens far from males along the dietary regime then introduced for 30 days and pregnancy was determined 30 days later.

2.2. Ultrasound examination

Animals were examined rectally using a Sono-Vet R3 ultrasound scanner (Madison, Samsung, South Korea) equipped with Linear Array 12MHz B-mode transducer for the measurement of the BF thickness (Ptáček *et al.,* 2021), ovarian follicles diameter and number, in addition, fetal early detection and counting their numbers. Depending on the BF thickness, goats were divided into lean (BF≤1.0 mm; *n*=12), moderate (BF>1.0 mm ≤2.0 mm; *n*=18), and obese (BF>2.0 mm; *n*=15). Ovarian follicles were categorized into small ovarian follicles having diameters <2mm, medium follicles having diameters >2 <5mm, and large follicles having diameters >5mm. The number and diameter of the ovarian follicles and corpus luteum were determined using ultrasound electronic calipers (Nogueira *et al.,* 2015). Total ovarian activity (TOA) was defined as the sum of total antral follicles and corpora lutea recorded in each animal (Meza-Herrera *et al.,* 2013 a, b). The corpus luteum area and the ovarian follicle areas are determined in pixels using Photoshop software.

2.3. Blood sampling and hormone assaying

Blood samples were collected from the jugular vein of goats in plain vacutainer tubes then serum was harvested and stored at -20°C for the hormone assay. Progesterone (P4, DRG® Progesterone ELISA (EIA-1561)) with a sensitivity of 0.1431 nmol/L. and intra- and inter-assay precisions of 6.99 and 4.34%; estradiol (E2, DRG® Estradiol ELISA (EIA-2693) which has a minimum detectable of 9.714 pg/mL, intra- and inter-assay precisions of 2.71 and 6.72%, DRG® Insulin Enzyme Immunoassay Kit EIA-2935 (DRG International, Inc. USA) with intra- and inter-assay precisions of 1.79% and 5.99% and sensitivity of 1.76 µIU/m. and DRG® Leptin (Sandwich) ELISA (EIA-2395) which has a minimum detectable limit of 0.44 ng/ml and intra- and inter-assay precisions' of 7.64% and 6.39%.

2.4. Analytical procedures of ration

Chemical analyses (Table 1) of ingredients (Yellow corn, soybean meal wheat bran and fresh green clover) were analyzed according to AOAC (2005) methods. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were also determined in the same previous ingredients according to Goering and Van Soest (1970) and Van Soest et al. (1991).

Table 1: Chemical analysis (as it is) of Berseem and concentrate feed mixture (CFM) fed to goats during the regime period.

Item	Fresh Berseem all goats	Concentrate feed mixture (CFM) moderate and lean
Moisture	84.89	9.05
Organic matter (OM)	13.52	83.50
Crude protein (CP)	2.07	15.41
Crude fiber (CF)	4.38	5.61
Ether extract (EE)	0.29	2.97
Nitrogen free extract (NFE)	6.78	59.51
Ash	1.59	7.45
Cell wall constituents		
Neutral detergent fiber (NDF)	5.24	32.91
Acid detergent fiber (ADF)	3.08	22.38
Acid detergent lignin (ADL)	0.44	3.17
Hemicellulose*	2.16	10.53
Cellulose**	2.64	19.21
Non-fibrous carbohydrates (NFC)	5.92	32.21
Gross energy (kcal/kg DM)	607	3853
Digestible energy (kcal/kg DM)	461.5	2928
Nutritive values (%)		
Total digestible nutrients (TDN)	10.42	66.09
Digestible crude protein (DCP)	0.74	10.60

* Hemicellulose = NDF – ADF, ** Cellulose = ADF – ADL.

2.5. Calculated values

Non-fibrous carbohydrates (NFC), were calculated according to Calsamiglia et al. (1995) using the following equation: NFC = 100 – (CP + EE + ash + NDF).
Gross energy (Kcal/ Kg DM) is calculated according to Blaxter (1968).
Where: Each g CP= 5.65 Kcal, g EE= 9.40 Kcal and g (CF & NFE) = 4.15 Kcal.
DE (Kcal/ kg DM): Calculated according to NRC (1977). DE = GE x 0.76.
TDN %: Calculated according to NRC (1977). TDN % = DE / 44.3.

DCP %: Calculated according to NRC (1977). DCP % = 0.85 X_1 – 2.5. Where X_1= CP% on a DM basis.

