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Original Article

Role of Fennel (*Foeniculum vulgare*) Seed Powder in Increasing Testosterone and *IGF1* Gene Expression in the Testis of Lamb

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Abstract

Background and objectives: The insulin/insulin-like growth factor (IGF) system has many receptors, signaling pathways, and ligands, making it highly complex. *IGF1* increases the function of germ cells by increasing the synthesis of spermatogonia DNA and slowing down apoptotic cells. One of the most important objectives of this study was to investigate the function of fennel on the expression of the *IGF1* gene in Kermani sheep testes.

Methods: The study involved three levels of fennel in the diets (20, 10, and zero g/kg dry matter), and tissue sampling was performed using testis tissue. Samples were rapidly placed in liquid nitrogen before being stored at -80°C. Then, total RNA was extracted and for the *IGF1* gene (target) and *GAPDH* gene (reference), the real-time polymerase chain reaction was applied.

Results: The outcomes displayed that increasing levels of fennel in the ration significantly ($p < 0.05$) increases the weight of the testis (0.35 kg and 0.36 kg at the levels of 1% and 2% fennel, respectively) compared to rations without fennel (0.29 kg at the levels of 0%). Association between testosterone and fennel feeding in studied lambs showed that adding fennel to the diet significantly ($p < 0.05$) increasesthe concentration of blood testosterone (3.5 ng/dL and 4.4 ng/dL at the levels of 1% and 2% fennel, respectively) compared with rations without fennel (1.7 ng/dL). The results show that adding fennel to the diet significantly ($p < 0.05$) increases the amount of *IGF1* gene expression in the testis (2.5 and 2.7 at the levels of 1% and 2% fennel, respectively) compared with rations without fennel (1 at the levels of 0% fennel).

Conclusions: Fennel has an affirmative effect on gene expression in the testis and can be added to sheep food rations to progress reproductive functions (by stimulating Leydig cell steroidogenesis, producing more sperm and testosterone, and growing and developing the testis).

Keywords: Fennel; *IGF1*; Gene expression; Testis; Testosterone.

Abbreviations: cDNA, copy DNA; DM, dry matter; *IGF*, insulin-like growth factor; PCR, polymerase chain reaction; RFU, relative fluorescence units; ROS, reactive oxygen species; ROX, carboxyrhodamine.

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Introduction

Evolutionary studies have shown that the processes of reproduction, growth, and metabolism are very closely related. In other words, organisms must first reach full maturity to reach the desired level of metabolism to be fertile.[1] Due to the direct connection of usual signaling and regulatory pathways and networks, growth, reproduction, and metabolism are linked.[2] The family of insulin-like growth factors (IGFs), or the insulin/IGF system, affects all organs in the body and regulates growth, reproduction, differentiation, proliferation, and cellular metabolism. The components of this system, by participating in various biochemical processes, connect

the three pathways of metabolic, reproductive, and mitogenic processes.[3] Various factors such as ligand biosynthesis, growth stage, nutrition, and hormonal interactions affect the activity of insulin, *IGF1*, and *IGF2*, and these factors must be coordinated with each other.[1] One of the main and influential factors in testicular growth and proper functioning is the insulin/IGF system.[4]

Even though, the hypothalamic-pituitary-gonadal axis regulates a person's ability to reproduce.[5] When the *IGF1* gene is inactivated in young males, they could become dwarf and infertile, and their serum testosterone levels and sperm production could drop by more than 80%.[6] Postnatal growth, maintaining reproductive function, overall body growth, and body size regulation are among the roles of *IGF1*.[4] The production of *IGF1* in any tissue (in addition to its production in the liver) is very important, as it represents a main task in the growth process of that tissue.[7] Compared to insulin or *IGF2*, the role of *IGF1* in postnatal development is greater and more important.[8] Various studies have shown that the natural activity of *IGF1* and its receptors is necessary for organisms to grow and develop normally.[9–11] Studying the testes of immature mice revealed that *IGF1* was equally present in Sertoli cells and spermatogenic cells by the age of three weeks, but was no longer present in Sertoli cells. Investigating the testes of adult mice showed that *IGF1* was present only in spermatocytes and not in Leydig and Sertoli cells and spermatogonia,[12] while Colón *et al.* showed that the phosphorylated *IGF1* receptor was present in both Sertoli and Leydig cells.[13] Yoon and Roser demonstrated that *IGF1* and *IGF1* receptor expression levels varied from pre-puberty to adulthood.[14] Immunoreactivity in adult stallions was positive and strong for spermatogonia and Leydig cells, weak for spermatocytes, and zero for Sertoli cells.[15] *IGF1* was also shown to motivate Leydig cells for steroidogenesis.[16] These events also depend on the stage of growth and examination of the steroidogenic process of equine Leydig cells during puberty and subsequently in vitro revealed that *IGF1* alone had no effect on the potential of this process. However, in combination with luteinizing hormone, and in a dose-dependent manner, it increased testosterone synthesis in post-pubertal stallions.[14] *IGF1* increases the function of germ cells by increasing the synthesis of spermatogonia DNA and slowing down apoptotic cells.[17] Researchers have shown that in late embryonic and early neonatal life, mouse Sertoli cell proliferation is highly dependent on *IGF1* signaling.[18] *IGF1* signaling also acts as a stimulus for Leydig cell maturation.[19] Treatment of adult and immature mice with *IGF1* in vitro showed a dose-dependent rise in the proliferation of immature Leydig cells and a reduction in the number of Leydig apoptotic cells.[13]

