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Polymorphism of insulin-like growth factor 1 gene and its relationship with reproductive performances and milk yield in Sarda dairy sheep

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ABSTRACT

The aim of this research was to detect polymorphisms within the *IGF-I* gene in dairy sheep and to verify their influence on milk yield and reproductive performances. Four hundred Sarda ewes in their second lactation were selected from 2 farms. Their reproductive traits (fertility rate, interval in days from ram introduction to lambing and litter size) and milk yield were recorded, from the second to the fourth lactation. DNA was extracted from individual blood samples and subjected to amplification and sequencing of the *IGF-I* gene 5′ UTR and Exon 3 regions. Three polymorphic sites were recorded at positions g184028491C>G and g184028489C>T of the 5′UTR, and g184023223G>A of the Exon 3. The C allele at position g184028491 showed a significant association with higher fertility rate ($P < 0.05$) and a shorter interval in days from ram introduction to lambing ($P < 0.01$). In addition, a significant effect of the CC genotype was found with higher milk yield for $P < 0.05$ in the second and third lactation, and $P < 0.01$ in the fourth lactation compared to the other genotypes. Even, AA genotype at position g184023223 of the exon 3 showed a positive significant effect on milk yield for $P < 0.05$, in the second and third lactation, and for $P < 0.01$ in fourth lactation compared to the other genotypes. In conclusion the found SNPs showed a significant influence on reproductive performances and milk yield in Sarda sheep breed suggesting a possible application in sheep selection plans.

1. Introduction

Insulin-like growth factor-1 is a low-molecular-weight peptide, produced by liver and by many other types of cells, and it plays an important role in regulating postnatal growth, mammary gland development, lactation and reproduction in male and female in several species (Jiang & Lucy, 2001; Lammers, Heinrichs & Kensinger, 1999; Renaville, Hammadi & Portetelle, 2002; Tabecka-Lonczynska, Mytych, Solek, Kowalewski & Koziorowski, 2019). For these important physiological actions, this molecule has received and still does receive considerable attention from researchers, as it can affect the animal's productive career. In fact, a rapid growth in young animal leads quickly to the adult body weight, with a greater chance of entering the production stage in a shorter time (Carcangiu et al., 2013). Furthermore, a more efficient reproduction is essential to reach an adequate production and, in recent years, it has been found that the action of the IGF-I on the ovary is important to improve the fertility of the animals (Gobikrushanth et al., 2018; Monte et al., 2019). In fact, alterations affecting the ovarian IGF-I receptors determine a high degree of apoptosis in large follicles as the FSH (follicle-stimulating hormone) cooperates with IGF-I in the control of growth, survival and maturation of ovarian follicles (Magalhães-Padilhaa et al., 2012). Recently, with the improvement of analytical tools and molecular biology, the study of the *IGF-I* gene and its expression has received considerable attention, and same polymorphisms in the *IGF-I* gene has been shown to be associated with many productive traits in different animal species. Particularly in cattle, a SNPs in the promoter region is linked with greater weight to calf weaning (de la Rosa Reyna et al., 2010; Ge, Davis, Hines, Irvin & Simmen, 2001). In chickens and pigs some polymorphisms in the coding region are associated with growth traits (Bhattacharya et al., 2015; Niu et al., 2013). In cashmere goats, some SNPs are linked to the wool growth (Wu-Jun et al., 2010). Finally, in sheep, variations of the *IGF-I* gene influence the litter size and the persistence of lactation (He et al., 2012; Scatà et al., 2010). So far, no research has been conducted on the sheep to evaluate association of IGF-1 gene polymorphisms with reproductive performances, with the exception of litter size. The aim of this research was to detect possible polymorphisms within the *IGF-I* gene and to verify their influence on reproductive

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performances and milk yield in Sarda breed sheep.

