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FSHβ and FSHR Genes Polymorphisms and their Effect on Productive Performance in Barki Sheep

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ABSTRACT

This study investigated the association among polymorphisms in the follicle-stimulating hormone beta subunit (*FSHβ*) and follicle-stimulating hormone receptor (*FSHR*) genes and their relationship with lamb growth traits. The studied traits including birth weight (BW), weaning weight at three months of age (WW), and body weights at 6, 9, and 12 months (yearling) and productive performance (mature body weight of each ewe at first mating (MW), the total lamb birth weight per ewe (TLBW) and total lamb weaning weight per ewe (TLWW) across four breeding seasons) of Egyptian Barki sheep. A total number of 67 ewes and 89 lambs (44 males and 45 females) were evaluated across four consecutive breeding seasons (2020–2023). The PCR-RFLP technique was employed to detect the genotypic variations in the *FSHβ* and *FSHR* genes using *ACCI* (*HinfI*) and *MSCI* restriction enzymes, respectively. No polymorphism was observed in the *FSHβ* gene, indicating that this locus is monomorphic. In contrast, three distinct genotypic variants (AA, AB, and BB) were identified in the *FSHR* gene. Association analysis revealed no significant effect of *FSHR* polymorphism on ewes reproductive traits, although ewes carrying the AA genotype showed slightly higher mature weight (MW) and total lamb birth weight (TLBW) than those with AB and BB genotypes. Conversely, the BB genotype was associated with increased total lamb weaning weight (TLWW). Moreover, the *FSHR* polymorphism exhibited a highly significant effect ($P < 0.01$) on lamb weaning weight at three months of age (WW), with BB genotype lambs averaging 16.90 kg, compared to 13.39 kg for the AA genotype. These results indicate that genetic variation in the *FSHR* genes may influence growth performance. Further investigations with larger sample size and additional molecular markers are warranted to elucidate the genetic mechanisms governing productive and growth traits in Barki sheep.

Keywords: Barki sheep, *FSHβ*, *FSHR*, polymorphism, productive performance, growth traits.

1. Introduction

Barki sheep are well adapted breed to the harsh environmental conditions of Egypt and are capable of producing high-quality meat and milk under such circumstances. The primary objectives of sheep breeding programs are to enhance reproductive performance, growth rate, and fleece characteristics, as these traits represent critical determinants of overall productivity. Among reproductive traits, litter size defined as the number of lambs born per ewe lambing is one of the most economically important and easily measurable traits (Yavarifard *et al*., 2015). Enhancing meat production in sheep can be achieved by increasing reproductive rate, improving meat quality, and promoting faster growth to achieve higher marketed weights.

Body weight is one of the most important traits used in livestock evaluation and management, serving as a primary criterion for breeding, marketing, and meat production (Lakew *et al*., 2017). Improving body weight has become a major target in breeding programs due to its substantial economic impact, particularly in meat-production systems (Kumar *et al*., 2017). Monitoring body weight also provides accurate insight into growth performance, enabling farmers to select superior replacement animals and enhance flock productivity (Shirzeyli *et al*., 2013). Furthermore, reliable body weight measurements contribute to better estimation of carcass yield and production efficiency, ensuring more

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effective utilization of genetic and nutritional resources (Iqbal *et al*., 2013; Yilmaz *et al*., 2013). In addition to direct additive genetic influence, livestock growth performance is also affected by maternal genetic factors and permanent environmental effects from the dam. Previous studies have shown that incorporating maternal effects into statistical models provides more accurate estimates of genetic parameters for growth-related traits (Zamani *et al*., 2008**;** Mohammadi *et al*., 2013).