Plants such as fodder plants, peas, and cereals contain phytoestrogens—plant compounds that have a structure similar to animal estrogens and occur in various segments of the seeds and plant.[20,21] Phytoestrogens have been shown to be beneficial and safe for animals: when added to animal feed, they are highly degradable, cannot be stored, and their shelf life in the body is very short.[22] The fennel plant, *Foeniculum vulgare* from the *Umbelliferae (Apiaceae)* family, contains important compounds such as anethole, camphene, pinene, fenchone, and phellandrene. The phytoestrogens in fennel have similar effects to synthetic 17-beta estradiol and can be used as an alternative to hypothalamic-pituitary-gonadal axis control.[23]

Considering the use of fennel as an antioxidant, antifungal, antibacterial, anti-tumor, anti-diabetic, anti-thrombotic, anti-cardiovascular hirsute, anti-inflammatory, antioxidant, liver dilator, bronchodilator, acaricide and insecticide, the importance of biomedical and pharmacological applications can be understood.[24,25] One of the estrogenic active agents of fennel is trans-anethole, which is the most abundant and important compound in fennel oil.[26] Some researchers have shown that fennel has been used in animal rations for a variety of aims, such as reducing the total number of bacteria, improving the oxidative quality of meat, increasing the volume of packed cells, increasing the speed of digestion and growth, increasing the weight and length of the small intestine, increasing hemoglobin and red blood cells, improving feed consumption and its conversion rate, and improving the health of the body.[27–31]

Sheep are one of the most important small ruminants in hot, dry, and desert areas.[32] Therefore, improving their genetic development using all available methods, both quantitatively and qualitatively, is a priority for livestock farmers and researchers in this field.[33] Improving reproductive processes and increasing yield are traditional ways of achieving higher herd yield.[34] Reports indicate that over 50 million sheep are bred in Iran and these sheep are composed of twenty-seven breeds and ecotypes.[35] One of these 27 breeds and ecotypes is the Kermani sheep; this fat-tailed dual-purpose meat-milk sheep with white wool and medium size has an important role in animal husbandry and supplying the needs of nomads and ranchers in this area of Iran.[36,37] Therefore, actualizing the needs of this breed through genetic and non-genetic improvement represents a significant task in the improvement of breeding this sheep and its conservation. In domestic species research, the identification of genes affecting important production traits is an essential area. Studies in the late 1980s revealed that molecular mechanisms including DNA replication, transcription, translation, and even how genes are regulated, are among the most important genetic processes.[38] Investigating the task of fennel on the expression of the *IGF1* gene in Kermani sheep testes was one of the most important objectives of this study.

Materials and methods

Animals

In this research, 30 male lambs of the Kermani breed weighing 27.5 ± 0.45 kg (eight months old) were used. Vaccination of all animals was performed using standard vaccines before the start of the experiment. The experiment was carried out in 1.2 × 1.5-meter pens with free access to food and water at the Animal Science Research and Training Station of Shahid Bahonar University of Kerman, Iran.

Ethical statement

All procedures related to animals were certified by the Animal Care and Use Committee of Bahonar University (IACUC Protocol #IR2018011) described by the Iranian Council of Animal Care. All guidelines and laws related to animal welfare policies for the animals used in this research were fully followed.

Feeding and data collection

The animals were fed twice a day with the same rations in terms of protein and energy (Table 1). The diets were grouped into 3 levels of fennel based on g/Kg dry matter (DM) (0, 10 or 1%, and 20 or 2% fennel g/Kg DM). The chemical formula of added fennel to rations was 91% dry matter, 15% crude protein, 87.03% organic matter, 9.76% ether extract, and 12.12 MJ/kg metabolizable energy. To analyze the diet and calculate its components, such as ether extract, nitrogen, ash, and DM, standard methods of AOAC were used.[39] The Van Soest method was applied to determine ash-free neutral detergent fiber (NDFom) and ash-free acid detergent fiber

Table 1. Ingredients of the diets and fennel used for the studied sheep

Ingredients of the diets	Amount of fennel seed powder (%)		
	0	1	2
Fennel seed powder (%)	0	1	2
Alfalfa hay (%)	30	30	30
Barley grain (%)	28	27	26
Wheat bran (%)	13	13	13
Wheat straw (%)	10	10	10
Corn grain (%)	9	9	9
Soybean meal (%)	8	8	8
Vitamins E, D, and A (%)	0.6	0.6	0.6
Trace-mineralized salt (%)	0.6	0.6	0.6
Sodium bicarbonate (%)	0.5	0.5	0.5
Limestone (%)	0.3	0.3	0.3

Ingredients of fennel	Amounts	Ingredients of fennel	Amounts
Metabolizable energy	12.12 MJ/kg	Ether extract	9.76%
Crude protein (%)	15	Dry matter	91
Organic matter (%)	87.03		

(ADFom).[40]

One day before slaughtering, at 3 hours after morning feeding, blood samples were collected. Centrifugation (10 min at 6000×rpm) was performed for collected samples and then for further analyses, blood serum samples were frozen at −20 °C. Calculation of testosterone concentration was done using an enzyme-linked immunosorbent assay kit (Stat Fax). It should be noted that a testosterone set (Patangostar-e-Eisar Co, Iran, Lot number: AC13476) was used with this kit. When the study period was over, the investigated animals were slaughtered for sampling and the weight of the testes was then recorded. Tissue sampling (90 samples containing 10 animals × 3 groups × 3 repeats for the tissue) was performed from testis tissue. Before storing the samples at −80 °C, they were quickly located in liquid nitrogen.

