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Effect of Diet Restriction and Polymorphism of Bone Morphogenetic Protein-15 and Growth Differentiation Factor-9 (*GDF9*) on Reproductive Performance of Three Egyptian Fat Tail Sheep Breeds

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ABSTRACT

Several genes are controlling prolificacy of sheep. The bone morphogenetic protein-15 and growth differentiation factor-9 (*GDF9*) control reproductivity of sheep. This study aimed to explore the diet restriction and the polymorphism in the bone morphogenetic protein-15 (*BMP15*) and growth differentiation factor-9 (*GDF9*) genes on ovulation and reproductive hormones in three sheep breeds. Ovaries of Rahmani ewes (R, n=56), Barki (B, n=56), and Ossimi ewes (O, n=50) were scanned to determine the preovulatory follicles and ovulation. The harvested sera were used for assaying progesterone, leptin, insulin-like growth Factor-I, and insulin. Whole blood samples were used for gene analysis using two PCR primers for amplifying the fragment 141-bp for *Fec*X^{G} site (exon-2) of the *BMP15* gene and 139-bp for *Fec*G^{H} locus (exon-1) of the *GDF9* gene. The output amplicons were digested (RFLP) with HinfI and DdeI endonucleases. Results revealed no mutation in the *Fec*X^{G} locus in all breeds. *Fec*G^{H} showed mutations but not in all breeds. Treated O received half dietary requirements for four weeks were the youngest (P=0.0001). Barki had the lightest bodyweight (P=0.017). Treated ewes had a higher (P=0.047) number of large follicles compared to their controls. Treated B and R got larger (P=0.0001) dominant follicles. The ovulation rate did not vary within the diet treated breeds but was lower than their control. Control O and R had higher (P=0.0001) ovulation rate, compared to B. Leptin concentrations were low (P=0.047) in treated B and R. The lowest (P=0.031) insulin concentrations were observed in treated O. In conclusion, the *BMP15* loci showed no polymorphism, while the *GDF9* loci were polymorphic in all breeds and treatments. Nutritional status and age modified ovulation rate in sheep.

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Introduction

Egyptian sheep are farmed for their meat, wool, and milk (Elshazly and Youngs, 2019). Barki ewes are bred in the northern coast and desert areas of Egypt. Ossimi ewes are raised in the south Delta and central Egypt. Rahmani ewes are raised in the central and northern delta (Elshazly and Youngs, 2019). These three main breeds are adapted to their environment and famed for high fertility but low prolificacy (Gabr *et al*., 2016). All these breeds possess fat tail. Barki ewes are considered the smallest breed in size with lighter fleece color, whereas Rahmani owns the largest size, and dark brown fleece color (Elshazly and Youngs, 2019).

The bone morphogenetic protein-15 (*BMP15*) gene controls fertility that exists on the X chromosome (*Fec*X) in sheep (Davis, 2005; Demars *et al*., 2013). The mutation in the *BMP15* gene *Fec*X[R] allele of the low-prolific Rasa Aragonesa Mediterranean ewes increased prolificacy when they got the heterozygous polymorphism and became sterile when that had the homozygotes polymorphism (Lahoz *et al*., 2011, 2014). Both *Fec*X^{GR} and *Fec*X^{O} mutations of the *BMP15* homozygotes demonates in high prolific animals (Wilson *et al*., 2001; Vacca *et al*., 2010; Ahlawat *et al*., 2015).

Growth Differentiation Factor-9 (*GDF9*) gene is localized on chromosome-5 [*Fec*G] (Liao *et al*., 2004) and has three phenotypes including the high fertility (*Fec*G^{H}), Embrapa (*Fec*G^{E}), and G1 (*Fec*G^{I}; Moradband *et al*., 2011). Both *BMP15* (Kona *et al*., 2016) and *GDF9* (Kona *et al*., 2016; Pan *et al*., 2018) are expressed in the ovarian tissues and oocytes to synthesize the *BMP15* protein that plays a synergistic role with *GDF9* protein, to control folliculogenesis and ovulation (McMahon *et al*., 2008; Qin *et al*., 2019), in addition to the ovarian functions and the oocyte maturation (Yan *et al*., 2001; Juengel and McNatty, 2005; Nagyova *et al*., 2017). The interactions of *BMP15* hormone with various factors are species-dependent, and its gene polymorphism controls estrogen levels (Peluso *et al*., 2017).

The ovulation rate as an indicator of prolificacy expresses several genetic and non-genetic factors (Alabart *et al*. 2016). Adequate nutrition is a non-genetic factor. Age and nutritional levels affected the diameter of the ovulating follicle and the ovulation rate (Junqueira *et al*., 2019). Increasing or decreasing the plane of nutrition during the rearing high prolific Belclare, Charmoise, Scottish Blackface and their crosses heterozygous to either *Fec*G^{H} or *Fec*X^{G} mutations influenced body weight but

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did not affect ovulation rate (Keady and Hanrahan, 2018).

Three metabolic hormones (leptin, insulin, and insulin-like growth factor-I) are secreted far away from the gonads but play vital roles in reproduction. Leptin is an adipocyte hormone that controls energy homeostasis and reproductive functions (Zieba *et al*., 2008). It regulates the ovulation rate and oocyte quality (Towhidi *et al*., 2007; Scaramuzzi *et al*. 2011). Leptin and its receptors are present in the sheep oocytes and granulosa cells (Pisani *et al*., 2008). Insulin-like growth factor-I (IGF-1) plays a role in the dominant follicle selection where its concentrations in the follicular fluid IGF-1 depend on its binding proteins (Fortune *et al*. 2004). It regulates the growth of small follicles (Mariana *et al*., 1998). Repotedly, insulin enhanced the reproductive performance of postpartum anoestrus sheep (Mann *et al*., 2003; Ferreira-Silva *et al*., 2017) and stimulated greater follicular development (Sarath *et al*., 2008).

This research aimed to identify the genetic polymorphism in the *Fec*X^G locus of *BMP15* gene and *Fec*G^H locus of *GDF9* gene for three Egyptian sheep lines (Barki, Ossimi, and Rahmani) with normal and reduced nutritional requirements via PCR-RFLP tool and finding out the influence of the plan nutrition (normal or reduced) on body weight, reproductive performance, and reproductive hormones.