Follicle-stimulating hormone (*FSH*) is a key regulator of mammalian reproduction, essential for puberty, follicular development in females, (Simoni and Nieschlag, 1995; Chappel and Howles, 1991). Together with luteinizing hormone (*LH*), it controls gonadal function through the hypothalamic pituitary gonadal axis (HPGA) (Pérez-Solis *et al*., 2010). *FSH* exerts its effects by binding to the *FSH* receptor (*FSHR*), a G-protein-coupled receptor responsible for activating intracellular signaling cascades that regulate *FSH* dependent gene expression (Ulloaaguirre *et al*., 2007). Mutations in the *FSHR* gene may modify receptor function and subsequently influence reproductive traits. In sheep, the *FSHR* gene is located on chromosome 3 and consists of ten exons and nine introns, with the final exon encoding conserved trans-membrane and intracellular domains. (Pan *et al*., 2014). Variation in *FSHR* expression has been linked to differences in ovarian responsiveness and prolificacy among breeds (Abdennebi *et al*., 1999; Chu *et al*., 2012). *FSH* consists of a common α subunit and a hormone-specific β subunit that determines receptor specificity (Fan and Hendrickson, 2005). Polymorphisms in the *FSHβ* subunit gene have been associated with variations in fertility in livestock (Dai *et al*., 2009; Lan *et al*., 2006). Beyond its well established role in reproduction, recent studies have suggested that *FSHR* polymorphisms may also influence growth traits, such as body weight (BW), weaning weight (WW), and daily gain (DG). For example, Zaghloul *et al*. (2024) reported significant differences among *FSHR* genotypes in buffalo. Also, the effect of *FSHR* gene polymorphisms on growth traits has been demonstrated, associated with better performance in the Arman sheep breed (Nazifi *et al*., 2015). Overall, genetic variations in *FSH* genes play crucial role in regulating productive and reproductive efficiency and represent promising molecular markers for fertility related traits in animals.

The aim of this study is to investigate polymorphisms in the *FSHβ* and *FSHR* genes and to examine their associations with productive and growth traits of Barki sheep, with the ultimate goal of incorporating these findings into genetic selection programs.

2. Materials and Methods

2.1. Phenotype measurements reproductive performance

A total number of 67 Barki ewes and 89 lambs (44 males and 45 females) maintained at the Desert Research Center were evaluated over four consecutive breeding seasons (2020–2023) to examine the association among genetic polymorphisms in the FSHβ and FSHR genes with productive and reproductive traits of Egyptian Barki sheep.

The mature body weight of each ewe was recorded at first mating (MW). Reproductive performance was evaluated by calculating the total lamb birth weight per ewe (TLBW) and total lamb weaning weight per ewe (TLWW) across four breeding seasons. Additionally, the body weights of ewe lambs were recorded at various growth stages, including birth weight (BW), weaning weight at three months (WW), and body weights at six, nine, and twelve months (yearling weight).

2.2. DNA Extraction and PCR Amplification of *FSHβ* and *FSHR* genes

Blood samples were collected from each ewe and lamb via the jugular vein into EDTA-coated vacationer tubes and stored at –20 °C until DNA extraction. DNA was extracted using commercial available kit according to the manufacturer's instructions. The DNA quality was assessed by Gel electrophoresis system (Biometra, USA) on 1% agarose in 1 X TBE buffer. The PCR assay was performed in a total volume of 12.5 µL containing 1 µL forward primer, 1 µL reverse primer, 1 µL DNA, 6.5 µL PCR red master mix and 3 µL of nuclease free water. For the FSHβ gene, the thermal profile consisted of initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 47 °C for 30 s, and extension at 72 °C for 45 s, with a final extension at 72 °C for 10 min. While, the *FSHR* gene, amplification was initiated at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 56 °C for 30 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min.PCR products were tested by using electrophoresis on 1.5% agarose gel, stained with ethidium bromide, and visualized using a Bio-Rad Gel Documentation System (USA). The primer sequences used for FSHβ and FSHR amplification are listed in Table 1.

Table 1. Primers sequences of *FSHβ* and *FSHR* genes

Gene Name	Primer sequence	PCR product	Enzyme	Reference
FSHβ	F: *ACTCAGGACTTGGTGTAC* R: *CTGCTGCTCTTTATTCTC*	247 bp	*ACCI* (*HinfI*)	An *et al.* (2010)
FSHR	F: *CCCATCTTTGGCATCAGC* R: *ACACAGTGATGAGGGGCAC*	304 bp	*MSCI*	

2.3. Restriction Fragment Length Polymorphism (RFLP) Analysis

Restriction fragment length polymorphism method was performed at 37 °C for 12 to 14 h for PCR products of *FSHβ* gene by *ACCI* and *HinfI* enzyme treatment. The ampliers of *FSHR* genes were digested by *MSCI* restriction enzyme. Digestion reactions were carried out by following profiles: a final volume of 15 µL containing 7 µL of each PCR products were treated with 0.2 µL of each enzyme, 1 µL Buffer and 6.8 µL distilled deionized water. Detection of the products has been done on 2% agaros gel in the presence of DNA size marker (Gene Ruller^{TM} 100 bp). Then the gel was stained with ethidium bromide and subsequently visualized by gel documentation system (BioRad, USA).