RNA expression analysis

Total RNA was extracted from the studied tissue (using the step-RNA Reagent kit made by Biobasic Company, Iran) and cDNA was then synthesized using commonly available kits (#K1631, Fermentase Company, Iran). The quality of RNA and cDNA was measured using electrophoresis on agarose gel. The characteristics of the primers used for amplification of two target and reference genes are given in Table 2. The information related to real-time polymerase chain reaction (PCR) is given in Table 3.

To ensure the amplification of the target fragments, the melting curves for the target gene and the reference gene at the end of the amplification were analyzed by PCR. In addition, the gradient protocol was used to determine the annealing temperature for the *IGF1*; target gene and the *GAPDH*; reference gene. For the evaluation of real-time PCR data, the Pfaffl method was applied.[41]

Statistical analysis

The data obtained from this research were analyzed in the mixed procedure with a completely randomized design using statistical analysis system software. The pairwise fixed reallocation randomization test was used to check the normality of the data distribution.[41] In addition, the least significant differences test was used to compare the means ($p < 0.05$).

Results

In this research, we studied the effects of different levels of fennel (0, 10, and 20 g/Kg DM) in diets on the weight of the testis of the Kermani sheep breed and the concentration of testosterone and expression of the *IGF1* gene in the testis of the Kermani sheep

Table 2. The characteristics of the primers used for amplification of *IGF1* target gene and *GAPDH* reference gene

Gene	Primer	Sequence	accession number in NCBI	Tm	product size
IGF1	forward	5′-ATTACAGCTGCCTGCCCCTT-3′	NM_001009774.3	57 °C	265 bp
	reverse	5′-CACATCTGCTTACACCTTACCCG-3′			
GAPDH	forward	5′-ACCACTTTGGCATCGTGGAG-3′	NM_001190390.1	57 °C	76 bp
	reverse	5′- GGCCATCCACAGTCTTCTG-3′			

IGF1, insulin-like growth factor 1.

Table 3. The information related to Real-Time PCR reactions for *IGF1* target gene and *GAPDH* reference gene

Final volume of each real-time PCR reaction	real-time PCR device	Contents of each real-time PCR reaction tube	Volume of each real-time PCR reaction tube	Program for real-time PCR reactions
15 µL	Rotor-Gene Q MDx device (QIAGEN Hilden, Germany)	template cDNA	1.5 µL	95 °C for 5 min, followed by a cycle of 95 °C 20 s, 57 °C 30 s and 72 °C 30 s for 38 cycles.
		2X SYBR Green PCR Master Mix (Fermentase Co., Tehran, Iran)	7.5 µL	
		ddH2O	4.7 µL	
		10 µM forward and reverse primers	1 µL	
		ROX	0.3 µL	

cDNA, copy DNA; *IGF1*, insulin-like growth factor 1; ROX, carboxyrhodamine; PCR, polymerase chain reaction.

breed. The currently obtained results demonstrate that including fennel in the ration significantly ($p < 0.05$) increases the weight of the testis (0.35 kg and 0.36 kg at the levels of 1% and 2% fennel, respectively) in comparison to diets without fennel (0.29 kg at the levels of 0%). Association between testosterone and fennel feeding in studied lambs showed that adding fennel to diets significantly ($p < 0.05$) increases the concentration of blood testosterone (3.5 ng/dL and 4.4 ng/dL at the levels of 1% and 2% fennel, respectively) in comparison to diets without fennel (1.7 ng/dL).

Evaluation of the quality of extracted RNA using electrophoresis on agarose gel showed that the total extracted RNA was healthy and complete and had 28S rRNA and 18S rRNA bands (Fig. 1). The observation of a single band on the agarose gel in the range of 265 bp for the *IGF1* gene in the testicular tissue and the presence of one band in the range of 76 bp for the *GAPDH* gene (Fig. 2) confirmed correct amplification of the fragments and the validity of the experiment. The information related to the IGF-I gene amplification curve in the testis is shown in Figure 3. According to the obtained amplification curve, treatments of 1% fennel (10 g/Kg DM) and 2% fennel (20 g/Kg DM) started to amplify in a lower

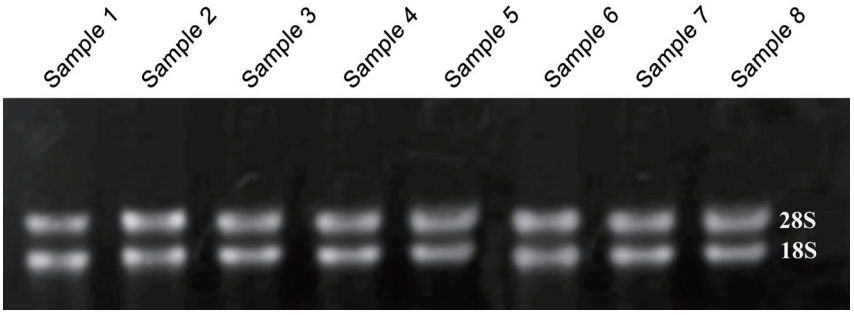

Fig. 1. Eight samples of total RNA extracted from testes of Kermani sheep on agarose gel.