Materials and methods

Animals

This study was approved by the Institutional Animal Care and Use Commeette of the National Research Centre (NRC-17-007) after taking the permission of the research farm of the Faculty of Agriculture; Cairo University (30.0276°N, 31.2101°E) was approved for blood sampling and the reproductive ultrasound examination. Multiparous Rahmani ewes of average body weight 37.88±5.81 kg (n =56; 3.36 years), Barki 36.21± 4.77 Kg (n=56; 3.68 years), and Ossimi 38.84± 5.99 kg (n =50; 2.82 years) were maintained under the regular farm feeding regime (0.25 Kg concentrate feed mixture and 4 Kg Egyptian clover), per head, according to NRC (1985). Of Rahmai (n =26), Barki (n =26), and Ossimi (n =25) ewes were fed 50% of their maintenance diet (Treated) for four weeks (Abo El-Maaty and Abd El-Gawad, 2014). Ewes (Control and Treated) were kept under the daylight and temperature and maintained in semi-shaded pens where clean drinking water is freely accessible.

Blood sampling

Blood samples were collected from the Jugular vein in plain vacuum tubes for the analysis of hormones. Blood samples collected with EDTA were used for DNA isolation. Sera were harvested and stored with the whole blood samples at -20°C until hormone assaying and DNA extraction.

Ultrasound examination

Ovarian ultrasonography was performed (Abdel-Mageed and Abo El-Maaty, 2012) using a modification of the large animals' endorectal real-time B-mode linear transducer 8-10 MHz to be handled externally (Scanner 100 SL, Pie Medical, Netherlands). Ewes were scanned in dorsal recumbence to avoid the fat tail. Corpora lutea and follicles were counted, and their diameters were determined at the pre-ovulatory wave following two submucosal doses of 65 µg Cloprostenol (Estrumate each of 1 ml or 250 µg Cloprostenol, Coopers Co., England) with 11 days apart. The count of the corpora lutae and their diameters were assessed seven days later to determine the ovulation rate. Visible follicles were counted and grouped respecting diameter into small (≤3 mm), medium (>3-5 mm), and large (≥5 mm). The number and diameters of corpora lutea on both ovaries were registered. The ovulation rates were detected ten days after ram introduction and pregnancy diagnosis 30 days later.

Hormonal assay

Serum progesterone (P4) was assayed, via Radioimmunoassay (RIA). Sensitivity, intra- and inter-assay coefficients were 0.05ng/ml, 3.6 and 3.9%. Multi-Species leptin RIA Kit (Linco Research Inc,t. Louis,MO, USA) was utilized where the limit of sensitivity, intra- and inter-assay coefficients of variation were 1.0 ng/ml, 2.8, and 8%, respectively. Insulin-like growth factor-1 (Biosource Europe, Belgium) commercial kit was used of the minimum detectable concentration, intra- and inter-assay coefficients of variation 1.10 ng/ml, 4.2%, and 11.5%. Insulin was assayed using a double-antibody, solid-phase enzyme-linked immunosorbent assay commercial kit (catalog number E29-072; Immunospec, Canoga Park, CA, USA) with a limit the assay sensitivity of 2.0 µIU/mL.

DNA isolation

For every 5 mL of whole blood, the genomic DNA has been extracted from white blood cells by the salting-out method and dissolved in TE buffer (Miller *et al*., 1988). A Nanodrop spectrophotometer (Thermo Fisher, USA) was employed to estimate DNA concentration and quality based on the absorbance of U.V. light at 260 and 280 nm. then kept at -20°C till use.

PCR-RFLP genotyping

Polymerase chain reaction (PCR) was performed in a mixture of 20 µl in C1000 Touch™ ThermalCycler (BIO-RAD, USA). The reaction mixture included 50-100 ng of genomic DNA,150 µMdNTPs, 1.2 mM $MgCl_2$, 2.0mM 10 × buffer, 30ng each forward and reverse primer (Table 1), and one Unit of TaqDNA polymerase. The PCR reaction protocol got an initial denaturation of 5 min. at 95°C; 30 cycles at 94°C for 45 sec. Annealing was performed at 58°C for 40 sec. (*BMP15*) or 60°C for 30 sec. (GDF-9) that was followed by an extension at 72°C for 45 sec. The amplicons were visualized in Gel DocTMEZ imager (BIO-RAD, USA) posterior to electrophoresis through 2% agarose gel stained with 2% ethidium bromide. Restriction fragment

Table 1. Primer sequence of candidate gene for prolificacy in Egyptian sheep breeds

Gene	Mutation site	Primer Sequence (5'-3')	PCR annealing temperature/ Time	Restriction nzyme	References
				*Hinf*I	
BMP15	*Fec*X^G	CACTGTCTTCTTGTTACTGTATTTCAATGAGAC	58°C/40 Sec.	5′…G↓ANTC…3′	Bahrami *et al*. (2018)
	Exon 2	GATGCAATACTGCCTGCTTG		3′…CTNA↓G…5′	
				DdeI	
GDF-9	*Fec*G^H	CTTTAGTCAGCTGAAGTGGGACAAC	60°C/30 Sec.	5′…C↓TNAG…3′	Paz *et al*. (2014)
	Exon 1	ATGGATGATGTTCTGCACCATGGTGTGAACCTGA		3′…GANT↓G…5′	

length polymorphism analysis (RFLP) was done by mixing each 10 µl of the PCR products for digestion with 5 units of the fast restriction enzyme including specific buffer (Fermentas, Germany) specific for each gene (Table 1) in a final reaction volume of 15 µl. The reaction mixture was incubated at 37°C in a water bath for 30 minutes. Afterwards, the digested fragments were separated by electrophoresis and imaged via the gel documentation system to detect the yielded genotypes.

Statistical analysis

Data are presented as mean± standard error of the mean. Statistical analysis of the data was performed using IBM SPSS 20.0 software (2016). Data were subjected to simple one-way ANOVA to determine the effect of the breed and the treatment, and Duncan's Multiple Range Test was used to differentiate between significant means. The univariate general linear model (3breeds x 2 treatments) was performed to study the effect of breeds (Barki, Rahmani, Ossisi), treatment (Control, nutritionally reduced), and their interaction, and to study the effect of age and dam body weight and their interactions on the ovarian and hormonal parameters.