2. Materials and methods

2.1. Animals management and experimental design

Domestic sheep is the most farmed species in the world, thanks to its adaptability to the difficult climatic and territorial conditions that characterize many less favored areas (poor vegetation, land not suitable for cultivation activity, reduced rainfall). In Italy, sheep have spread more widely in the southern and insular regions because they are able to maximize production even in adverse climatic conditions. Sarda is the most farmed and the best Italian breed of dairy sheep, and Sardinia (with more than 3 million of farmed ewes) has the highest density of sheep in Italy. This breed produces the highest amount of sheep milk in Italy and a considerable quantity of sheep milk at world level. Thus the improvement of their reproductive and productive performances is an important goal for the development of this breed. For the present study, 400 lactating Sarda ewes were selected from two farms located in North Sardinia. Each farm had approximately 800 adult Sarda ewes that were kept under the same management conditions, with grazing on pasture mixture of leguminous and gramineous grasses during the day. Moreover, they received 300 g per head/daily of concentrate commercial food (crude protein 20.4% and 12.5 MJ ME/kg DM) during the milking time. During the night sheep were penned and had free access to water and hay (crude protein 11.1% and 7.2 MJ ME/kg DM). The engaged ewes were all at their second lactation, in a good state of health and nutrition, and had been followed also for the two subsequent lactations. For each animal the reproductive performances and the milk yield of three consecutive lactations were recorded, from the second to the fourth. The ewes at their first lambing and lactation were excluded because they do not express the full reproductive and productive potential (Mura et al., 2019). According to the commonly practiced sheep management, males, generally separated from females, were introduced in the flocks, between May 20th and June 1st, to trigger the mating period. Males were removed after 60 days of cohabitation with females. The individual rumen bolus was recorded and each ewe, selected for this study, was individually marked with a numbered collar to avoid identification mistakes. In order to evaluate the reproductive performances over three years, the fertility rate (as percentage of lambed ewes), the interval in days from ram introduction to lambing (IRIL) and the litter size (as number of newborn lambs per ewes) were recorded. Milk yield produced during three years were registered for each animal by ASSONAPA (Associazione Nazionale della Pastorizia).

2.2. Blood sampling and DNA analysis

An individual blood sample (10 ml) was collected, before the males introduction in the first years of the trial, from the jugular vein of the engaged ewes using sterile vacuum tubes with EDTA (ethylenediamine tetraacetic acid) as an anticoagulant (BD Vacutainer Systems, Belliver Industrial Estate, Plymouth, UK). The DNA was extracted from whole blood using a genomic DNA extraction kit (NucleoSpin® Blood, Macherey-Nagel, Germany). An amount of 150 ng genomic DNA was subjected to polymerase chain reaction (PCR) using specific primers (Thermo Fisher Scientific Massachusetts, USA) according to He et al. (2012) and Scatà et al. (2010) for 5′ UTR and exon 3, respectively. According to the Ovis aries *IGF-I* gene sequence (GenBank accession number X69472.1), the primers were designed to amplify the 5′ UTR sequence: sense primer 5′ – TGA GGG GAG CCA ATT ACA AAG C – 3′; antisense primer: 5′ – CCG GGC ATG AAG ACA CAC ACA T – 3′. Likewise, as regards of Exon 3, the primers were designed using the sequence X69473.1: sense primer: 5′ – CAC ACA CCT TGT TGC ACT CC – 3′; antisense primer: 5′ – AGA GCA TCC ACC AAC TCA GC – 3′. PCR analysis for 5′UTR was carried out in 25 μl total volume containing 2.5 μL of 10X PCR Buffer [200 mM Tris HCl (pH 8.4), 500 mM KCl]; 0.75 μl of 50 Mm $MgCl_2$; 4 μl of 1.25 mM dNTPs; 1 μl of 10 μM of both Primers (Forward and Reverse), 0.1 μl of Platinum Taq DNA Polymerase (Thermo Fisher Scientific, Massachusetts, USA). The PCR analysis for exon 3 was carried out in 25 μl total volume containing 2.5 μL of 10X PCR Reaction Buffer B (Mg2+ free) [0.8 M Tris HCl (pH 8.4), 0.2 M $(NH_4)_2$ SO4, 0.2% w/v Tween-20]; 2.5 μl of 25 Mm $MgCl_2$, 4 μl of 0.2 mM dNTPs; 1 μl of 0.1 mM of each forward and reverse primers, and 2 U of FIREPol DNA Polymerase (5 U/μl) (Solis BioDyne, Tartu, Estonia). The PCR programs (performed on a Mastercycle ep gradient S, Eppendorf AG, Hamburg, Germany) consist in the following steps: heating lid at 105 °C, Taq activation at 94 °C for 2 min, followed by 30 cycles comprising denaturation at 94 °C for 30 s, annealing at 61 °C for 30 s for 5′ UTR and 30 s at 59 °C for exon 3, extension 72 °C for 30 s, and a final extension at 72 °C for 10 min. PCR products were separated by electrophoresis on a 1.5% (w/v) agarose gel (GellyPhor, Euroclone, Milan, Italy) in parallel with a 100-bp DNA ladder (Thermo Fisher Scientific, Massachusetts, USA). Following electrophoresis (at a constant voltage of 100 V for 30 min) gel was stained with ethidium bromide for 20 min and viewed on a UV Trans-illuminator (UVItec, Cambridge, UK).