In addition to the concentrate ration formula and chemical analysis of different ingredients presented in Table (2), 2% limestone, 0.50% sodium chloride,0.30% vitamins and mineral mixtures and 0.10% antitoxic compounds are added. Each kilogram of Vitamin and Mineral mixture contains: 2000.000 IU Vitamin A, 150.000 IU Vitamin D, 8.33 g Vitamin E, 0.33 g Vitamin K, 0.33 g Vitamin B_1, 1.0 g Vitamin B_2, 0.33g Vitamin B_6, 8.33 g Vitamin B_5, 1.7 mg Vitamin $B1_2$, 3.33 g Pantothenic acid, 33 mg Biotin, 0.83g Folic acid, 200 g Choline chloride, 11.7 g Zn, 12.5 g Fe, 16.6 mg Se, 16.6 mg Co, 66.7 g Mg and 5 g Mn.

Table 2: Formula of concentrate feed mixture (CFM) and chemical analysis of different ingredients and calculated analysis of concentrate feed mixture (CFM) and total mixed ration (TMR).

Item	Determined				Calculated	
	Yellow corn	Soybean meal	Wheat bran	Fresh Berseem	CFM	TMR
%	48.50	19.50	29.10	*Ad libitum*		
Moisture	9.78	7.15	9.71	84.89	9.05	31.80
Chemical analysis (%) on DM basis						
Organic matter (OM)	98.50	94.38	87.79	89.47	91.81	91.11
Crude protein (CP)	9.00	44.00	13.72	13.68	16.94	15.96
Crude fiber (CF)	4.60	4.93	10.25	29.00	6.17	13.02
Ether extract (EE)	4.80	0.60	2.81	1.92	3.27	2.87
Nitrogen-free extract (NFE)	80.10	44.85	61.01	44.87	65.43	59.26
Ash	1.50	5.62	12.21	10.53	8.19	8.89
Cell wall constituents						
Neutral detergent fiber (NDF)	34.52	34.77	43.54	34.68	36.19	35.74
Acid detergent fiber (ADF)	21.38	26.13	31.42	20.36	24.61	23.34
Acid detergent lignin (ADL)	2.24	6.33	3.98	2.93	3.48	3.32
Hemicellulose*	13.14	8.64	12.12	14.32	11.58	12.40
Cellulose**	19.14	19.80	27.44	17.43	21.13	20.02
Non-fibrous carbohydrates (NFC)	50.18	15.01	27.72	39.19	35.41	36.54
Gross energy (kcal/kg DM)	4475	4608	3997	4019	4236	4171
Digestible energy (kcal/kg DM)	3401	3502	3038	3054	3219	3170
Nutritive values (%)						
Total digestible nutrients (TDN)	76.77	79.05	68.58	68.94	72.66	71.56
Digestible crude protein (DCP)	5.15	34.90	9.16	9.13	11.90	11.07

Total mixed ration (TMR) is composed of 70% CFM + 30% fresh berseem.

3. Results

Obese goats had the highest ($P<0.0001$) BF (2.69±0.39) thickness compared to goats with moderate (1.58±0.22) and lean (0.78±0.18) BF (Table 3). After depriving concentrates for 30 days, obese goats (1.53±0.57) had nearly BF thickness as moderate BF ones. Regarding to P4 concentration in all experimental groups, obese goats before depriving concentrates had the lowest ($P<0.05$) P4 concentrations (9.52±0.66) and after depriving concentrates, they attained the highest concentration (14.15±0.48) with no difference of P4 in goats with lean as well as moderate BF (Table 3). Goats with extreme BF either obese (135.11±21.66) or lean (103.53±7.78) had low estradiol (E2, $P<0.001$) compared to the moderate (181.95±0.32) and obese (219.84±0.17) subjected to a dietary regime free from concentrates (table 3).

Obese goats had the highest ($P<0.001$) leptin concentrations (1.79±0.13) compared to lean, moderate, and obese after concentrates restriction (Table 3). While goats with moderate BF had the lowest insulin ($P<0.01$) compared to lean, obese before the dietary regime, and obese after depriving concentrates (Table 3).

Table 3: Mean± SEM body BF, estradiol (E2), progesterone (P4), insulin and leptin hormones in obese goats before and after concentrate diet restriction, moderate BF, and lean goats.