Results

The treated Ossimi ewes were the youngest (P=0.0001), whereas the Ossimi and Rahmani ewes were heavier than Barki ewes throughout the study (Table 2). Control Barki had insignificantly the highest number of small and medium follicles (P=0.09) that associated with the lowest (P=0.047) number of large follicles. The reduced nutritional level decreased the total number of follicles and the small ones of both Rahmani and Barki ewes (Table 2). Compared to control Ossimi ewes, control Barki and Rahmani ewes had the lowest diameter of the dominant follicles (P=0.0001). Treated Ossimi and Rahmani ewes obtained insignificantly low dominant follicle diameters than Barki ewes. Control Ossimi ewes had the largest corpus luteum diameter but treated Rahmani ewes had insignificantly the lowest corpus luteum diameter (Table 3). Treated Rahmani ewes had the lowest corpus luteum diameter. The low nutritional level decreased the diameter of the dominant follicles of Ossimi ewes but increased it for Barki and Rahmani ewes (Table 3). Both control Rahmani and Ossimi ewes had higher ovulation rate (P=0.0001) than Barki. Lowered nutritional level decreased (P=0.0001) the ovulation rate of Rahmani and Ossimi ewes but did not decline those of Barki ewes (Table 3).

Progesterone concentrations of treated Rahmani ewes declined slightly compared to either treated or controls Barki and treated or controls Ossimi ewes (Table 4). Nutritional treatment decreased (P=0.047) the concentrations of leptin of Barki and Rahmani ewes with no significant variation between the three control breeds. Control Barki ewes had the highest (P=0.031) insulin and tended to have the highest (P=0.09) insulin-like growth factor-I (IGF-1) concentrations. Treated Ossimi ewes had the lowest insulin, but the control Ossimi tended to have the lowest IGF-1 (Table 4). Sheep breed influenced the number of small follicles (P=0.051), ovulation rate (P=0.046), and insulin (P=0.017) concentrations. Nutritional treatment influenced the ovulation rate ((P=0.0001) with a tendency to influence the number of medium (P=0.067) and large (P=0.095) follicles. Both breed and nutritional treatment interactions tended to affect the diameter of the large follicle (P=0.088) and the ovulation rate (P=0.082). The age of the ewe affected the number of the small follicles (P=0.048), the diameter of the large follicle (P=0.001) and tended (P=0.070) to affect the number of large follicles. The body weight of the ewes-affected leptin hormone concentrations (P=0.053) but tended to influence the number of total follicles (P=0.098), the diameter of the large follicle (P=0.070), and the corpus luteum diameter (P=0.094).

Table 2. Mean ± Standard deviation of the body weight, number of small, medium, large, total follicles in the sheep breeds

Treatment	Control			Treated			P-value
Breeds	Barki	Ossimi	Rahmani	Barki	Ossimi	Rahmani	
Number	30	25	30	26	25	26	162
Age/year	3.70±0.12^{c}	3.28±0.18^{bc}	3.41±0.13^{bc}	3.67±0.09^{c}	2.38±0.19^{a}	3.20±0.14^{b}	0.0001
Initial Body weight/kg	39.40±1.85^{ab}	44.69±3.43^{bc}	40.30±1.50^{ab}	37.50±0.90^{a}	41.22±1.60^{ab}	42.80±1.61^{ab}	0.149
Final Body weight/kg	35.30±1.62	39.13±3.07	38.30±1.32	34.61±0.86	36.06±1.49	37.11±1.42	0.401
N. Small follicles	2.38±0.42	1.52±.26	1.99±.16^{ab}	1.95±0.22	1.84±0.22	1.65±0.16	NS
N. Medium follicles	2.77±0.26^{b}	1.94±0.27^{a}	2.24±0.29^{ab}	2.08±0.21^{a}	2.30±0.26^{ab}	2.18±0.18^{ab}	0.09
N. Large follicles	1.57±0.21^{a}	1.94±0.24^{ab}	1.78±2.4^{ab}	2.00±0.24^{ab}	2.33±0.32^{b}	2.41±0.26^{b}	0.047
N. Total follicles	5.21±0.48	4.56±0.42	5.03 ±0.34	5.04±0.39	5.13±0.34	4.52 ±0.31	NS

Number (N.), Means with different superscripts within row (a, b) are significantly different at P< 0.05

Table 3. Mean ± Standard deviation of diameter of the dominant follicles, Diameter of the corpus luteum, and the ovulation rate.

Treatment	Control			Treated			P-Value
Breeds	Barki	Ossimi	Rahmani	Barki	Ossimi	Rahmani	
Diameter Dom./mm	5.78±0.33^{a}	6.63±0.20^{b}	5.67±0.21^{a}	6.67±0.26^{b}	5.98±0.24^{ab}	6.09±0.26^{ab}	0.0001
CL diameter/mm	8.35±0.4^{ab}	9.29±0.39^{b}	8.07±0.46^{ab}	8.33±0.50^{ab}	8.09±0.39^{ab}	7.77±0.26^{a}	NS
Ovulation rate	1.11±0.05^{a}	1.33±0.09^{b}	1.47±0.08^{b}	1.07±0.05^{a}	1.06±0.04^{a}	1.08±0.04^{a}	0.0001

Dominant follicles (Dom.), corpus luteum (CL), Means with different superscripts within row (a, b) are significantly different at P< 0.05

Table 4. Effect of breed on the reproductive hormones

Treatment	Control			Treated			P-Value
Breeds	Barki	Ossimi	Rahmani	Barki	Ossimi	Rahmani	
Progesterone ng/ml	2.24±0.79	2.19±0.45	1.72±0.17	2.52±0.45	2.26±0.99	1.49±0.12	NS
Leptin ng/ml	3.01±0.29^{b}	2.77±0.16^{ab}	2.87±0.08^{ab}	1.92±0.14^{a}	2.67±0.45^{ab}	1.97±0.25^{a}	0.047
Insulin µIU/mL	7.16±1.69^{b}	3.38±0.99^{a}	4.56±0.59^{b}	4.51±1.03^{ab}	1.95±0.48^{a}	4.67±0.43^{ab}	0.031
IGF-1 ng/ml	440±54	229±65	346±89	426102±	354±87	405±41	0.09

Means with different superscripts within row (a, b) are significantly different at P< 0.05

PCR successfully amplified DNA fragments of 141 bp (*Fec*X^{G}) from *BMP15* exon 2, and 139 bp (*Fec*G^{H}) from *GDF9* exon 1. These fragments were digested with HinfI for *Fec*X^{G} and DdeI for *Fec*G^{H}. *Fec*X^{G}-HinfI/RFLP output two genotypes; one homozygote (++), in which only two bands exist; 111 and 30-bp (30 bp band was not distinguished on gel because of its small size). The other genotype is the heterozygote +G, having three bands: 141, 111, and 30-bp, with the absence of homozygous (GG) genotype (Fig. 1). For *Fec*G^{H}-DdeI/RFLP, also two genotypes appeared; one homozygote (BB), with two bands; 108 and 31-bp, and the other is the heterozygote (AB), with 139, 108, and 31-bp bands, while the homozygote (AA) was absent (Fig. 2).