2.3. Sequencing

All the 400 PCR products were sequenced in the forward and reverse directions, using a commercial service. Sequencing was carried out using Applied Biosystems 3730 DNA Analyzer (Perkin-Elmer Applied Biosystems, Foster City, CA, USA), with Dye Terminator 3.1 chemistry. Nucleotide sequence alignments, translations and comparisons were carried out using the Bioedit software (http://www.mbio.ncsu.edu/bioedit/bioedit.html).

2.4. Statistical analysis

Allelic frequencies were determined by direct counting of the observed genotypes. R statistical software (Version 3.6.1 R Core Team 2019 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/) was used to analyze the associations among genotype and milk yield (individual daily milk production, g/die) or reproductive traits, measured as fertility rate, litter size and interval in days from ram introduction to lambing (IRIL). The following linear model was used:

$Y_{jk} = \mu + G_j + e_{jk}$ Where Y_{jk} is the trait measured for each animal, μ is the overall mean, G_j is the fixed effect of the genotype and e_{jk} is the random residual effect of each observation. The farm effect did not show statistical significance and thus, were not included in the model. When P was <0.05 it was considered statistically significant. In this case, multiple comparisons of the means were performed using Tukey's method (library Agricolae, R package version 1.3-1. Felipe de Mendiburu (2019): Statistical Procedures for Agricultural Research. https://CRAN.R-project.org/package=agricolae) (de Medinburu, 2019).

3. Results

The PCR analysis allowed to detect, in all the 400 tested samples, a single band of 294 bp in length for *IGF-I* gene 5′ UTR, and a single band of 264 bp in length for *IGF-I* gene Exon 3 amplification. Using the Genomic Context section of NCBI (National Center for Biotechnology Information) we found that the obtained fragments, in reverse complement, correspond to nucleotides g184028587–g184028294 (5′UTR) and g184023450–g184023187 (Exon 3) of the latest ovine genome version, within the chromosome 3 (Oar_rambouillet_v1.0, GenBank accession number NC_040254.1). The sequencing analysis revealed three polymorphic sites, ordered according to the above said genome version, at positions g184028491C>G and g184028489C>T within

Table 1.
Allele and genotype frequencies at the SNPs identified in the *IGF-I* gene in the Sarda breed sheep ($n = 400$).

SNP position	SNP location	Allele	Allele frequency (%)	Genotype	Genotype frequency (%)
g184028491	5′ UTR	C	90	CC	85
		G	10	CG	11
				GG	4
g184028489	5′ UTR	C	90	CC	85
		T	10	CT	11
				TT	4
g184023223	Exon 3	G	54	GG	45
		A	46	GA	18
				AA	37

Table 2.
Fertility rate (%), litter size (%.) and distance in days from ram introduction to lambing (IRIL) in the three considered years regardless of genotypes in Sarda sheep ($n = 400$).

	Fertility rate	Litter size	IRIL
Second lambing	81.4	1.18	190.12 ± 20.60
Third lambing	83.7	1.20	193.17 ± 20.53
Fourth lambing	84.4	1.18	192.60 ± 20.48

the 5′UTR, and at position g184023223G > A at the Exon 3 region. The polymorphisms at position g184028491C > G and g184028489C > T were always associated each other. Therefore, 3 genotypes for each SNP were detected in the tested ewes but considering that nucleotide variations at positions g184028491C > G and g184028489C > T were always associated in this study, they can be considered as a unique genotype and referred from now on as position g184028491C > G. Genotype distribution within the studied population was not in Hardy–Weinberg equilibrium for both SNPs (GG, $n = 340$; GC, $n = 44$; CC, $n = 16$, at position g184028491; GG, $n = 180$; GA, $n = 72$; AA $n = 148$, at position g184023223). Accordingly, allele frequency was 90% for G and 10% for C allele, for g184028491C > G, and 54% for G and 46% for A allele for g184023223G > A (Table 1). The fertility rate, the litter size and the interval in days from male introduction to lambing in all 400 animals, regardless of genotypes, in the three recorded years is shown in Table 2. These three reproductive parameters showed no significant differences among years. The SNP at position g184028491C > G showed a significant association with reproductive traits (Table 3). Mainly, ewes carrying the C allele showed a higher fertility rate ($P < 0.05$) and a shorter interval in days from ram introduction to lambing ($P < 0.01$) in all the three recorded years. Also, the association study between the SNP at position g184028491C > G and milk production levels showed a significant effect of the C allele. Indeed, ewes carrying CC genotype produced higher daily milk yield in all their recorded lactations, with a significant P value ($P < 0.05$) in their 2nd and 3rd lactation, and an even greater significance in their 4th lactation ($P < 0.01$) compared to those carrying CG or GG genotype (Table 4). Even the g184023223G > A SNP within the Exon 3 of the *IGF-I* gene showed a significant effect of AA genotype on the recorded milk yield. Precisely, ewes carrying AA genotype showed higher milk yield with $P < 0.05$, in the second and third lactation, and with $P < 0.01$ in fourth lactation compared to the other genotypes (Table 5).