Condition	Obese		Moderate BF	Lean	P-value
	Before	after			
Number	15		18	12	
BF/mm	2.69±0.39^{c}	1.53±0.57^{b}	1.58±0.22^{b}	0.78±0.18^{a}	0.0001
P4 nmol/L	9.52±0.66^{a}	14.15±0.48^{b}	12.95±0.23^{b}	12.42±0.28^{ab}	0.015
E2 pg/ml	135.11±21.66^{b}	219.84±0.17^{d}	181.95±0.32^{c}	103.53±7.78^{a}	0.001
Insulin µIU/ml	47.12±9.13^{b}	48.58±0.98^{b}	19.05±0.87^{a}	43.08±0.51^{b}	0.006
Leptin ng/ml	1.79±0.13^{b}	1.60±0.06^{a}	1.63±0.14^{a}	1.69±.08 ^{ab}	0.001

Superscript letters (a, b, c) within a row are significant at $P<0.05$

Concerning the effect of obesity on ovarian follicle and corpus luteum parameters, the results showed that, the total ovarian follicles ($P<0.0001$) and large ($P<0.05$) follicles increased in obese goats (Table 4). The number of medium follicles ($P<0.01$) increased in obese goats before and after a dietary regime free from concentrates. Obese goats subjected to depriving concentrates had high dominant (DF; $P<0.05$) and medium (MF; $P<0.01$) follicle diameters. Obese goats before and after the dietary regime obtained the lowest DF area ($P<0.01$). Obese goats before and after the dietary regime had the highest corpus luteum diameter (CL; $P<0.01$). After the dietary regime, obese goats still have the maximum CL area ($P<0.05$).

Table 4: Mean± SEM ovarian follicles population in obese, moderate and lean goats

Condition	Obese		Moderate	Lean	P-value
	Before	after			
N. total follicles	5.89±0.27^{b}	2.75±.44^{a}	1.40±0.14^{a}	1.83±0.23^{a}	0.0001
N. Large follicles	1.45±1.24^{b}	1.04±0.48^{ab}	0.55±0.01^{a}	0.60±.03^{a}	0.025
N. MF	1.55±0.74^{b}	1.46±0.52^{b}	1.00±.00^{a}	1.00±0.00^{a}	0.003
DF diameter /mm	0.58±0.07^{ab}	0.63±0.08^{b}	0.55±0.04^{a}	0.60±0.07^{ab}	0.035
MF diameter /mm	0.37±0.05^{a}	0.42±0.06^{b}	0.39±0.05^{ab}	0.35±0.02^{a}	0.004
DF area /pixel	824±197^{a}	981±234^{a}	1245±101^{b}	1213±100^{b}	0.001
MF area /pixel	448.3±155.9	428.3±172.8	484.3±132.3	552.0±164.7	NS
CL number	1.00±0.00	1.00±0.00	1.00±0.00	0.00±0.00	NS
CL diameter/mm	1.05±0.12^{c}	0.90±0.05^{b}	0.78±0.74^{a}	0.0±0.00^{a}	0.001
CL area/ pixel	2002±191.89^{a}	3276±101^{b}	2200±52^{a}	2296±728^{a}	0.003
Ovarian activity	6.71±0.35^{c}	2.78±0.49^{b}	1.6±0.22^{a}	1.83±0.25^{ab}	0.001

Different superscripts (a, b, c, d) are significantly different at $P<0.05$, number (N.), Dominant follicle (DF), medium follicle (MF), non-significant (NF)