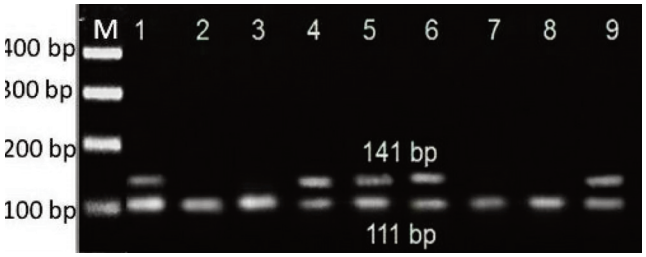

Fig. 1. PCR product of the *Fec*X^{G} locus of the *BMP15* gene digested with Hinf1. Lane 1, M: 100 bp DNA ladder, lanes: 1,4 ,5, 6 and 9; genotype G+ (141bp-111bp). While, lanes: 2, 3, 7, and 8; genotype ++ (111bp-111bp).

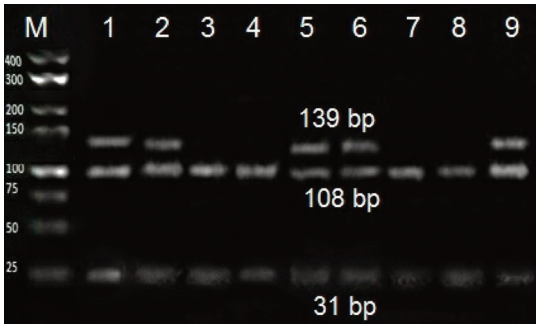

Fig. 2. DNA electrophoretic pattern of *Fec*G^{H} locus after digestion with DdeI endonuclease. Lane 1: 25 bp DNA ladder, lanes: 1, 2, 5 ,6 and 9; genotype AB (139, 108 and 31 bp). Lanes: 3, 4, 7 and 8.

In treated or diet-restricted animals of this study, allele (+) frequency for *BMP15* (*Fec*X^{G}) was much higher than allele (G), two times higher, for all treated and control sheep, except for the non-treated Rahmani, which was four times higher (Table 5). Also, the heterozygote (+G) frequency is considerably higher than the homozygote (++), in both treated and control, for all breeds (Table 5). Regarding *GDF9* (*Fec*G^{H}), the allele (B) frequency is higher than that of (A), and the homozygote (BB) frequency is markedly higher than the heterozygote (AB), in both control and treated ewes, all over the three studied breeds (Table 6).

Discussion

The breed, nutritional treatment, age, and body weight affected the reproductive performance and the hormonal status in sheep underwent in this study. In agreement with the obtained results, the ovulation rate increases with increasing age from two to six years (Shorten *et al*., 2013). The diameters of the dominant follicle and the corpus luteum are not only age, nutritional level, or breed dependant but also the larger the number of ovulatory follicles, the smaller of their diameter within the same breed (Souza *et al*., 2003) which explain the decrease in the ovulation rate and the increase in the dominant follicle diameter due to reducing nutrition level in Rahmani ewes. Similar to the small size of Barki ewes, the ovulation rate did not differ between control and fasted ewes (Kiyma *et al*., 2004).

Regardless of the difference of breed, body weight, and age of ewes in this study, no difference among breeds in the progesterone, leptin, and IGF-1 concentrations were noticed in control or treated ones. The nutritional levels of this study did not affect the regulatory effect of leptin on ovarian function (Smith *et al*., 2002) because of the storage of fat in the tail compensated any dietary shortage. IGF-I increased granulosa cell proliferation and enhanced progesterone secretion (Mariana *et al*., 1998). Similar to the current results, Towhidi *et al*. (2007) observed a difference in the ovulation rate with no significant difference in the circulating leptin concentrations between Mehraban and Sanjabi sheep synchronized with cloprostenol. Nutritional treatment influenced both leptin and insulin concentrations in the three strains of this study. Similar to results from this study, the increased expression of leptin receptors in the granulosa cells of the ovaries of sheep in response to reducing their nutritional level was not associated with any difference in leptin gene expression when compared

Table 5. Allele and genotypes frequencies of *BMP15* (*Fec*X^{G}) in Egyptian sheep breeds.

Treatment	Breed	Allele frequency		Genotype frequency		
		G	+	GG	+G	++
Control	Barki	0.29	0.71	0	0.59	0.41
	Ossimi	0.32	0.68	0	0.64	0.36
	Rahmani	0.18	0.82	0	0.56	0.44
Treated	Barki	0.3	0.7	0	0.6	0.4
	Ossimi	0.33	0.67	0	0.66	0.34
	Rahmani	0.29	0.71	0	0.59	0.41

Table 6. Allele and genotypes frequencies of *GDF9* (*Fec*G^{H}) in Egyptian sheep breeds.

Treatment	Breed	Allele frequency		Genotype frequency		
		A	B	AA	AB	BB
Control	Barki	0.24	0.76	0	0.49	0.51
	Ossimi	0.18	0.82	0	0.36	0.64
	Rahmani	0.17	0.83	0	0.35	0.65
Treated	Barki	0.21	0.79	0	0.42	0.58
	Ossimi	0.2	0.8	0	0.4	0.6
	Rahmani	0.2	0.8	0	0.4	0.6