Table 4
Means ± standard deviation (SD) of the individual daily milk production (g/die) in Sarda sheep, based on the different genotypes at position g184028491 of the 5′UTR of the *IGF-I* gene ($n = 400$).

	CC	CG	GG
Second lactation	1580 ± 33^{b}	1370 ± 21^{b}	1160 ± 16^{a}
Third lactation	1800 ± 40^{b}	1540 ± 34^{a}	1500 ± 19^{a}
Fourth lactation	1990 ± 52^{B}	1640 ± 30^{A}	1580 ± 5^{A}

Different lowercase letters within the row differ significantly for $P < 0.05$; Different capital letters within the row differ significantly for $P < 0.01$.

Table 5
Means ± standard deviation (SD) of the individual daily milk production (g/die) in Sarda sheep, based on the different genotypes at position g184023223 Exon 3 of *IGFI* gene.

	GG	GA	AA
Second lactation	1340 ± 225^{a}	1330 ± 250^{a}	1430 ± 245^{b}
Third lactation	1565 ± 300^{a}	1585 ± 285^{a}	1692 ± 350^{b}
Fourth lactation	1650 ± 305^{A}	1675 ± 295^{A}	1885 ± 340^{B}

Different lowercase letters within the row differ significantly for $P < 0.05$; Different capital letters within the row differ significantly for $P < 0.01$.

4. Discussion

In the present study three single nucleotide variations were found in Sarda sheep breed, two located at position g184028491C > G and g184028489C > T in the 5′ UTR and one located in Exon 3 at position g184023223G > A of the *IGF-I* gene. The SNPs found at the 5′UTR sequence resulted always associated each other and a clear prevalence of the CC genotype at both positions emerged. The GG genotype is the most represented in the SNP at Exon 3. The allelic and genotypic frequencies for the two SNPs found in the 5′ UTR agree with what found by Scatà et al. (2010) in the Sarda breed and in two other Italian breeds. Instead, He et al. (2012) in Chinese and European breeds found different genotypic and allelic frequency for the two polymorphisms at the 5′ UTR region. In Texel and Dorset breed indeed a similar frequency than in this study was reported, while Chinese breeds have shown a lower G frequency, i.e., in agreement with observation reported by Darwish, El-Shorbagy, Abou-Eisha, El-Din and Farag (2017) and by Bakhtiar et al. (2017) in Egyptian and Iranian sheep, respectively. Also, about Exon 3, the allelic and genotypic frequencies found in the present

Table 3.
Fertility rate, litter size, and interval in days from ram introduction to lambing (IRIL) according to genotypes at position g184028491 of the *IGFI* gene in Sarda sheep ($n = 400$).

		CC	CG	GG
Second lambing	Fertility rate	86^{b}	83^{b}	75^{a}
	Litter size	1.21	1.19	1.13
	IRIL	186.20 ± 20.25^{A}	186.19 ± 20.12^{A}	198.97 ± 21.41^{B}
Third lambing	Fertility rate	88^{b}	85^{b}	78^{a}
	Litter size	1.27	1.20	1.14
	IRIL	188.37 ± 20.08^{A}	189.55 ± 20.32^{A}	201.59 ± 21.18^{B}
Fourth lambing	Fertility rate	87^{b}	86^{b}	77^{a}
	Litter size	1.24	1.18	1.13
	IRIL	187.43 ± 20.04^{A}	186.39 ± 20.22^{A}	203.41 ± 21.19^{B}

Different lowercase letters within the row differ significantly for $P < 0.05$; Different capital letters within the row differ significantly for $P < 0.01$.

study agree with what found by Scatà et al. (2010) in Italian breeds. Instead, Bakhtiar et al. (2017) in Iranian sheep found an allelic and genotypic frequency different from Italian sheep. These differences among breeds are certainly linked to the genetic selection operated over time.