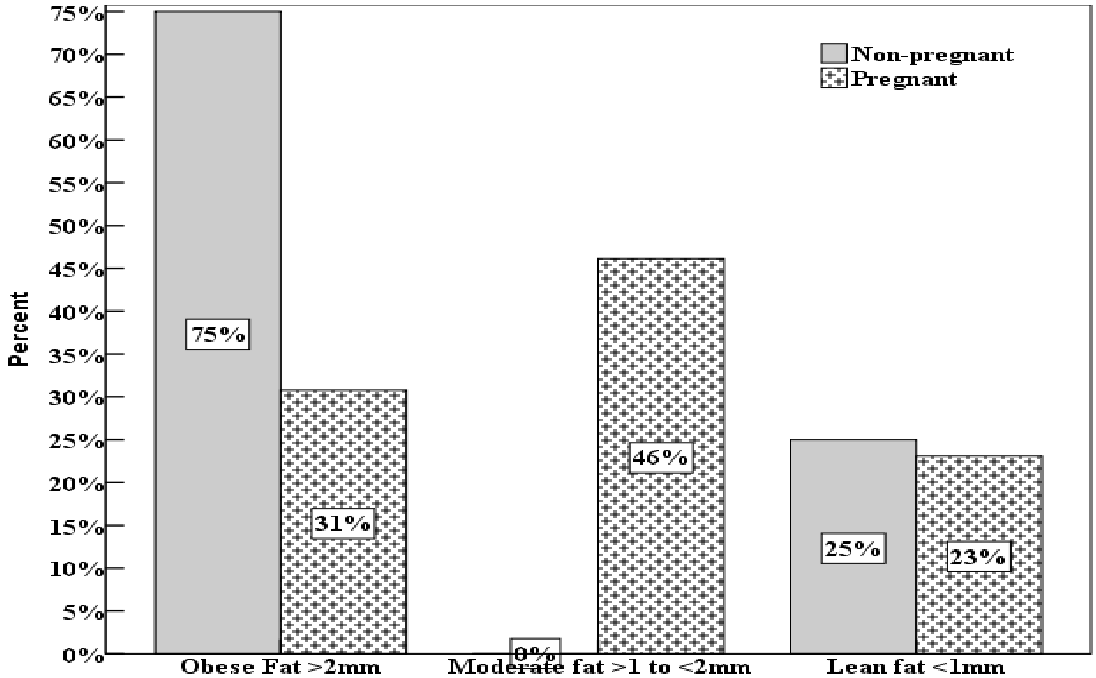

Fig. 1: The percentage of pregnant and non-pregnant goats with different BF

Of all non-pregnant goats, 75% were obese goats and 25% were lean. All goats with moderate BF got pregnant and constituted 46% of pregnant goats. Pregnant obese goats constituted 31% and pregnant lean goats constituted 23% of pregnant goats (Fig. 1). Obese non-pregnant goats obtained BF >2.7mm but those of lean had BF of 0.50mm (Fig. 2).

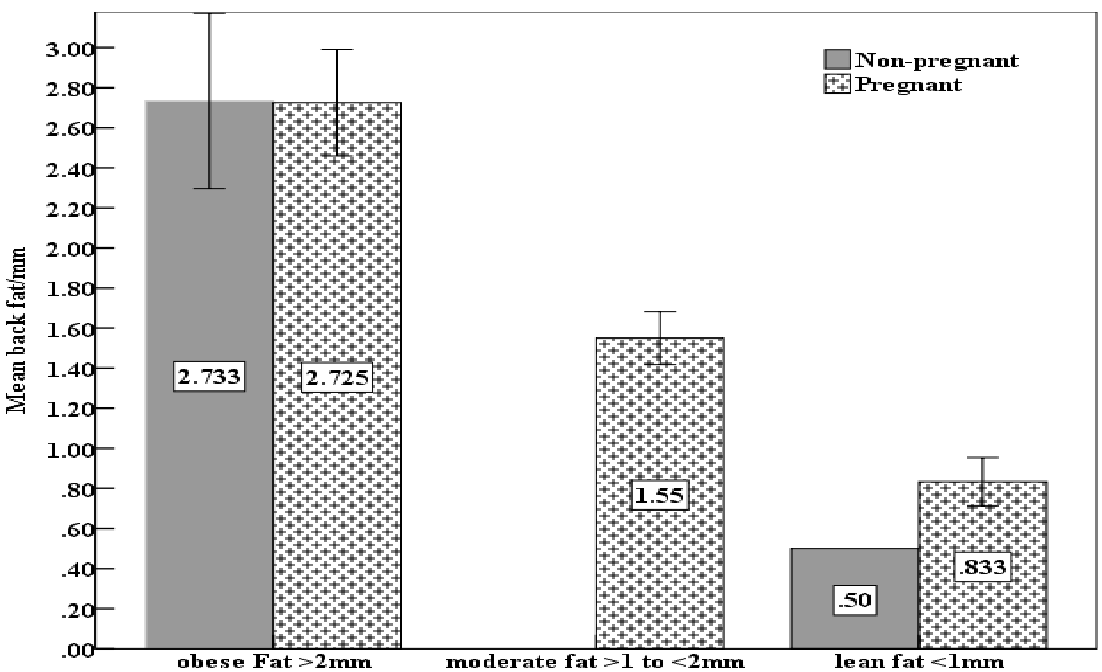

Fig. 2: Mean BF thickness in pregnant and non-pregnant goats

4. Discussion

Obesity and /or overweight attracted great attention in humans and is considered the recent global epidemic. In our study, the concentrated ration was deprived totally from the diet of obese goats with BF >2.0 mm and was replaced by common green clover rich in carotenoids, antioxidants such as tocopherols, and minerals (Singh *et al.,* 2019) simulating the calorific restriction trials to reduce body weight. The replacement of concentrated ration with antioxidant supplementation in the form of good quality green clover aimed to enhance the mitochondrial functions in caloric-restricted obese goats (Abad-Jiménez *et al.,* 2024). The replacement of concentrates by the green clover in this study improved the ovarian, hormonal, and pregnancy rates of obese goats subjected to withdrawn concentration for 30 days. The increase of leptin in obese goats in this study was also reported in obese sows (Jamal *et al.,* 2024). The decreased pregnancy rates in obese goats with hyperleptinemia even after withdrawn concentrates were attributed to the association of hyperleptinemia with irregular estrous behavior characterized by increased inter-estrous interval (Jamal *et al.,* 2024). The decreased fertility was referred to the hormonal imbalances resulting in increased ovarian, thyroid, FSH, and LH hormones (Jamal *et al.,* 2024).