to those with increased nutritional level (Pisani *et al*., 2008). In sheep, the fluctuations in the circulating concentrations of IGF-1 and insulin coincided with the phase of the estrous cycle (Sosa *et al*., 2006; 2009). The decreased insulin concentrations, in the treated Ossimi ewes of this study could be related to the reduced dietary level, their younger age, or high body weight (Abecia *et al*., 2015). Similar to the reduced ovulation rate in the treated ewes in the present study, acute fasting reduced ovulation rate, oocyte quality, embryos, leptin, insulin, and IGF-1 in sheep (Kosior-Korzecka *et al*., 2006). The decreased dietary level in large-sized ewes of this study was not associated with any significant decrease in their IGF-1, leptin, and insulin. That could be referred to the mobilization of fat from their fat tail. Whereas the declined leptin, insulin, and IGF-1 levelswith lowered dietary level in Barki ewes may be attributed to their small size and lowered fat storage in the small fat tail. Similar to Barki ewes, fasting did not affect the ovulation rate in ewes (Kiyma *et al*., 2004). The increased IGF-1 concentrations in treated Ossimi and Rahmani ewes were associated with lowered ovulation rate. Though the increased blood IGF-1 levels augmented the number of recruited follicles (Gong *et al*., 1994) but did not improve the ovulation rate (Bister *et al*., 1999). The nutritional levels influence on reproduction may be linked to variations in the IGF-I system (IGF-I and IGFBP) level secreted from the liver or present in other reproductive tissues (Roberts *et al*., 2001). Thus, acute nutrient restriction to the point of decreasing IGF-I secretion may affect the ability to develop follicles in response to a reduction in FSH-receptor expression (Minegishi *et al*. 2000). The lowered dietary level raised the number of large follicles but dropped the ovulation rate in the three studied breeds. That could be attributed to the suppressed estradiol secretion that either prolonged (Viñoles *et al*., 2005; Scaramuzzi *et al*., 2006), shortened the existence of dominant follicles and ovulatory wave (Murphy *et al*., 1991), or lengthened the estrous cycle (Abo El-Maaty *et al*., 2008).

Limited or over nourishment can determine the reproductive efficiency to an extent dependent on the degree and reproductive status at the time of feeding (Nie *et al*., 2019). The difference of insulin between the three breeds did not affect their reproductive status but their body condition and nutritional status. In accordance with our observations, live weight at first oestrus was related positively to insulin concentration and negatively to GH concentration (Viñoles *et al*., 2010).

GDF9 gene was found to boost the development of the ovarian primordial follicles and the proliferation of granulosa cells (Liao *et al*. 2004). *Fec*G^{H} polymorphism of C1184T mutation caused a substitution of Serine with a Phenylalanine at position-77 of the mature peptide. This polymorphism altered the interaction of the protein type-1 domain linker receptor by what ovarian development from the pre-antral stage even that of follicular growth was different in all genotypes (Mora-Luna *et al*. 2014). Homozygous ewes for this mutation did not ovulate and were infertile (i.e. sterile). Heterozygote's ones had the average ovulation rate compared to the wild type, so better fertility, prolificacy, and ovulatory rate were only for heterozygotes (Hanrahan *et al*. 2004; Barzegari *et al*. 2010). The absence of any difference among the different BMPs stimulated estradiol (E2) production in the cultured granulosa cells and had a clear interaction with IGF-I. BMPs had no effect on granulosa cells proliferation BMP-6 protein expression in the oocyte, granulosa, and thecal layers of antral follicles from both genotypes. These results confirm the role of BMPs in controlling ovarian somatic cell function in sheep and provides evidence to support the hypothesis that *Fec*B mutation increases the BMPs' response of somatic cells when stimulated to differentiate by gonadotropins (Campbell *et al*., 2006).

In agreement with our results, ewes with two homozygous inactive copies of the *BMP15* gene were sterile (Galloway *et al*., 2002; Hanrahan *et al*. 2004) but the heterozygotes having a single inactive copy of the *BMP15* gene were fertile and had higher ovulation rate and lambing (Hanrahan *et al*., 2004; Davis, 2005). Moreover, the immunization against the bone morphogenetic protein-15 and growth differentiation factor-9 disturbed the ovarian function (Davis *et al*., 2018). Parallel to outcomes from this study, the similarity in the genetic polymorphism, between the three sheep strains, output nearly similar reproductive performance not withstanding the size of the breed or the bodyweight of the dam before breeding. Any observed decrease in the reproductive performance depended on their nutritional status before breeding (Debus *et al*., 2012). Additionally, ewes got one allele (heterozygote) from two copies of the mutated *GDF9* (*Fec*G^{H}) gene were fertile and had a high rate of ovulation (Hanrahan *et al*., 2004). Otherwise, the homozygous ewes for this mutation were infertile in addition to the elementary ovary failure. Heterozygous individuals for mutations of *GDF9* and *BMP15* genes are fertile, and these mutations had additional effects on the ovulation rate (Hanrahan *et al*., 2004).

The TGFß family member *BMP15* was the first gene to be associated with prolificacy. *GDF9* and *BMP15* genes play a crucial role in folliculogenesis and ovulation rate control, where their different mutations like *Fec*X^{G} and *Fec*G^{H}, boost the proliferation of granulosa cells, reduced the expression levels of FSH receptor and steroid hormones (Mottershead *et al*., 2015), and raised the ovulation rate and lamb size (Bodin *et al*., 2007; Monteagudo *et al*., 2009). All known *BMP15* mutations (named *Fec*XI, H, B, G, L, R), produced the same phenotype. In other words, heterozygous females were highly prolific whereas homozygous ones are infertile because of the blockage of follicular development at the primary stage (Almahdy *et al*., 2000; Kumar *et al*., 2006; Guan *et al*., 2007; Polley *et al*., 2009). *GDF9* (*Fec*G) is also a member of the TGFß family. A mutation in *GDF9* (*Fec*G^{E}) associated with prolificacy was identified in the Brazilian Santa Ineˆs strain (Liao *et al*., 2004). Interestingly, Silva *et al*. (2011) showed for the first time a novel phenotype since *Fec*G^{E} homozygous ewes were not sterile but exhibited a significant increase compared to non-mutated individuals in their ovulation rate and litter size.

Conclusion

BMP15 gene loci has no polymorphism, while *GDF9* gene loci are polymorphic in Barki, Ossimi, and Rahmani sheep breeds. The fertility rate in these breeds is related to the *GDF9* (*Fec*G^{7} and *Fec*G^{H}) gene, but not related to the mutants of the *BMP15* (*Fec*X^{G}) and *BMP15* (*Fec*X^{B}) loci. The non-genomic factors such as breed, animal size, age, and body condition, and dietary level control the reproductive performance when their genomes are similar.

Conflict of interest

The authors declare that they do not have any conflict of interest.

References

Abdel-Mageed, I.I., Abo El-Maaty, A.M., 2012. The effect of backfat thickness at mating on the reproductive and productive performances of ewes. Small Rum. Res. 105, 148-153.

Abecia, J.A., Forcada, F., Palacín, I., Sánchez-Prieto, L., Sosa, C., Fernández-Foren, A., Meikle, A., 2015. Undernutrition affects embryo quality of superovulated ewes. Zygote 23, 116-124.