In the current study the 5′ UTR SNPs are associated with the Sarda sheep reproductive performance. In fact, animals carrying the C allele, either in homozygous or in heterozygous state, exhibited a greater fertility and a shorter interval in days from male introduction to lambing in all the studied three years. These results agree with what found in Chinese sheep and Chinese and Indian goats, where, precisely, these two polymorphisms improve reproductive performances (He et al., 2012; Thomas, Venkatachalapathy, Aravindakshan & Raghavan, 2016; Wang et al., 2011). Nucleotide variations at the 5′ UTR of the *IGF-I* gene were also found in cattle and had been correlated with reproductive traits (Mullen et al., 2011; Nicolini, Carriquiry & Meikle, 2013). Also, in other animals' species a positive effect of some polymorphisms of the *IGF-I* gene on reproductive activity has been found and many times this effect has been linked to the type of animal management (Hax et al., 2017). The important role of IGF-I hormone has been recognized in several reproductive stages, in fact, the higher levels of this hormone in pregnancy are necessary for fetal development and in postpartum favor the recovery of ovarian function (Kawashima et al., 2006; Satue, Marcilla, Medica, Cravana & Fazio, 2018). In addition, in bovine granulose cell, FSH acts in synergy with IGF-I to increase cell number and aromatase expression (Silva, Figueiredo & van den Hurk, 2009). Moreover, the IGF-I enhances the stimulatory effects of FSH on progesterone production, aromatase/estradiol, luteinizing hormone receptor and inhibin-a (Adashi, Esnick, Svoboda & Van Wyk, 1985). Indeed, studies demonstrated that ovaries lacking IGF-I receptor, had higher levels of apoptosis in follicles from the primary to the large secondary stages (Cox, Navarrete, Carrasco, Dorado & Saravia, 2019). Therefore, it is plausible to think that these polymorphisms can in some way influence the mechanism of action of IGF-I thus determining an improvement in reproductive activity. In this regard it would be crucial to highlight the precise role of the *IGF-I* gene polymorphisms on the follicular development.

The CC genotypes at the 5′ UTR and the AA genotypes at Exon 3 showed to influence the daily milk yield in the three recorded years. These results agree with what found by Scatà et al. (2010) both in the Sarda and in other Italian breeds. Indeed, Authors found that the SNP at Exon 3 caused a greater persistence of milk production during lactation period. Furthermore, several authors have found positive effects on different productive traits in different animal species thus confirming the important role played by this gene in several physiological mechanisms (Abdolmohammadi & Zamani, 2014; Ge et al., 2001; Gobikrushanth et al., 2018; Niu et al., 2013; Sharma et al., 2013).

Regulatory regions are sections in the genome that do not code for proteins but exert a control on the expression of other district, which have coding function. Despite the lack of coding function, the regulatory regions play a crucial role in govern gene activity. Most of them exhibit binding sites for the transcription factors (TFs), proteins able to start and regulate DNA transcription. Therefore, given their crucial role, it is not surprising the great effect that a SNP that occur in the regulatory region can have. The nucleotide substitutions g184028491C > G and g184028489C < T detected in the 5′ UTR in the present research fall within the following sequence: TGACGG**C**G**C**TGGGATGACCCCTCGT. This sequence is recognized to be the core consensus binding sequence of the retinoid X receptor heterodimers (RXR) (Scatà et al., 2010). A consensus binding sequence is a short sequence of nucleotides that are shared or similar among multiple distinct DNA sites and is thought to play the same role in its different locations. In the specific case of our research, the consensus binding sequence of the retinoid X receptor heterodimers (RXR), above mentioned, is a member of the superfamily of nuclear factors that includes the vitamin D receptor (VDR). RXR and VDR form a heterodimeric complex and bind to the vitamin D responsive elements (VDREs) to activate or repress a great number of genes, involved in a variety of physiological functions (Lemon, Fondell & Freedman, 1997). This means that, the *IGF-I* gene could have different functional activity in the different genotypes, as hypothesized also by Scatà et al. (2010). In the present study, a significant effect of the CC genotype was found on milk yield in Sarda sheep.

