

Research Article



Investigation of Litter Size in Awassi Sheep and their Correlation to Adipose Tissue Biomarkers

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Abstract | Adipose tissue produces multiple adipokines, including leptin, and visfatin. The expression and/or secretion of adipokines could play a role in regulating fertility. Thus, this study aimed to investigate the association of litter size with the adipose tissue biomarkers in Awassi ewes. In the current study, 100 sexually mature, not pregnant or lactating, and healthy ewes between the ages of 3 and 4 years were evaluated. The live body weight and age of each animal were determined. Serum was collected to measure leptin and visfatin hormones using ELISA kits made by SunLong Biotech. This study revealed that litter size significantly ($P \leq 0.05$) influences the live body weight and adipose tissue hormones of Awassi ewes. Ewes with twin births had higher live body weight, leptin, and visfatin (50.41 ± 0.92 kg), (192.16 ± 27.63 pg/mL), and (3.56 ± 0.96 ng/mL), respectively, compared to single births ewes. There was the highest correlation ($P \leq 0.05$) between litter size with live body weight and adipose tissue hormones of ewes with single and twin births. Logistic regression analyses further explored the association of the progeny type with adipose tissue biomarkers. In conclusion, Awassi ewes with twin births exhibited an increase in the leptin and visfatin levels. This study provided valuable information about the association of litter size with adipose tissue hormones. This finding makes it a potential candidate for further research and application in sheep breeding and reproduction to improve prolificacy in the field.

Keywords | Adipose tissue, Biomarkers, Leptin, Sheep, Twinning Rate, Visfatin

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INTRODUCTION

Sheep are small ruminants that provide high levels of animal protein which contributes to human diet protein content worldwide (Goldansaz et al., 2022). Sheep producers benefit economically and biologically from increasing ewe productivity and reproduction (Habtegiorgis et al., 2022). Many factors affect reproductive performance, including litter size, season, photoperiod, age, nutrition, management, male behavior, and genetics (Ajafar et al., 2022; Al-Thuwaini and Kareem, 2022). The litter size is influenced by various factors, such as the rate of ovulation, the capacity of the uterus, the length of gestation, parity,

the mortality of lambs before weaning, and the body fat of the ewe (Sarvinda et al., 2022; Alkhammas and Al-Thuwaini, 2023). Live weight and body fat reserves of ewes at mating affect their reproductive and productive behavior. Low body fat reserves and low live weight at mating affect sheep's productivity and reproductive ability (Ptacek et al., 2014; McNamara and Huber, 2018). Multiple studies have been conducted to analyze fat's impact on fat-tailed sheep productivity, reproductive traits, and physiological functions. Furthermore, these studies focus on adipose biomarkers that have a significant impact on productive traits. Notably, Xu et al. (2015), Yue et al. (2016), and Ibrahim (2021) have greatly contributed to our

understanding of this area. Adipose biomarkers, including metabolites, proteins, hormones, and DNA can be used to measure or predict traits indirectly in livestock. As well, biomarkers are particularly useful when measuring traits in a short period, or if direct measurements of traits require lengthy trials, are labour-intensive, result in animal loss, or are expensive (Fontanesi, 2016; Goldansaz et al., 2017).

There are multiple adipokines produced and secreted by adipose tissue, such as leptin, visfatin, adiponectin, chemerin, and many others (Häussler et al., 2022; Al-Jumaili et al., 2023). Adipose tissue-secreted hormones, namely adipokines, could contribute to fertility regulation (Estienne et al., 2020; Mohammed et al., 2022). Leptin, produced by adipose tissue, regulates various physiological processes including energy balance, metabolism, fat oxidation, and reproductive processes (Kuznicka et al., 2020). A second adipokine called Visfatin or (nicotinamide phosphoribosyl-transferase) is expressed in the uterus, ovary, and testis. There is evidence that visfatin increases the gonad expression of chickens and buffaloes, as well as is involved in the regulation of ovarian steroidogenesis in these species (Diot et al., 2015; Thakre et al., 2021). Kaminski et al. (2021) conducted a study that revealed the hypothalamus of porcine-expressed visfatin. This expression is found to be intricately linked to the hormonal environment associated with early pregnancy and the estrous cycle. According to these data, information regarding these biomarkers could be of considerable interest concerning potential associations between sheep breeds and reproductive traits. Middle Eastern countries are predominantly home to the Awassi sheep breed (Al-Thuwaini and Al-Hadi, 2022). While it is capable of surviving harsh conditions (Al-Thuwaini, 2021), it is generally regarded as having lower reproduction rates than Karakuls and Assaf (Ajafar et al., 2022). There is a lot of concern from Middle Eastern breeders about this breed's low reproductive capacity, which drives efforts to enhance it. However, no publications have used adipose tissue biomarkers to characterize litter size in Awassi ewes. This study aimed to examine the relationship between the number of lambs born to ewe and biomarkers found in adipose tissue, emphasizing the significant role of the Awassi breed in sheep reproduction.