2.4. Statistical Analysis

Association analysis among the different genotypes of *FSHR* gene and the phenotypes of the studied traits was performed using the General Linear Model (GLM) procedure of Analysis of Variance (ANOVA) in SPSS Version 20 (IBM, New York, NY, USA). The following statistical model was used to evaluate the effect of genotype on;

1- Lamb body weights traits:

$Y_{ijk}= \mu + G_i + S_j + e_{ijk}$,

Where Yijk is the analysed trait, µ is the overall mean, G_i is the effect of *FSHR* genotypes (i = 3 levels),

S_j is the effect of sex (j = 2 levels), and e_{ijk} is the error effect. $P < 0.05$ was considered significant.

2- Ewe productive traits:

$Y_{ik}= \mu + G_i + e_{ik}$,

Where Yik is the analysed trait, µ is the overall mean, G_i is the effect of *FSHR* genotypes (i = 3 levels), and e_{ik} is the error effect. $P < 0.05$ was considered significant

3. Results

3.1. Phenotypic data of ewe productive and lamb growth traits

An overview of descriptive statistics of ewe productive traits terms of mature weight (MW), and the total lamb birth weights per ewe (TLBW) and total lamb weaning weights per ewe (TLWW). The ewe lamb's body weights at different ages, including birth weight, weaning weight at 3 months, weight at 6 months, 9 months, and 12 months (yearling weight), are shown in Table 2.

Table 2: Descriptive statistics of the studied traits.

Trait	N	Mean	S.D	Min	Max
Ewe					
MW	67	36.77	5.18	23.50	46.50
TLBW	67	11.59	4.25	3.00	19.10
TLWW	67	39.78	17.18	11.20	100.60
Lamb					
BW	89	3.61	0.47	2.50	4.50
WW	89	15.67	3.02	10.00	23.00
6 months	89	20.27	4.55	10.50	34.00
9 months	89	25.12	5.90	11.50	37.50
12 months	89	31.96	9.23	13.00	55.00

MW = Ewe Mature weight (kg), TLBW = Total Lamb Birth Weight (kg), TLWW =Total Lamb Weaning Weight (kg), BW = Birth Weight, WW = Weaning Weight (kg).

3.2. Allelic and genotypic frequencies of *FSHβ* and *FSHR* genes

RFLP-PCR analysis of the *FSHβ* gene revealed no detectable polymorphism among ewes and lambs (Figure 1). In contrast, digestion of the *FSHR* gene fragment (304 bp) with the *MSCI* restriction enzyme produced three distinct genotypes. Samples lacking the restriction site were identified as BB genotype (304pb), while those in which the cleavage site was present yielded two fragments (214 and 90 bp), corresponding to the AA genotype. The heterozygous AB genotype exhibited three bands (304, 214 and 90 bp) (Figure 2). The allele and genotype frequencies, along with the observed heterozygosity for the *FSHR* locus, are summarized in Table 3.

Table 3: Allelic and genotypic frequencies of *HSF1* and *HSPA6* genes.

Gene	Alleles	Allelic frequency	Genotypes	Genotypic frequency
FSHR	A	0.45	AA	0.28
	B	0.55	AB	0.36
			BB	0.36

3.3. Association of *FSHR* genotypes and ewe productive traits

Association analysis between ewes productive traits and genotypes (Table 4) revealed that no significant effect of the *FSHR* gene polymorphism on ewe's productive traits but AA genotype show a slight increase in mature weight (37.06 kg) and total weaning weight (41.75 kg) than AB (36.88 and 37.73 kg) and BB (36.43 and 40.42 kg) genotypes. While, there was a slight increase in total birth weight (12.42 kg) associated with the BB genotype.

Table 4: Effect of *FSHR* genotypes on the ewe productive traits (Means ±SEM).

Trait	Genotype			*P* value
	AA	AB	BB	
MW	37.06±1.24	36.88±1.07	36.43±1.12	0.926 (N.S)
TLBW	10.55±1.00	11.62±0.87	12.42±0.91	0.388 (N.S)
TLWW	41.75±4.10	37.73±3.55	40.42±3.71	0.743 (N.S)

MW = Ewe Mature weight (kg), **TLBW** = Total Lamb Birth Weight (kg), **TLWW =**Total Lamb Weaning Weight (kg), N.S = not significant.

3.4. Association of *FSHR* genotypes and lambs' sex on body weights traits

Association analysis between phenotypic traits and genotypes revealed a highly significant effect ($P < 0.01$) of the *FSHR* gene polymorphism on weaning weight at 3 months. Ewes carrying the BB genotype had a relatively higher Lamb's weaning weight (16.90 kg) compared to AA lambs (13.39 kg) (Table 5). Additionally, Lambs' sex was highly significantly associated with birth weight, 9 months and yearling weight ($P<0.01$), while weights at 6 months was significantly associated ($P<0.05$).