Abo El-Maaty, A.M., Abd El-Gawad, M.H., 2014. Follicle growth, ovulation rate, body weight change, and antioxidant and metabolic status in three fattailed sheep breeds fed a

half-maintenance diet. Open Access Anim. Physiol. 6, 21-31.
Abo El-Maaty, A.M., Mansour, M.M., Ezzo, O.H., Hamam, A.M., 2008. Some reproductive and metabolic responses to food restriction and re-feeding in Egyptian native goats. Global Vet. 2, 225-232.
Ahlawat, S., Sharma, R., maitra, A., Raja, K.N., Verma, N.K., Tantia, N.K., 2015. Prolificacy in Indian goat breeds is independent of *Fec*B mutation. Indian J. Anim. Sci. 85, 617-620.
Alabart, J.L. , Lahoz, B. , Calvo, J.H. , Jurado J.J. , Fantova, E. , Folch, J., 2016. Studies and state of the art of the prolific genetic variant ROA (*Fec*X^{R}) in the Rasa Aragonesa sheep breed. Archivos de Zootecnia 65, 449-452.
Almahdy, H., Tess, M.W., El-Tawil E., Shehata, E., Mansour, H., 2000. Evaluation of Egyptian sheep production systems: II. Breeding objectives for purebred and composite breeds. J. Anim. Sci. 78, 288-295.
Barzegari, A., Atashpaz, S, Ghabili, K., Nemati, Z., Rustaei, M., Azarbaijani, R., 2010. Polymorphisms in *GDF9* and *BMP15* Associated with Fertility and Ovulation Rate in Moghani and Ghezel Sheep in Iran. Reprod. Dom. Anim. 45, 666-669.
Bister, J.L., Noel, B., Perrad, B., Mandiki, S.N., Mbayahaga, J., Paquay, R., 1999. Control of ovarian follicles activity in the ewe. Dom. Anim. Endocrinol. 17, 315-328.
Bodin, L., Di Pasquale, E., Fabre, S., Bontoux, M., Monget, P., 2007. A novel mutation in the bone morphogenetic protein 15 gene causing defective protein secretion is associated with both increased ovulation rate and sterility in Lacaune sheep. Endocrinol. 148, 393-400.
Campbell, B.K., Souza, C.J., Skinner, A.J., Webb, R., Baird, D.T., 2006. Enhanced response of granulosa and theca cells from sheep carriers of the *Fec*B mutation in vitro to gonadotropins and bone morphogenic protein-2, -4, and -6. Endocrinol. 147, 1608-1620.
Davis, G.H., 2005. Major genes affecting ovulation rate in sheep. Genet. 37, 11-23.
Davis, K.A., Klohonatz, K.M., Mora, D.S.O., Twenter, H.M., Graham, P.E., Pinedo, P., Eckery, D.C., Bruemmer, J.E., 2018. Effects of immunization against bone morphogenetic protein-15 and growth differentiation factor-9 on ovarian function in mares. Anim. Reprod. Sci. 192, 69-77.
Debus, N., Chavatte-Palmer, P., Viudes, G., Camous, S., Roséfort, A., Hassoun, P., 2012. Maternal periconceptional undernutrition in Merinos d'Arles sheep: 1. Effects on pregnancy and reproduction results of dams and offspring growth performances. Theriogenology 77, 1453-1465.
Demars, J., Fabre, S., Sarry, J., Rossetti, R., Gilbert, H., 2013. Genome-Wide Association Studies Identify Two Novel *BMP15* Mutations Responsible for an Atypical Hyperprolificacy Phenotype in Sheep. PLoS Genet 9, e1003482.
Elshazly, A.G., Youngs, C.R., 2019. Feasibility of utilizing advanced reproductive technologies for sheep breeding in Egypt. Part 1. Genetic. Egyptian J. Sheep and Goat Sci. 14, 39-52.
Ferreira-Silva, J.C., Basto, S.R.L., Tenório Filho, F., Moura, M.T., Silva Filho, M.L., Oliveira, M.A.L., 2017. Reproductive performance of postpartum ewes treated with insulin or progesterone hormones in association with ram effect. Reprod. Dom. Anim. 52, 610–616.
Fortune, J.E., Rivera, G.M., Yang, M.Y., 2004. Follicular development: the role of follicular microenvironment in selection of the dominant follicle. Anim. Reprod. Sci. 82-83, 109-126.
Gabr, A.A., Shalaby, N.A., Ahmed, M.E., 2016. Effect of ewe born type, growth rate and weight at conception on the ewe subsequent productivity of Rahmani sheep. Asian J. Anim. Vet. Adv. 11, 732-736.
Galloway, S.M., Gregan, S.M., Wilson, T., McNatty, K.P., Juengel, J.L., Ritvos, O., Davis, G.H., 2002. Bmp15 mutations and ovarian function. Mol. Cell. Endocrinol. 191, 15-18.
Gong, J.G., Campbell, B.K., McBride, D., Webb, R., 1994. The effect of recombinant bovine somatotrophin (rGH) on ovarian follicular populations in mature ewes. J. Reprod. Fertil. Abstr. Ser. 13, 9.
Guan, F., Shou-ren, L., Shi, G.Q., Yang, L.G., 2007. Polymorphism of *Fec*B gene in nine sheep breeds or strains and its effects on litter size, lamb growth and development. Anim. Reprod. Sci. 99, 44-52.
Hanrahan, J.P., Gregan, S.M., Mulsant, P., Mullen, M., Davis, G.H., Powell, R, Galloway, S., 2004. Mutations in the genes for oocyte derived growth factors *GDF9* and *BMP15* are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovis aries*). Biol. Reprod. 70, 900-909.
IBM SPSS Data Collection Divestiture. 2 February 2016. Retrieved 7 June 2017, PC software, Version (20.0).
Juengel, J.L., McNatty, K.P., 2005. The role of proteins of the transforming growth factor-β superfamily in the intraovarian regulation of follicular development. Human Reproduction Update, 11, 144-161.
Junqueira, F.B., Souza, J.C., Campos, J.P.A., Faria, L.R., Silva, D.R.D., Ascari, I.J., Lima, R.R., Furusho-Garcia, I.F., Nogueira, G.P., Alves, N.G., 2019. Ovarian follicular development, hormonal and metabolic profile in prepubertal ewe lambs with moderate dietary restriction and lipid supplementation. Anim. Reprod. Sci. 204, 152-164.
Keady, T.W.J., Hanrahan, J.P., 2018. Plane of nutrition during the rearing phase for replacement ewes of four genotypes: I- effects on growth and development, and on ovulation rate at first joining. Anim. 12, 713-721.
Kiyma, Z., Alexander, B.M., Van Kirk, E.A., Murdoch, W.J., Hallford, D.M., Moss, G.E., 2004. Effects of feed restriction on reproductive and metabolic hormones in ewes. J. Anim. Sci., 82, 2548-2557.
Kona, S.S., Praveen Chakravarthi, V., Siva Kumar, A.V., Srividya, D., Padmaja, K., Rao, V.H., 2016. Quantitative expression patterns of *GDF9* and *BMP15* genes in sheep ovarian follicles grown in vivo or cultured in vitro. Theriogenology. 85, 315-322.
Kosior-Korzecka, U., Bobowiec, R., Lipecka, C., 2006. Fasting-induced Changes in Ovulation Rate, Plasma Leptin, Gonadotropins, GH, IGF-I and Insulin Concentrations During Oestrus in Ewes. J. Vet. Med. Series A 53, 5-11.
Kumar, S., Kolte, A.P., Mishra, A.K., Arora, A.L., Singh, V.K., 2006. Identification of the *Fec*B mutation in Garole x Malpura sheep and its effect on litter size. Small Rumin. Res. 64, 305-310.
Lahoz, B., Alabart, J.L., Jurado, J.J., Calvo, J.H., Martínez-Royo, A., Fantova, E., Folch J., 2011. Effect of the *Fec*X(R) polymorphism in the bone morphogenetic protein 15 gene on natural or equine chorionic gonadotropin-induced ovulation rate and litter size in Rasa Aragonesa ewes and implications for on-farm application. J. Anim. Sci. 89, 3522-3530.
Lahoz, B., Alabart, J.L., Cocero, M.J., Monniaux, D., Echegoyen, E., Sánchez, P., Folch, J. 2014. Anti-Müllerian hormone concentration in sheep and its dependence of age and independence of *BMP15* genotype: an endocrine predictor to select the best donors for embryo biotechnologies. Theriogenology. 81, 347-357.
Liao, W.X., Moore, R.K., Shimasaki, S., 2004. Functional and molecular characterization of naturally occurring mutations in the oocyte secreted factors bone morphogenetic protein-15 and growth and differentiation factor-9. J. Biol. Chem. 17, 17391-1796.
Mann, G.E., Green, M.P., Sinclairb, K.D., Demmers, K.J., Fray, M.D., Gutierrez, C.G., Garnsworthy, P.C., Webb, R., 2003. Effects of circulating progesterone and insulin on early embryo development in beef heifers. Anim. Reprod. Sci. 79, 71-79.
Mariana, J.C., Monniaux, D., Caraty, A., Pisselet, C., Fontaine, J., Solari, A., 1998. Immunization of sheep against GnRH early in life: effects on gonadotropins, follicular growth and responsiveness of granulosa cells to FSH and IGF-I in two breeds of sheep with different prolificacy (Romanov and Ile-de-France). Domest. Anim. Endocrinol. 15, 195-207.
McMahon, H.E., Sharma, S., Shimasaki, S., 2008. Phosphorylation of Bone Morphogenetic Protein-15 and Growth and Differentiation Factor-9 Plays a Critical Role in Determining Agonistic or Antagonistic Functions. Endocrinol. 149, 812-817.
Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16, 1215.
Monteagudo, L.V., Ponz, R., Tejedor, M.T., Lavina, A., Sierra, I., 2009. A 17-bp deletion in the Bone Morphogenetic Protein 15 (*BMP15*) gene is associated to increased prolificacy in the Rasa Aragonesa sheep breed. Anim. Reprod. Sci. 110, 139-146.
Moradband, F., Rahimi, G., Gholizadeh, M., 2011. Association of polymorphism in fecundity genes of *GDF9*, *BMP15* and BMPR-1B with litter size in Iranian Baluchi sheep. Asian- Aust. J. Anim. Sci. 9, 1179-1183.
Mora-Luna, R.E., Chicco, C.F., Herrera-Angulo, A.M., Godoy, S., Garmendia, J., 2014. Supplementation with degradable and undegradable protein sources in rumen on cows fed Urochloa humidicola. I. Body weight changes, body score condition,