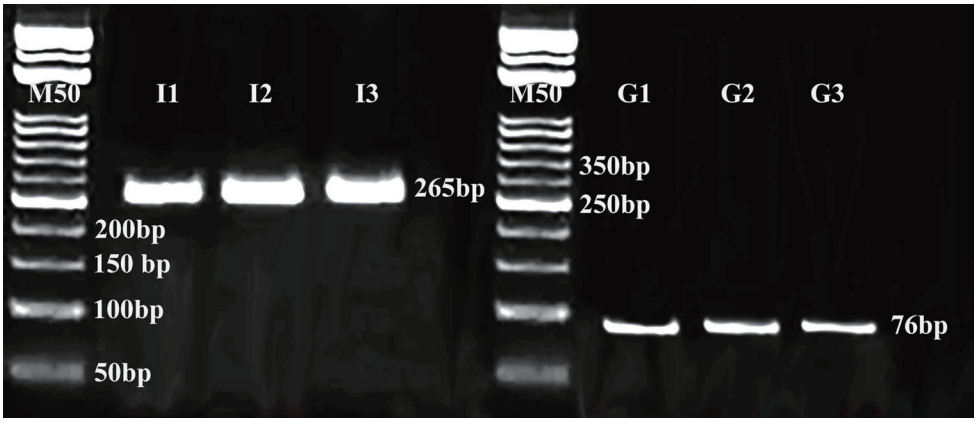

Fig. 2. Electrophoresis of amplified products for *IGF1* and *GAPDH* genes in the testes of Kermani lambs on agarose gel. Lanes I1-I3 belong to *IGF1* gene, Lanes G1-G3 belong to *GAPDH* gene and M50 is size marker. *IGF1*, insulin-like growth factor 1.

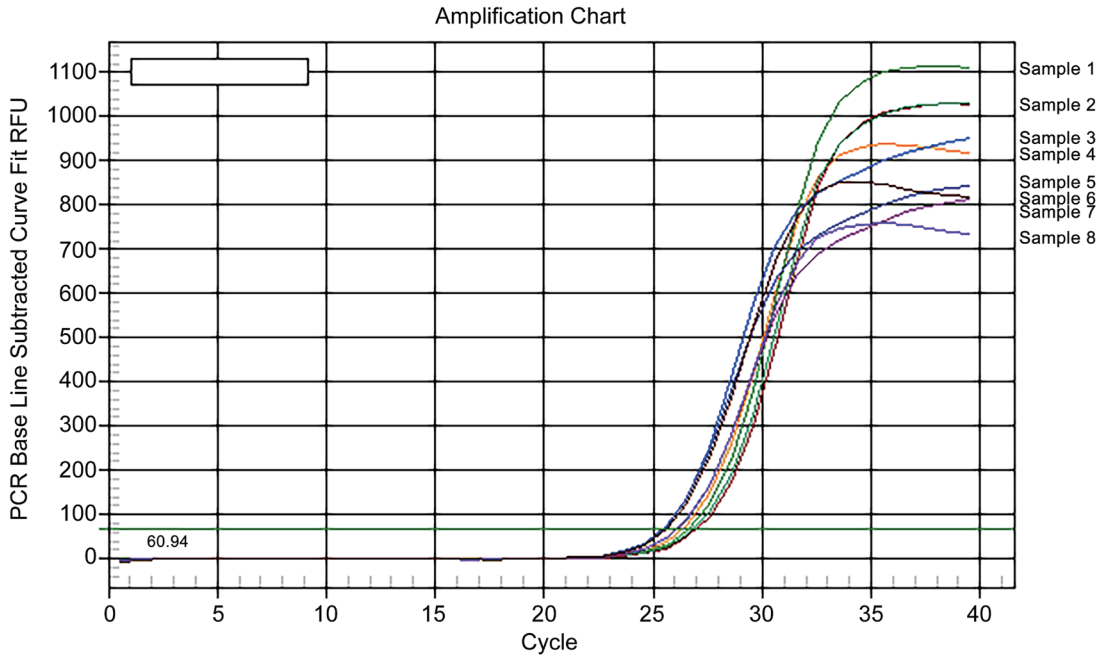

Fig. 3. PCR amplification curve of *IGF1* gene in testicular tissue of Kermani lambs. *IGF1*, insulin-like growth factor 1; PCR, polymerase chain reaction; RFU, relative fluorescence units.

cycle than the control (0 g/Kg DM fennel).

The results obtained from the melting curve of the *IGF1* gene showed that the curve produces only one peak at 86 °C. This result indicates the production of a specific product in this reaction.

The obtained results displayed that including fennel in the ration significantly ($p < 0.05$) increases the amount of *IGF1* gene expression (2.5 and 2.7 at the levels of 1% and 2% fennel, respectively) in comparison to diets without fennel (1 at the levels of 0% fennel) in the testis.

Discussion

The results of our investigation showed that adding fennel seed powder to diets significantly increases the weight of the testis in comparison to diets without fennel. In confirmation of this result, according to Sotoudeh and Yeganeh, a significant increase in fertility and number of eggs, gonad weight, gonad index, fertility, fertilization percentage, and hatching percentage as well as a decrease in average sexual maturity were observed in ornamental fish, Convict cichlid (Cichlasoma nigrofasciatum), fed with fennel essential oil.[31] El-Garawani *et al.* reported that the consumption of fennel oil in etoposide-defective rats caused a reduction of sperm defects and increased mature sperm production, which was due to the anti-oxidant properties of the fennel extract.[42] One of the most important and vital factors in optimizing the fertility rate and production of sperm cells is the reduction of reactive oxygen species (ROS), which is possible using plant antioxidants because these antioxidants make ROS formation impossible and, therefore, suppress it.[43,44] Hajalizadeh *et al.* studied the effects of adding fennel (Foeniculum vulgare) to the diet of sheep on some characteristics and reported that feeding fennel significantly increases the weight of the testis in comparison to diets without fennel.[23]