As stated above, the found allele distribution at positions g184028491 and g184028489 and the almost total lack of animals carrying minor homozygous genotypes could be the results of the intense genetic selection aimed to increase milk production in this breed. Despite further investigations on a higher study population would help to confirm the importance of the *IGF-I* gene on reproductive efficiency and milk yield in a high production dairy sheep breed. However these results provide new insights into its crucial role in dairy performance. In this research a genotype/phenotype association was found that, carefully detailed and studied, could become an important reference to be used in sheep breeding programs.

5. Conclusions

Considering the *IGF-I* as a potential candidate gene for reproductive and milk yield, its polymorphisms were investigated in dairy sheep. In Sarda breed, three nucleotide variations in the 5′ UTR region and Exon 3 of the *IGF-I* gene, were found. The three SNP investigated have shown to be involved in regulating the reproductive activity and milk production in Sarda sheep breed. So, these SNPs can be useful in planning selection programs that can improve the reproductive and productive performances in sheep. However, it will be interesting to conduct further research on a higher number of animals to confirm the effect of the found SNPs and to highlight other SNPs in the sequence of this gene that can also be associated with productive and/or productive parameters in sheep.

Ethical statement

All the animals in this research had veterinary care by the National Health Veterinary Service in accordance with the Animal Welfare Act. Blood samples were collected by veterinarians of the National Health Service for routine health assessments. The farmers involved in the research were informed and provided consent to use the animals in the present study. The approval of the ethics committee was not considered necessary because we did not handle the animals, the biological material was provided by National Health Veterinary Service.

CRediT authorship contribution statement

Luridiana Sebastiano: Data curation, Investigation, Methodology, Software, Validation, Writing - original draft, Writing - review & editing. **Mura Maria Consuelo:** Formal analysis, Methodology, Supervision, Validation, Writing - original draft, Writing - review & editing. **Di Stefano Maria Veronica:** Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Pulinas Luisa:** Formal analysis, Writing - review & editing. **Cosso Giovanni:** Formal analysis, Writing - review & editing. **Nehme Michella:** Formal analysis, Writing - review & editing. **Carcangiu Vincenzo:** Conceptualization, Funding acquisition, Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

None of the authors have any conflicts of interest to declare.

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References

Abdolmohammadi, A., & Zamani, P. (2014). SNP exploring in the middle and terminal regions of the IGF-1 gene and association with production and reproduction traits in Holstein cattle. *Gene, 540*, 92–95. https://doi.org/10.1016/j.gene.2014.02.011.

Adashi, E. Y., Esnick, C. E., Svoboda, M. E., & Van Wyk, J. J. (1985). Somatomedin-C enhances induction of luteinizing hormone receptors by follicle-stimulating hormone in cultured rat granulosa cells. *Endocrinolog, 116*, 2369–2375. https://doi.org/10.1210/endo-116-6-2369.

Bakhtiar, R., Abdolmohammadi, A., Hajarian, H., Nikousefat, Z., & Kalantar-Neyestanaki, D (2017). Investigation of the 5′ flanking region and exon 3 polymorphisms of IGF-1 gene showed moderate association with semen quality in Sanjabi breed rams. *Theriogenology, 104*, 186–191. https://doi.org/10.1016/j.theriogenology.2017.08.023.

Bhattacharya, T. K., Chatterjee, R. N., Dushyanth, K., Paswan, C., Shukla, R., & Shanmugam, M. (2015). Polymorphism and expression of insulin-like growth factor 1 (IGF1) gene and its association with growth traits in chicken. *British Poultry Science, 56*, 398–407. https://doi.org/10.1080/00071668.2015.1041098.

Carcangiu, V., Mura, M. C., Daga, C., Luridiana, S., Bodano, S., Sanna, G. A., et al. (2013). Association between SREBP-1 gene expression in mammary gland and milk fat yield in Sarda breed sheep. *Meta Gene, 1*, 43–49. https://doi.org/10.1016/j.mgene.2013.10.001.

Cox, J. F., Navarrete, F., Carrasco, A., Dorado, J., & Saravia, F. (2019). Effect of bST administration on plasma concentrations of IGF-I and follicular dynamics and ovulation during the interovulatory cycle of sheep and goats. *Theriogenolog, 123*, 159–166. https://doi.org/10.1016/j.theriogenology.2018.10.003.