pregnancy and blood chemistry on Brahman first calf heifers on grazing. Revista Cient.-Facultad Cienc.Vet. 24, 563-576.

Mottershead, D.G., Sugimura, S., Sara, L., Al-Musawi, Li, J.J., Richani, D., White, M.A., Martin, G.A., Trotta, A.P., Ritter, L.J., Shi, J., Mueller, T.D., Harrison C.A., Gilchrist, R.B., 2015. Cumulin, an Oocyte-secreted Heterodimer of the Transforming Growth Factor-β Family, is a Potent Activator of Granulosa Cells and Improves Oocyte Quality. J. Biol. Chem. 290, 24007-24020.

Murphy, M.G., Enright, W.J., Crowe, M.A., McConnell, K., Spicer, L.J., Boland, M.P., Roche, J.F., 1991. Effect of dietary intake on pattern of growth of dominant follicles during the oestrous cycle in beef heifers. J. Reprod. Fertil. 92, 333-338.

Nagyova, E., Nemcova, L., Bujnakova Mlynarcikova, A., Blaha, M., Prochazka, R., Scsukova, S., 2017. Effect of bone morphogenetic protein-15 on gonadotropin-stimulated synthesis of hyaluronan and progesterone in porcine ovarian follicle. J. Physiol. Pharmacol. 68, 683-691.

NRC. 1985. National Academy of Sciences. Nutrient Requirements of Sheep. 6th ed. Washington, DC: National Academy Press.

Nie, H.T., Wang, Z.Y., Zhang, G.M., Wang, F., 2019. Amino acids profile within peripheral blood and follicular fluid based on high-performance liquid chromatography methods may explain differences in folliculogenesis between short-term under/over-fed treatments during luteal phase of Hu sheep. Reprod Domest Anim. 54, 72-82.

Pan, Z., Wang, X., Di, R., Liu, Q., Hu, W., Cao, X., Guo, X., He, X., Lv, S., Li, F., Wang, H., Chu, M.A., 2018. 5-Methylcytosine Site of Growth Differentiation Factor 9 (*GDF9*) Gene Affects its Tissue-Specific Expression in Sheep. Anim. (Basel) 8, pii: E200.

Peluso, C., Goldman, C., Cavalcanti, V., Gastaldo, G., Trevisan, C.M., Christofolini, D.M., Barbosa, C.P., Bianco, B., 2017. Use of Bone Morphogenetic Protein 15 Polymorphisms to Predict Ovarian Stimulation Outcomes in Infertile Brazilian Women. Genet. Test Mol. Biomarkers 21, 328-333.