The association between testosterone and fennel seed powder in the diets of lambs in our study showed that adding fennel to diets significantly ($p < 0.05$) increases the concentration of blood testosterone in comparison to diets without fennel. Abbas *et al.* studied how serum sex hormones and fertility in mice and rabbits are affected by Foeniculum vulgare.[45] They demonstrated that the use of 2% and 4% Foeniculum vulgare in the diet of male rabbits causes a significant increase in serum testosterone levels. One of the compounds of Foeniculum vulgare is isoflavones; this compound inhibits the activity of 5α-reductase and aromatase P450 enzymes. The first enzyme stimulates the conversion of testosterone to 5α-dihydrotestosterone and the second enzyme converts testosterone to estradiol.[46] Another effect of isoflavones is to increase the synthesis of sex hormone-binding globulin. When sex hormone binding globulin levels rise, it binds to more testosterone, lowering free testosterone levels and promoting its synthesis.[47]

The obtained results demonstrated that the levels of *IGF1* gene expression in the studied sheep testes were high. The results of this study are in line with the results of other researchers who have demonstrated the expression of the *IGF1* gene in the testes of different animals.[12,18,48–51] Testis size and sperm production are directly correlated to the total number of adult Sertoli cells. Pitetti *et al.* showed that growth factors of the insulin family played an essential role in regulating the final number of Sertoli cells, testis size, and daily sperm output.[18] Weller *et al.* investigated the effects of maternal overnutrition on gonadal development and pituitary-gonadal gene expression in cattle fetuses at mid- and late gestation.[49] They demonstrated that *IGF1* gene expression was higher in fetal testes derived from moderate intake than in high (ad libitum) intake cows, irrespective of the day of gestation. Moreover, testicular expression of *IGF1* was higher at day 139 of gestation than at day 199 of gestation and at day 241 of gestation than at day 199 of gestation in both maternal intake groups. As Abd–Elmaksoud-Ahmed explained, the greater expression of *IGF1* at day 139 of gestation compared with day 199 of gestation may be related to the different ages of the fetus, which is correlated to the stage

of testicular development.[52] Additionally, it may be related to the stimulatory role of growth factors during testis enlargement, which increases testis size with further proliferation and differentiation. Similar to this trend, as the pregnancy progresses from 199 to 241 days, when the expression of the *IGF1* gene increases, the growth of the fetus also increases due to the production of more connective tissue. It has also been proven that *IGF1* affects the expression of key testicular steroidogenic enzymes during mouse development before birth.[53]

Müller *et al.* studied *IGF1* gene expression in the testes of prepubertal and adult dogs and concluded that in adult dogs *IGF1* can mediate Leydig cell function and support spermatogenesis.[12] *IGF1* can cause the growth of gonocytes and Leydig and Sertoli cells in the immature testis. It also has positive effects on the function of germ cells, increases spermatogonia DNA synthesis, and reduces the rate of apoptotic cells.[17]

When adult and immature mice were treated with *IGF1* in vitro, the number of Leydig apoptotic cells decreased and the proliferation of immature Leydig cells increased.[13] A study by Peters *et al.* demonstrated that the expression of IGF-binding protein 1 and IGF 1 genes in Sertoli cell tumors and seminomas was lower than in normal testes.[54] Research by Pitetti *et al.* on rodents showed that *IGF1* for the growth of immature Sertoli cells is necessary.[18] If insulin signaling and *IGF1* are lacking in the murine testis, Sertoli cell proliferation in the late embryonic and early neonatal periods decreases. The consequence of this reduction in proliferation in adults will be a decrease in sperm production and testicular weight. Leydig cells, with supervision and control of IGF, manufacture insulin-like hormone 3 and androgens, which are responsible for the masculinization of the testicular descent and urogenital system. Cannarella *et al.* investigated the role of *IGF1* in testicular growth during embryonic development and showed that it increases Sertoli proliferation and germ cells and promotes germ cell differentiation.[55]

Various studies have shown that sperm production and reproductive function of males are regulated by the insulin/IGF signaling pathway in Sertoli and Leydig cells.[1,56,57]

Since, in this study, the inclusion of fennel in the ration enhanced *IGF1* gene expression in the testis and on the other hand, the role of *IGF1* in the stimulation of Leydig cell steroidogenesis, the increased production of sperm and testosterone, and the growth and development of the testis, it can be affirmed that, including fennel in sheep diets can be applied to progress testis function and reproduction of sheep. However, to draw conclusions with 100% certainty, it is necessary to conduct studies with a wider scope.

Conclusions

Referring to the findings of the present study, it can be affirmed that fennel with a useful effect on the expression of the *IGF1* gene in the testis can be applied to sheep diets to progress reproductive functions (by stimulating Leydig cell steroidogenesis, producing more sperm and testosterone, and growing and developing the testis). Although referring to the findings of the present study, it can be affirmed that fennel can be applied for various goals in sheep breeding, but in future investigations, it would be better to consider various and more complex physiological and epigenetic conditions in order to hold out for a definitive outcome.

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Conflict of interest

MM has been an editorial board member of *Gene Expression* since September 2022. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Conceptualization, funding acquisition, investigation, methodology, resources, supervision, and experimental validation (MM); data curation (SMHS, BM, and OOB); formal analysis (MM, OB, and VA); project administration (MM, OK, and NK); software (MM and OB); manuscript drafting (MM, OB, SMHS, OK); manuscript review and revise (MM, BM, OOB, VA, NK). All authors read and approved the final manuscript.