Table 5: Effect of *FSHR* genotypes and lambs' sex on the lamb weight traits (Means ±SEM).

Trait	Genotype			*P* value	Sex		*P* value
	AA	AB	BB		Males	Females	
BW	3.62±0.11	3.57±0.07	3.73±0.10	0.405 (N.S)	3.83±0.08	3.49±0.07	0.001 (**)
WW	13.39±0.67	16.15±0.40	16.90±0.59	0.000 (**)	16.12±0.49	14.84±0.42	0.052 (N.S)
6 months	18.10±1.18	20.65±0.71	21.28±1.02	0.106 (N.S)	21.50±4.48	18.52±4.87	0.011(*)
9 months	23.21±1.19	26.04±0.72	26.30±1.02	0.093 (N.S)	28.42±0.88	21.95±0.74	0.000 (**)
12 months	31.17±1.81	33.69±0.99	35.70±1.32	0.132 (N.S)	40.43±1.36	26.61±0.91	0.000 (**)

BW = birth weight, **WW** = weaning weight, **N.S** = not significant. Significance (1<0.05). High Significance (P< 0.01).

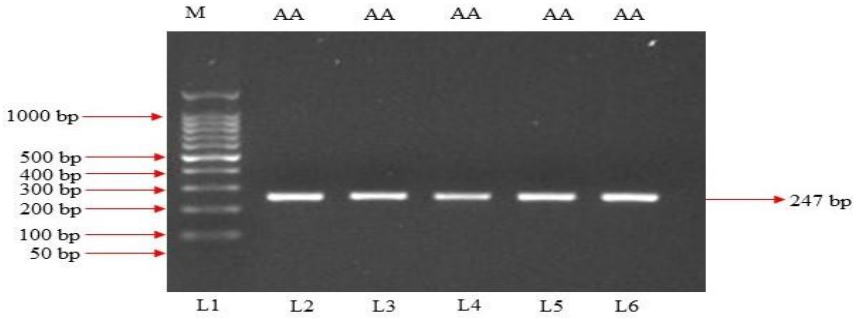

Fig. 1: PCR-RFLP analysis of Barki dams, *FSHβ* gene: the DNA marker was shown in Lane 1, AA genotype in lanes2-6.

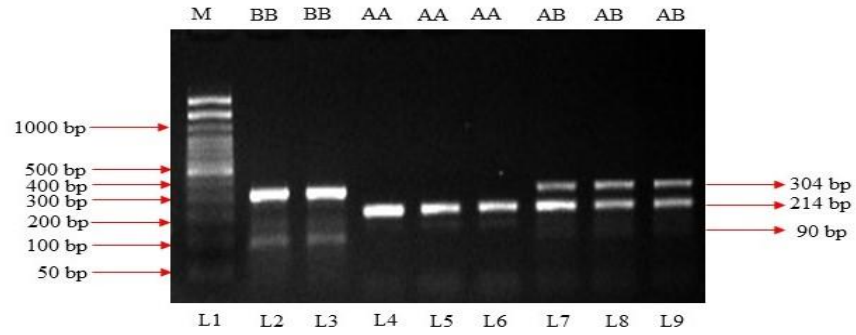

Fig. 2. PCR-RFLP analysis of Barki dams, *FSHR* gene: the DNA marker was shown in Lane 1, BB genotype in Lanes 2–3, AA genotype in Lanes 4, 5, 6 and AB genotype in Lane 7,8,9.

4. Discussion

In the present study the wide range in ewe mature weight (MW) (23.5–46.5 kg; mean 36.77 kg) suggests variation in body condition and nutrition at first mating, influencing ovulation and conception success, consistent with El-Wakil and El-Sayed (2013). However, the variation in total lamb birth weight TLBW (3.0–19.10 kg; mean 11.59 kg) and total lamb weaning weight TLWW (11.20–100.6 kg; mean 39.78 kg) indicates differences in prolificacy and maternal ability, particularly milk yield and nurturing behavior. These averages were lower than those reported by Ibrahim (2021), likely due to differences in sample size, and number of breeding season. Variation in productive traits of Barki ewes, including mature weight (MW), total lamb birth weight (TLBW), and total lamb weaning weight (TLWW), reflects the combined effects of genetic and environmental factors. However, the productivity and profitability of sheep farming are significantly influenced by the ewe's productive performance throughout its lifetime (Pinto, *et al*., 2025).