Pisani, L.F., Antonini, S., Pocar, P., Ferrari, S., Brevini, T.A.L., Rhind, S.M., Gandolfi, F., 2008. Effects of pre-mating nutrition on mRNA levels of developmentally relevant genes in sheep oocytes and granulosa cells. Reproduction 136(3), 303-312.

Polley, S., De, S., Brahma, B., Mukherjee, V., Batabyal, S., Arora, J.S., Pan, S., Samanta, A.K., Datta, T.K., Goswami, S.L., 2009. Polymorphism of BMPR1B, *BMP15* and *GDF9* fecundity genes in prolific Garole sheep. Trop. Anim. Health Prod. 42, 985-993.

Qin, Y., Tang, T., Li, W., Liu, Z., Yang, X., Shi, X., Sun, G., Liu, X., Wang, M., Liang, X., Cong, P., Mo, D., Liu, X., Chen, Y., He, Z., 2019. Bone Morphogenetic Protein 15 Knockdown Inhibits Porcine Ovarian Follicular Development and Ovulation. Front. Cell Dev. Biol. 7, 286.

Roberts, C.T., Sohlstrom, A, Kind, K.L, Grant, P.A., Earl, R.A. Robinson, J.S, Khong, T.Y., Owens, P.C., Owens J.A., 2001. Altered Placental Structure Induced by Maternal Food Restriction in Guinea Pigs: A Role for Circulating IGF-II and IGFBP-2 in the Mother? Placenta 22(A), S77-S82.

Sarath, T., Mehrotra, S., Agarwal, S.K., Varshney, V.P., Hoque, M., Shankar, U., Singh, S.K., 2008. Effect of insulin administration on ovarian function and estrus induction in acyclic goats. Animal Reproduction Science 108, 216–225.

Scaramuzzi, R.J., Baird D.T., Campbell, B.K., Driancourt, M.A., Dupont, J., Fortune, J.E., Gilchrist, R.B., Martin, G.B., McNatty, K.P., McNeilly, A.S., Monget, P., Monniaux, D., Viñoles, C., Webb, R., 2011. Regulation of folliculogenesis and the determination of ovulation rate in ruminants. Reprod. Fertil. Dev. 23, 444-467.

Scaramuzzi, R.J., Campbell, B.K., Downing, J.A., Kendall, N.R., Khalid, M., Muñoz-Gutiérrez, M., Somchit, A., 2006. A review of the eīects of supplementary nutrition in the ewe on the concentrations of reproductive and metabolic hormones and the mechanisms that regulate folliculogenesis and ovulation rate. Reprod. Nutr. Dev. 46, 339–354.

Shorten, P.R., O'Connell, A.R., Demmers, K.J., Edwards, S.J., Cullen, N.G., Juengel, J.L., 2013. Effect of age, weight, and sire on embryo and fetal survival in sheep. J. Anim. Sci. 91, 4641-4653.

Silva, B.D., Castro, E.A., Souza, C.J., Paiva, S.R., Sartori, R., Franco, M.M., Azevedo, H.C., Silva, T.A.S.N., Vieira, A.M. C., Neves, J.P., Melo, E.O., 2011. A new polymorphism in the Growth and Differentiation Factor 9 (*GDF9*) gene is associated with increased ovulation rate and prolificacy in homozygous sheep. Anim. Genet. 42, 89-92.

Smith, G.D., Jackson, L.M., Foster, D.L., 2002. Leptin regulation of reproductive function and fertility. Theriogenology 57, 73-86.

Sosa, C., Abecia, J.A., Carriquiry, M., Forcada, F., Martin, G.B., Palacin, I., Meikle, A. 2009. Early pregnancy alters the metabolic responses to restricted nutrition in sheep. Domest. Anim. Endocrinol. 36, 13-23.

Sosa, C., Abecia, J.A., Forcada, F., Vinoles, C., Tasende, C., Valares, J.A., Palacin, I., Martin, G.B., Meikle, A., 2006. Effect of undernutrition on uterine progesterone and oestrogen receptors and on endocrine profiles during the ovine oestrous cycle. Reprod. Fertil. Dev. 18, 447-58.

Souza, C.J., Campbell, B.K., McNeilly, A.S., Baird, D.T., 2003. Bone morphogenetic proteins and folliculogenesis: lessons from the Booroola mutation. Reprod Suppl. 61, 361-370.

Towhidi, A., Masoumi, R., Moeini, M.M., Solgi, H., Moravej, H., 2007. The relationship between plasma leptin and FSH concentrations with ovulation rate in Iranian native sheep. Pak. J. Biol. Sci. 10, 363-367.

Vacca, G., Dhaouadi, A., Rekik, M., Carcangiu, V., Pazzola, M., Dettori, M.L., 2010. Prolificacy genotypes at BMPR 1B, *BMP15* and *GDF9* genes in North African sheep breeds. Small Rum. Res. 88, 67–71.

Viñoles, C., Forsberg, M., Martin, G.B., Cajarville, C., Repetto, J., Meikle, A., 2005. Short term nutritional supplementation of ewes in low body condition affects follicle development due to an increase in glucose and metabolic hormones. Reprod. 129, 299-309.

Viñoles, C., Paganoni, B., Glover, K.M., Milton, J.T., Blache, D., Blackberry, M.A., Martin, G.B., 2010. The use of a 'first-wave' model to study the effect of nutrition on ovarian follicular dynamics and ovulation rate in the sheep. Reprod. 140, 865-874

Wilson, T., Wu, X.Y., Juengel, J.L., Ross, I.K., Lumsden, J.M., Lord, E.A., Dodds, K.G., Walling, G.A., McEwan, J.C., O'Connell, A.R., McNatty, K.P., Montgomery, G.W., 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biol. Reprod. 64, 1225-1235.

Yan, C., Wang, P., DeMayo, F.J., Elvin, J.A., Carino, C., Prasad, S.V., Skinner, S.S., Dunbar, B.S., Dube, J.L., Celeste, A.J., Matzuk, M.M., 2001. Synergistic roles of bone morphogenetic protein 15 and growth differentiation factor 9 in ovarian function. Mol. Endocrinol. 15, 854-866.

Zieba, D.A., Szczesna, M., Klocek-Gorka, B., Williams, G.L., 2008. Leptin as a nutritional signal regulating appetite and reproductive processes in seasonally-breeding ruminants. J. Physiol. Pharmacol. 59, 7-18.