Ethics statement

All procedures related to animals were certified by the Animal Care and Use Committee of Bahonar University (IACUC Protocol #IR2018011) described by the Iranian Council of Animal Care. All guidelines and laws related to animal welfare policies for the animals used in this research were fully followed.

Data sharing statement

The data sets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

References

[1] Neirijnck Y, Papaioannou MD, Nef S. The insulin/IGF system in mammalian sexual development and reproduction. Int J Mol Sci 2019;20(18):4440. doi:10.3390/ijms20184440, PMID:31505893.

[2] Hansen M, Flatt T, Aguilaniu H. Reproduction, fat metabolism, and life span: what is the connection? Cell Metab 2013;17(1):10–19. doi:10.1016/j.cmet.2012.12.003, PMID:23312280.

[3] Kaprara A, Huhtaniemi IT. The hypothalamus-pituitary-gonad axis: Tales of mice and men. Metabolism 2018;86:3–17. doi:10.1016/j.metabol.2017.11.018, PMID:29223677.

[4] Griffeth RJ, Bianda V, Nef S. The emerging role of insulin-like growth factors in testis development and function. Basic Clin Androl 2014;24:12. doi:10.1186/2051-4190-24-12, PMID:25780585.

[5] Roth MY, Amory JK. Pharmacologic development of male hormonal contraceptive agents. Clin Pharmacol Ther 2011;89(1):133–136. doi:10.1038/clpt.2010.103, PMID:20827265.

[6] Baker J, Hardy MP, Zhou J, Bondy C, Lupu F, Bellvé AR, *et al.* Effects of an IGF1 gene null mutation on mouse reproduction. Mol Endocrinol 1996;10(7):903–918. doi:10.1210/mend.10.7.8813730, PMID:8813730.

[7] Stratikopoulos E, Szabolcs M, Dragatsis I, Klinakis A, Efstratiadis A. The hormonal action of IGF1 in postnatal mouse growth. Proc