In currant study the average of birth and weaning weight (3.61 and 15.67 kg, respectively). This is consistent with previous results obtained by Sallam *et al*. (2019), confirming the breed's moderate growth potential under semi-arid condition. The relatively lower 6-month weight (20.27 kg) compared with Baluchi and Iran Black breeds (Nazifi *et al*., 2015) may reflect breed and management differences. The large variation in 9-month body weight (11.50–37.50 kg) indicates that some individuals possess superior growth potential that can be exploited through selection. Early growth traits such as birth, weaning, and yearling weights are valuable predictors of future reproductive performance (Gabr *et al*., 2016). Faster-growing lambs generally develop into more productive adults, with birth weight reflecting maternal environment and neonatal viability, weaning weight indicating early growth and nutritional status, and yearling weight representing maturity and reproductive capacity (Gabr *et al*., 2025). Previous studies have reported positive genetic correlations between growth and productive traits in sheep. Selection for growth traits, especially weaning weight, can indirectly enhance performance (Snyman *et*

al., 1998; Mohammadi *et al*., 2013). Substantial variation in body weight traits among Barki lambs emphasizes the strong genetic and environmental effects on growth.

In the present study, the *FSHβ* gene was observed to be monomorphic among Barki ewes, indicating the absence of detectable genetic variation at this locus. This result aligns with the findings of Nazifi *et al*. (2015), who also observed no polymorphism in the *FSHβ* gene while investigating its association with litter size and body weight traits in Baluchi, Iran Black, and Arman sheep breeds. Similarly, Andreas *et al*. (2014) observed monomorphic of the *FSHβ* gene in the Angus breed, further supporting the current results. Conversely, Dai *et al*. (2009) identified nine single nucleotide polymorphisms (SNPs) across the full β-subunit sequence of the *FSH* gene in bulls, suggesting that genetic variability at this locus may be species specific, with cattle showing greater polymorphic diversity compared to sheep.

The *FSHR* gene, positioned on chromosome 3, plays a crucial role in regulating follicular development and reproductive efficiency through its interaction with follicle-stimulating hormone (FSH) (Pan *et al*., 2014). In the current study, three genotypes (AA, AB, and BB) were identified, but no significant association was observed among these genotypes and ewe productive traits. Nevertheless, ewes with the AA genotype showed a slight increase in mature weight (MW) and total lamb birth weight (TLBW) compared with heterozygous and homozygous BB carriers, suggesting that the A allele might contribute marginally to improve reproductive performance. Conversely, a minor increase in total weaning weight (TLWW) associated with the B allele could reflect its potential role in enhancing postnatal growth or maternal milk production. These results are consistent with those reported by Abdel-Rahman *et al*. (2019), who identified two alleles (A and B) and two genotypes (AA and AB) at the *FSHR* locus in Ossimi sheep and found no significant effect of genotype on litter size.

However, association analysis between lamb body weight traits and genotypes revealed a highly significant effect ($P < 0.01$) of *FSHR* gene polymorphism on weaning weight at 3 months. Lambs carrying the BB genotype exhibited higher weaning weights (16.90 kg) compared with those carrying the AA genotype (13.39 kg). These findings are consistent with recent evidence from Nazifi *et al*. (2015), who reported significant associations between *FSHR* gene polymorphisms and growth as well as productive traits in Arman sheep, suggesting that specific alleles may influence growth regulation and follicular function. Similarly, a survey of polymorphisms at the *FSHR* locus indicated age-specific differences in body weight, with the wild-type allele being associated with superior growth performance. In contrast, other studies have documented weak or non-significant associations between FSHR variants and reproductive performance across different ovine and caprine populations (Li *et al*., 2010; Yun *et al*., 2007; Lan *et al*., 2006). Collectively, these results indicate that the impact of *FSHR* polymorphism on growth and productive traits is breed-dependent, likely influenced by differences in genetic background, selection pressure, and environmental conditions.

Conclusion

Productive efficiency and growth performance represent critical economic determinants in sheep production programs. The *FSHR* and *FSHβ* genes are known to play pivotal roles in the regulation of productive traits. In the present study, no polymorphism was detected within the *FSHβ* gene, indicating a monomorphic state at this locus, whereas three distinct genotypes (AA, AB, and BB) were identified for the *FSHR* gene. The *FSHR* genotypes exhibited favorable associations with lamb body weight traits in the Barki sheep population, suggesting a potential influence on growth performance. Nonetheless, these findings should be interpreted with caution and further investigations incorporating larger populations and additional molecular markers are warranted to elucidate the precise contribution of these genes to the reproductive and productive performance of Barki sheep.

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