Darwish, H. R., El-Shorbagy, H. M., Abou-Eisha, A. M., El-Din, A. E., & Farag, I. M. (2017). New polymorphism in the 5′ flanking region of IGF-1 gene and its association with wool traits in Egyptian Barki sheep. *Journal of Genetic Engineering and Biotechnology, 15*, 437–441. https://doi.org/10.1016/j.jgeb.2017.08.001.

de la Rosa Reyna, X. F., Montoya, H. M., Castrellón, V. V., Rincón, A. M. S., Bracamonte, M. P., & Vera, W. A (2010). Polymorphisms in the IGF1 gene and their effect on growth traits in mexican beef cattle. *Genetics and Molecular Research, 9*, 875–883. https://doi.org/10.4238/vol9-2 gmr745.

de Medinburu, F. (2019). R package version 1.3-1. *Statistical Procedures for Agricultural Research,* 1–86. https://CRAN.R-project.org/package = agricolae.

Ge, W., Davis, M. E., Hines, H. C., Irvin, K. M., & Simmen, R. C. M. (2001). Association of a genetic marker with blood serum insulin-like growth factor-I concentration and growth traits in Angus cattle. *Journal of Animal Science, 79*, 1757–1762. https://doi.org/10.2527/2001.7971757x.

Gobikrushanth, M., Purfield, D. C., Colazo, M. G., Wang, Z., Butler, S. T., & Ambrose, D. J (2018). The relationship between serum insulin-like growth factor-1 (IGF-1) concentration and reproductive performance, and genome-wide associations for serum IGF-1 in Holstein cows. *Journal of Dairy Science, 101*, 9154–9167. https://doi.org/10.3168/jds.2018-14535.

Hax, L. T., Schneider, A., Jacometo, C. B., Mattei, P., da Silva, T. C., Farina, G., et al. (2017). Association between polymorphisms in somatotropic axis genes and fertility of Holstein dairy cows. *Theriogenolog, 88*, 67–72. https://doi.org/10.1016/j.theriogenology.2016.03.044.

He, J. N., Zhang, B. Y., Chu, M. X., Wang, P. Q., Feng, T., Cao, G. L., et al. (2012). Polymorphism of insulin-like growth factor 1 gene and its association with litter size in Small Tail Han sheep. *Molecular Biology Reports, 39*, 9801–9807. https://doi.org/10.1007/s11033-012-1846-y.

Jiang, H., & Lucy, M. C. (2001). Variants of the 5′-untranslated region of the bovine growth hormone receptor mRNA: Isolation, expression and effects on translational efficiency. *Gene, 265*, 45–53. https://doi.org/10.1016/S0378-1119(01)00356-0.

Kawashima, C., Kaneko, E., Amaya Montoya, C., Matsui, M., Yamagishi, N., Matsunaga, N., et al. (2006). Relationship between the first ovulation within three weeks postpartum and subsequent ovarian cycles and fertility in high producing dairy cows. *Journal of Reproduction and Development, 52*, 479–486. https://doi.org/10.1262/jrd.18003.

Lammers, B. P., Heinrichs, A. J., & Kensinger, R. S. (1999). The effects of accelerated growth rates and estrogen implants in prepubertal Holstein heifers on growth, feed efficiency, and blood parameters. *Journal of Dairy Science, 82*, 1746–1752. https://doi.org/10.3168/jds.S0022-0302(99)75405-6.

Lemon, B. D., Fondell, J. D., & Freedman, L. P (1997). Retinoid X receptor:Vitamin D3 receptor heterodimers promote stable preinitiation complex formation and direct 1,25-dihydroxyvitamin D3- dependent cell-free transcription. *Molecular and Cellular Biology, 17*, 1923–1937. https://doi.org/10.1128/MCB.17.4.1923.

Magalhães-Padilhaa, D. M., Duartea, A. B. G., Araújoa, V. R., Saraivaa, M. V. A., Almeidaa, A. P., Rodriguesa, G. Q., et al. (2012). Steady-state level of insulin-like growth factor-I (IGF-I) receptor mRNA and the effect of IGF-I on the in vitro culture of caprine preantral follicles. *Theriogenolog, 77*, 206–213. https://doi.org/10.1016/j.theriogenology.2011.07.036.

Monte, A. P. O., Barros, V. R. P., Santos, J. M., Menezes, V. G., Cavalcante, A. Y. P., Gouveia, B. B., et al. (2019). Immunohistochemical localization of insulin-like growth factor-1 (IGF-1) in the sheep ovary and the synergistic effect of IGF-1 and FSH on follicular development in vitro and LH receptor immunostaining. *Theriogenology, 129*, 61–69. https://doi.org/10.1016/j.theriogenology.2019.02.005.