In agreement with the low pregnancy rate could be referred to the hyperleptinemia, absence of ovulation, decreased body weight associated with neutrophil infiltrations in the ovaries and reduced breeding efficiency (Jamal *et al.,* 2024). Similar to the increased insulin in obese goats after 30 days of withdrawn-concentrates-diet and supplementing the green clover rich in carotenes, goats supplemented with short-term beta carotene presented high insulin and total follicles compared to non-supplemented ones (Meza-Herrera *et al.,* 2013 b). Also, goats shortly supplemented with maize to reach 1.5 times their maintenance requirements for 9 days showed increased insulin and leptin after supplementation (Nogueira *et al.,* 2016). In contrast to the decreased insulin in goats with moderate BF in this study, insulin decreased in goats during the interval of diet restriction compared to its increase after supplementation with 1.5 of their requirements (Thammasiri *et al.,* 2016). The decreased progesterone in lean goats in this study compared to those of high and moderate BF agree with the increase of P4 on Day 3 and Day 8 of the estrous cycle in goats subjected to a restricted diet for 42 days (Thammasiri *et al.,* 2016). In agreement with the increased progesterone in obese goats of this study, diets composed of crushed maize for 9 days increased progesterone in goats (Nogueira *et al.,* 2016).

Berseem is the main green clover during winter in our locality and each 1.0 kg contains 240 mg B-carotene, 16% CP, and 60% TDN (Chawla *et al.,* 2001). The increased CL area in goats with high BF after depriving concentrates of their ration for 30 days agrees with the increased CLs weight and DNA content on day 8 of the estrous cycle in goats subjected to diet restriction and re-supplementation with 1.5 of their requirements (Thammasiri *et al.,* 2016). Though obese goats in this study obtained the highest number of total follicles most of these follicles were small. In agreement with the increase in the number of total, large, and medium follicles in addition to TOA in obese goats supplemented with green clover for 30 days, goats supplemented with beta carotene for 34 days had a greater number of antral follicles and TOA compared to controls (Meza-Herrera *et al.,* 2013 a, b). The improvement in ovarian follicles due to the effect of beta carotenoids present in our green clovers simulated the effect of exogenous beta carotenoids in increasing total follicles and ovulation rate in yearling anestrous goats (Lopez-Flores *et al.,* 2020). The similar effect of exogenous beta carotene supplemented to yearling anestrous and cyclic goats (Meza-Herrera *et al.,* 2013 a, b) and our goats could be referred to the highest carotenoids and beta carotenes and tocopherols present in the available green clover used in this study to replace all concentrated ration (Maxin *et al.,* 2020). The increase in the number of total, small and large follicles in obese goats of this study was also reported in goats supplemented 1.5 of their requirements by adding maize for the short term during their non-breeding season (Nogueira *et al.,* 2016).

All goats of this study with different BF thicknesses had the same ovulation rate except lean goats showed anestrum. Similarly, Damascus does supplement with concentrated ration with either rice straw, green acacia, or berseem (*Trifolium alexandrinum*) clover hay did not influence the percentage of kidding and the litter weight at birth (Shetaewi *et al.,* 2001). The decrease in the pregnancy rate in goats, with high BF after replacing concentrated ration with the green clover, could be attributed to that 30 days were not enough to decrease BF thickness to the normal level. Also, BF <0.5mm is associated with lower pregnancy rates and could be referred to the alterations in insulin and leptin.

4. Conclusions

Our hypothesis came true. Maximum and minimum BF are associated with low fertility expressed by the increased number of small follicles in goats with high BF and an absence of ovulation in goats with low BF. Berseem (*Trifolium alexandrinum*) replaced efficiently the concentrates in the diet of obese goats with high BF restored ovarian activity and improved conception and pregnancy rates. Short-term substitution of concentrates with green clover berseem could be used as a safe and effective dietary regime in obese goats. Leptin can be used as a marker of increased subcutaneous fat in goats. Both high and low BF deposition in goats were associated with hyperinsulinemia. A dietary regime depending on good quality green clover for 30 days was not enough for reducing subcutaneous fat in goats.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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