Natl Acad Sci U S A 2008;105(49):19378–19383. doi:10.1073/pnas.0809223105, PMID:19033454.
[8] Dupont J, Holzenberger M. Biology of insulin-like growth factors in development. Birth Defects Res C Embryo Today 2003;69(4):257–271. doi:10.1002/bdrc.10022, PMID:14745968.
[9] van Duyvenvoorde HA, van Setten PA, Walenkamp MJ, van Doorn J, Koenig J, Gauguin L, *et al*. Short stature associated with a novel heterozygous mutation in the insulin-like growth factor 1 gene. J Clin Endocrinol Metab 2010;95(11):E363–E367. doi:10.1210/jc.2010-0511, PMID:20668042.
[10] Wit JM, Kiess W, Mullis P. Genetic evaluation of short stature. Best Pract Res Clin Endocrinol Metab 2011;25(1):1–17. doi:10.1016/j.beem.2010.06.007, PMID:21396571.
[11] Wit JM, Walenkamp MJ. Role of insulin-like growth factors in growth, development and feeding. World Rev Nutr Diet 2013;106:60–65. doi:10.1159/000342546, PMID:23428682.
[12] Müller L, Kowalewski MP, Reichler IM, Kollár E, Balogh O. Different expression of leptin and IGF1 in the adult and prepubertal testis in dogs. Reprod Domest Anim 2017;52(Suppl 2):187–192. doi:10.1111/rda.12896, PMID:28101891.
[13] Colón E, Zaman F, Axelson M, Larsson O, Carlsson-Skwirut C, Svechnikov KV, *et al*. Insulin-like growth factor-I is an important antiapoptotic factor for rat leydig cells during postnatal development. Endocrinology 2007;148(1):128–139. doi:10.1210/en.2006-0835, PMID:17023532.
[14] Yoon MJ, Roser JF. A synergistic effect of insulin-like growth factor (IGF-I) on equine luteinizing hormone (eLH)-induced testosterone production from cultured Leydig cells of horses. Anim Reprod Sci 2011;126(3-4):195–199. doi:10.1016/j.anireprosci.2011.05.008, PMID:21676562.
[15] Yoon MJ, Berger T, Roser JF. Localization of insulin-like growth factor-I (IGF-I) and IGF-I receptor (IGF-IR) in equine testes. Reprod Domest Anim 2011;46(2):221–228. doi:10.1111/j.1439-0531.2010.01643.x, PMID:20546172.
[16] Manna PR, Chandrala SP, King SR, Jo Y, Counis R, Huhtaniemi IT, *et al*. Molecular mechanisms of insulin-like growth factor-I mediated regulation of the steroidogenic acute regulatory protein in mouse leydig cells. Mol Endocrinol 2006;20(2):362–378. doi:10.1210/me.2004-0526, PMID:16166197.
[17] Ozkurkcugil C, Yardimoglu M, Dalcik H, Erdogan S, Gokalp A. Effect of insulin-like growth factor-1 on apoptosis of rat testicular germ cells induced by testicular torsion. BJU Int 2004;93(7):1094–1097. doi:10.1111/j.1464-410X.2004.04787.x, PMID:15142170.
[18] Pitetti JL, Calvel P, Zimmermann C, Conne B, Papaioannou MD, Aubry F, *et al*. An essential role for insulin and IGF1 receptors in regulating sertoli cell proliferation, testis size, and FSH action in mice. Mol Endocrinol 2013;27(5):814–827. doi:10.1210/me.2012-1258, PMID:23518924.
[19] Wang GM, O'Shaughnessy PJ, Chubb C, Robaire B, Hardy MP. Effects of insulin-like growth factor I on steroidogenic enzyme expression levels in mouse leydig cells. Endocrinology 2003;144(11):5058–5064. doi:10.1210/en.2003-0563, PMID:12959969.
[20] Masoudzadeh SH, Mohammadabadi MR, Khezri A, Kochuk-Yashchenko OA, Kucher DM, Babenko OI, *et al*. dlk1 gene expression in different Tissues of lamb. Iran J Appl Anim Sci 2020;10(4):669–677.
[21] Shahsavari M, Mohammadabadi M, Khezri A, Borshch O, Babenko O, Kalashnyk O, *et al*. Effect of fennel (foeniculum vulgare) seed powder consumption on insulin-like growth factor 1 gene expression in the liver tissue of growing lambs. Gene Expr 2022;21(2):21–26. doi:10.14218/GE.2022.00017.
[22] Shahsavari M, Mohammadabadi M, Khezri A, Asadi Fozi M, Babenko O, Kalashnyk O, *et al*. Correlation between insulin-like growth factor 1 gene expression and fennel (Foeniculum vulgare) seed powder consumption in muscle of sheep. Anim Biotechnol 2023;34(4):882–892. doi:10.1080/10495398.2021.2000997, PMID:34783639.
[23] Hajalizadeh Z, Dayani O, Khezri A, Tahmasbi R, Abadi MRM. The effect of adding fennel (Foeniculum vulgare) seed powder to the diet of fattening lambs on performance, carcass characteristics and liver enzymes. Small Ruminant Res 2019;175:72–77. doi:10.1016/j.smallrumres.2019.04.011.
[24] Badgujar SB, Patel VV, Bandivdekar AH. Foeniculum vulgare Mill: a review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. Biomed Res Int 2014;2014:842674. doi:10.1155/2014/842674, PMID:25162032.
[25] Rather MA, Dar BA, Sofi SN, Bhat BA, Qurishi MA. Foeniculum vulgare: A comprehensive review of its traditional use, phytochemistry, pharmacology, and safety. Arab J Chem 2016;9:1574–1583. doi:10.1016/j.arabjc.2012.04.011.
[26] Khazaei M, Montaseri A, Khazaei MR, Khanahmadi M. Study of foeniculum vulgare effect on folliculogenesis in female Mice. Int J Fertil Steril 2011;5(3):122–127. PMID:25101154.
[27] EL-Deek AA, Attia YA, Hannfy MM. Effect of anise (Pimpinella anisiumj), ginger (Zingiber officinale roscoe) and fennel (Foeniculum vulgare) and their mixture of performance of Broilers. Archiv fur Geflugelkunde 2003;67(2):92–96.
[28] Mohammed A, Abbas R. The effect of using fennel seeds (Foeniculum vulgare L.) on productive performance of broiler chickens. Int J Poultry Sci 2009;8:642–644. doi:10.3923/ijps.2009.642.644.
[29] Gharaghani H, Shariatmadari F, Torshizi K. Comparison of oxidative quality of meat of chickens feed corn or wheat based diets with fennel (Foeniculum vulgare Mill.), antibiotic and probiotic as feed additive, under different storage conditions. Archiv Fur Geflugelkunde 2013;77(3):199–205.
[30] Aćimović MG, Ljiljana M, Kostadinović NM, Puvača SJ, Popović MIU. Phytochemical constituents of selected plants from apiaceae family and their biological effects in poultry. Food Feed Res 2016;43(1):35–41. doi:10.5937/FFR1601035A.
[31] Sotoudeh A, Yeganeh S. Effects of supplementary fennel (Foeniculum vulgare) essential oil in diet on growth and reproductive performance of the ornamental fish, Convict cichlid (Cichlasoma nigrofasciatum). Aquac Res 2016;10(36):1–8. doi:10.1111/are.13249.
[32] Amiri Roudbar M, Mohammadabadi MR, Mehrgardi AA, Abdollahi-Arpanahi A. Estimates of variance components due to parent-of-origin effects for body weight in Iran-Black sheep. Small Ruminant Res 2017;149:1–5. doi:10.1016/j.smallrumres.2017.01.002.
[33] Safaei SMH, Dadpasand M, Mohammadabadi M, Atashi H, Stavetska R, Klopenko N, *et al*. An origanum majorana leaf diet influences myogenin gene expression, performance, and carcass characteristics in lambs. Animals (Basel) 2022;13(1):14. doi:10.3390/ani13010014, PMID:36611623.
[34] Amiri Roudbar M, Abdollahi-Arpanahi R, Ayatollahi Mehrgardi A, Mohammadabadi MR, Taheri Yeganeh A, Rosa GJM. Estimation of the variance due to parent-of-origin effects for productive and reproductive traits in Lori-Bakhtiari sheep. Small Ruminant Res 2018;160:95–102. doi:10.1016/j.smallrumres.2018.01.022.
[35] Ghotbaldini H, Mohammadabadi MR, Nezamabadi-pour H, Babenko OI, Bushtruk MV, Tkachenko SV. Predicting breeding value of body weight at 6-month age using Artificial Neural Networks in Kermani sheep breed. Acta Sci Anim Sci 2019;41(1):e45282. doi:10.4025/actascianimsci.v41i1.45282.
[36] Jafari Ahmadabadi SAA, Askari-Hemmat H, Mohammadabadi M, Asadi MF, Mansouri M. The effect of Cannabis seed on DLK1 gene expression in heart tissue of Kermani lambs. Agr Bio J 2023;15(1):217–234. doi:10.22103/jab.2023.21265.1471.
[37] Shokri S, Khezri A, Mohammadabadi M, Kheyrodin H. The expression of MYH7 gene in femur, humeral muscle and back muscle tissues of fattening lambs of the Kermani breed. Agr Bio J 2023;15(2):217–236. doi:10.22103/jab.2023.21524.1486.
[38] Barazandeh A, Mohammadabadi MR, Ghaderi-Zefrehei M, Rafeied F, Imumorin IG. Whole genome comparative analysis of CpG islands in camelid and other mammalian genomes. Mamm Biol 2019;98:73–79. doi:10.1016/j.mambio.2019.07.007.
[39] AOAC. Official Methods of Analysis, 17th ed. Arlington: Association of Official Analytical Chemists; 2000.
[40] Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 1991;74(10):3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2, PMID:1660498.
[41] Pfaffl MW, Horgan GW, Dempfle L. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res 2002;30(9):e36. doi:10.1093/nar/30.9.e36, PMID:11972351.
[42] El-Garawani I, El-Nabi SH, El-Ghandour E. The protective effect of (Foeniculum vulgare) oil on etoposide-induced genotoxicity on male