Mullen, M. P., Lynch, C. O., Waters, S. M., Howard, D. J., O'Boyle, P., Kenny, D. A., et al. (2011). Single nucleotide polymorphisms in the growth hormone and insulin-like growth factor-1 genes are associated with milk production, body condition score and fertility traits in dairy cows. *Genetics and Molecular Research, 10*, 1819–1830. https://doi.org/10.4238/vol10-3 gmr1173.

Mura, M. C., Luridiana, S., Pulinas, L., Bizzarri, D., Cosso, G., & Carcangiu, V (2019). Melatonin treatment and male replacement every week on the reproductive performance in sarda sheep breed. *Theriogenology, 135*, 80–84. https://doi.org/10.1016/j.theriogenology.2019.06.008.

Nicolini, P., Carriquiry, M., & Meikle, A. (2013). A polymorphism in the insulin-like growth factor 1 gene is associated with postpartum resumption of ovarian cyclicity in Holstein-Friesian cows under grazing conditions. *Acta Veterinaria Scandinavica, 55*, 11. https://doi.org/10.1186/1751-0147-55-11.

Niu, P., Kim, S.-. W., Choi, B.-. H., Kim, T.-. H., Kim, J.-. J., & Kim, K.-. S. (2013). Porcine insulin-like growth factor 1 (IGF1) gene polymorphisms are associated with body size variation. *Genes and Genomic, 35*, 523–528. https://doi.org/10.1007/s13258-013-0098-0.

Renaville, R., Hammadi, M., & Portetelle, D. (2002). Role of the somatotropic axis in the mammalian metabolism. *Domestic Animal Endocrinology, 23*, 351–360. https://doi.org/10.1016/S0739-7240(02)00170-4.

Satue, K., Marcilla, M., Medica, P., Cravana, C., & Fazio, E. (2018). Temporal relationships of GH, IGF-I and fructosamine concentrations in pregnant Spanish Purebred mares: A substantial contribution from the hormonal standpoint. *Theriogenology, 118*, 164–171. https://doi.org/10.1016/j.theriogenology.2018.05.040.

Scatà, M. C., Catillo, G., Annicchiarico, G., De Matteis, G., Napolitano, F., Signorelli, F., et al. (2010). Investigation on lactation persistency and IGF-I gene polymorphisms in dairy sheep. *Small Ruminant Research, 89*, 7–11. https://doi.org/10.1016/j.smallrumres.2009.10.014.

Sharma, A., Dutt, G., Sivalingam, J., Singh, M. K., Pathodiya, O. P., Khadda, B. S., et al. (2013). Novel SNPs in IGF1, GHR and IGFBP-3 genes reveal significant association with growth traits in Indian goat breeds. *Small Ruminant Research, 115*, 7–14. https://doi.org/10.1016/j.smallrumres.2013.08.001.

Silva, J. R. V., Figueiredo, J. R., & van den Hurk, R (2009). Involvement of growth hormone (GH) and insulin-like growth factor (IGF) system in ovarian folliculogenesis. *Theriogenology, 71*, 1193–1208. https://doi.org/10.1016/j.theriogenology.2008.12.015.

Tabecka-Lonczynska, A., Mytych, J., Solek, P., Kowalewski, M. P., & Koziorowski, M. (2019). Seasonal expression of insulin-like growth factor 1 (IGF-1), its receptor IGF-1R and klotho in testis and epididymis of the eurOpean bison (Bison bonasus, Linnaeus 1758). *Theriogenology, 126*, 199–205. https://doi.org/10.1016/j.theriogenology.2018.12.016.

Thomas, N., Venkatachalapathy, T., Aravindakshan, T., & Raghavan, K. C. (2016). Molecular cloning, SNP detection and association analysis of 5' flanking region of the goat IGF1 gene with prolificacy. *Animal Reproduction Science, 167*, 8–15. https://doi.org/10.1016/j.anireprosci.2016.01.016.

Wang, P.-. Q., Tan, Y., Zhang, B.-. Y., Chu, M.-. X., Deng, L-M., Fan, Q., et al. (2011). DNA polymorphisms of 5'-flanking region of insulin-like growth factor 1 gene and their association with reproduction traits in goats. *Agricultural Sciences in China, 10*, 1609–1617. https://doi.org/10.1016/S1671-2927(11)60158-2.

Liu, W. J., Fang, G. X., Fang, Y., Tain, K. C., Huang, X. X, Yao, X. K., et al. (2010). The polymorphism of a mutation of IGF-1 gene on two goat breeds in China. *Journal of Animal and Veterinary Advances, 9*, 790–794. https://doi.org/10.3923/javaa.2010.790.794.