albino rats. Eur J Pharm Med Rese 2017;4(7):180–194.
[43] Kierszenbaum AL. Histology & cell biology: an introduction to pathology, 2nd ed. Philadelphia, PA: Saunders; 2006.
[44] Bordbar H, Esmaeilpour T, Dehghani F, Panjehshahin MR. Stereological study of the effect of ginger's alcoholic extract on the testis in busulfan-induced infertility in rats. Iran J Reprod Med 2013;11(6):467–472. PMID:24639780.
[45] Abbas A, Ikram R, Hasan F, Sarfaraz S, Bukhari QUA. Fennel fortified diet: New perspective with regard to fertility and sex hormones. Pak J Pharm Sci 2020;33(6):2595–2600. PMID:33867335.
[46] Brooks JD, Thompson LU. Mammalian lignans and genistein decrease the activities of aromatase and 17beta-hydroxysteroid dehydrogenase in MCF-7 cells. J Steroid Biochem Mol Biol 2005;94(5):461–467. doi:10.1016/j.jsbmb.2005.02.002, PMID:15876411.
[47] Celec P, Ostatníková D, Cagánová M, Zuchová S, Hodosy J, Putz Z, *et al*. Endocrine and cognitive effects of short-time soybean consumption in women. Gynecol Obstet Invest 2005;59(2):62–66. doi:10.1159/000081895, PMID:15528926.
[48] Hu GX, Lin H, Chen GR, Chen BB, Lian QQ, Hardy DO, *et al*. Deletion of the Igf1 gene: suppressive effects on adult Leydig cell development. J Androl 2010;31(4):379–387. doi:10.2164/jandrol.109.008680, PMID:20203337.
[49] Weller MMDCA, Fortes MRS, Marcondes MI, Rotta PP, Gionbeli TRS, Valadares Filho SC, *et al*. Effect of maternal nutrition and days of gestation on pituitary gland and gonadal gene expression in cattle. J Dairy Sci 2016;99(4):3056–3071. doi:10.3168/jds.2015-9673, PMID:26874426.
[50] Özdamar MY, Şahin S, Zengin K, Seçkin S, Gürdal M. Detection of insulin-like growth factor receptor-1 in the human cremaster muscle and its role in the etiology of the undescended testis. Asian J Surg 2019;42(1):290–296. doi:10.1016/j.asjsur.2018.02.005, PMID:29615365.
[51] Wang H, Shan B, Duan Y, Zhu J, Jiang L, Liu Y, *et al*. Effects of Heshouwuyin on gene expression of the insulin/IGF signalling pathway in rat testis and spermatogenic cells. Pharm Biol 2020;58(1):1199–1210. doi:10.1080/13880209.2020.1839511, PMID:33264567.
[52] Abd-Elmaksoud A. Morphological, glycohistochemical, and immunohistochemical studies on embryonic and adult bovine testis [Dissertation]. Munich: Ludwig-Maximilians University; 2005.
[53] Villalpando I, López-Olmos V. Insulin-like growth factor I (IGF-I) regulates endocrine activity of the embryonic testis in the mouse. J Steroid Biochem Mol Biol 2003;86(2):151–158. doi:10.1016/s0960-0760(03)00265-6, PMID:14568566.
[54] Peters MA, Mol JA, van Wolferen ME, Oosterlaken-Dijksterhuis MA, Teerds KJ, van Sluijs FJ. Expression of the insulin-like growth factor (IGF) system and steroidogenic enzymes in canine testis tumors. Reprod Biol Endocrinol 2003;1:22. doi:10.1186/1477-7827-1-22, PMID:12646054.
[55] Cannarella R, Condorelli RA, La Vignera S, Calogero AE. Effects of the insulin-like growth factor system on testicular differentiation and function: a review of the literature. Andrology 2018;6(1):3–9. doi:10.1111/andr.12444, PMID:29195026.
[56] Cannarella R, Mancuso F, Condorelli RA, Arato I, Mongioì LM, Giacone F, *et al*. Effects of GH and IGF1 on basal and FSH-Modulated porcine sertoli cells in-vitro. J Clin Med 2019;8(6):811. doi:10.3390/jcm8060811, PMID:31174315.
[57] Neirijnck Y, Kühne F, Mayère C, Pavlova E, Sararols P, Foti M, *et al*. Tumor suppressor PTEN regulates negatively sertoli cell proliferation, testis size, and sperm production in vivo. Endocrinology 2019;160(2):387–398. doi:10.1210/en.2018-00892, PMID:30576429.