MATERIALS AND METHODS

SHEEP POPULATION AND PHENOTYPIC MEASUREMENTS

The study was performed between July 2022 and September 2023 at Al-Qasim Green University with approval from the ethics committee (Agri, No. 01, 7, 22). There were 100 ewes of the Awassi breed, aged 3 to 4 years, mature sexually, not pregnant or lactating, and healthy. A singleton ewe and a twin ewe were randomly selected from Babylon and Karbala stations and classified as 50 and 50, respectively, after parturition. The animals were given a concentrated

feed consisting of barley, bran, and salt in the proportions of 59%, 40%, and 1%, respectively, equivalent to 2.5% of their body weight. Additionally, 3 kilograms of alfalfa and 1 kilogram of straw were given to the animals. Throughout the day, all animals had access to fresh water.

The sheep's external jugular vein was punctured with a disposable needle of 18 gauge to collect blood (5 ml). An analysis of hormone levels was made by centrifuging blood for 15 minutes at 2,000 xg and storing the serum at -20°C. An ELISA kit provided by SunLong Biotech Co., LTD (Hangzhou, China) was used to measure leptin and visfatin levels in the adipose tissue (SL00009Sp and SL00147Sp, respectively). Leptin sensitivity was 8 pg/mL, and the intra- and inter-assay coefficients of variation were 10% and 12%, respectively. The assay range of leptin concentrations was 30 pg/mL to 2000 pg/mL. The sensitivity of the visfatin assay was 0.1 ng/ml, with intra-assay and inter-assay coefficients of variation of 10% and 12%, respectively. Assay visfatin concentrations ranged from 0.5 ng/mL to 40 ng/mL. A suspended spring balance was used in the morning to measure the weight of Awassi ewes (kg) (Kadhem and Al-Thuwaini, 2022).

DATA ANALYSIS

An investigation was conducted to determine the effects of birth type on various animal traits. Based on the general linear model, SPSS version 23.0 was used to conduct this study:

$$Y_{ijk} = \mu + L_i + P_j + e_{ijk}$$

Where Y_{ijk} = characteristics phenotypically, μ = overall mean, L_i = fixed effect of i^{th} birth type ($i = 1, 2$), P_j = fixed effect of j^{th} parity ($j = 1, 2, 3$), and e_{ijk} = random error associated with Y_{ijk} observation and assumed to be NID ($0, \sigma^2e$). Bonferroni test with a significance level of 0.05 was used to compare means. The results of preliminary statistics showed that seasonal, station, and factor interactions did not significantly influence phenotypic traits. Thus, the general linear model excluded these variables.

RESULTS AND DISCUSSION

This study examined the associated progeny type with adipose tissue biomarkers in Awassi ewes. The least-square means of adipose tissue biomarkers affected by the progeny type are presented in Table 1. The live body weight, leptin, and visfatin levels were significantly higher ($P \leq 0.05$) in Awassi ewes with twin births than in ewes with single births. However, the age of Awassi ewes did not show a significant difference ($P \geq 0.05$). The correlation coefficient between progeny type and phenotypic characteristics of Awassi ewes is presented in Table 2. The highest and strongly positive correlations ($P \leq 0.05$) were recorded

between twin ewes with live body weight ($r = 0.45$, $P = 0.001$), leptin ($r = 0.37$, $P = 0.001$), and visfatin ($r = 0.33$, $P = 0.02$), respectively. Univariate regression analyses further explored the association of the progeny type with adipose tissue biomarkers (Table 3).

Table 1: The effect of progeny type on adipose tissue biomarkers in Awassi ewes.

Indices	Progeny type (LSM ± SE)		P value
	Single (52)	Twin (48)	
Live body weight (Kg)	47.19 ± 0.85 ^b	50.41 ± 0.92 ^a	0.01
Age (year)	3.53 ± 0.09	3.50 ± 0.10	0.79
Leptin (pg/ml)	192.16 ± 27.63 ^b	286.41 ± 30.51 ^a	0.02
Visfatin (ng/ml)	3.56 ± 0.96 ^b	6.51 ± 0.91 ^a	0.03

LSM±SE, Least square means±Standard error. ^{a,b} Significant differences in means represent differences in the same row within each classification.

Table 2: Correlation between litter size and other variables in Awassi ewes.

Variables	Single		Twin	
	r	P-value	r	P-value
Live body weight (Kg)	0.33	0.004	0.45	0.001
Age (year)	-0.03	0.75	-0.12	0.46
Leptin (pg/ml)	0.30	0.008	0.37	0.001
Visfatin (ng/ml)	0.29	0.01	0.33	0.02

$P \leq 0.05$: Significant, $P \geq 0.05$: Not significant.

Table 3: Logistic regression analysis of progeny type with phenotypic parameters in Awassi ewes.

Characteristic	Univariate logistic regression		
	Estimate	Odds ratio (95% CI)	P value
Live body weight (Kg)	0.43	1.53 (0.98-4.58)	0.04
Age (year)	0.12	1.12 (0.91-3.98)	0.43
Leptin (pg/ml)	0.93	2.53 (1.21-5.83)	0.003
Visfatin (ng/ml)	0.89	2.43 (1.01-5.67)	0.01

The P -value with statistical significance is indicated in bold numbers; CI: confidence interval.

The economic viability of livestock exploitation heavily relies on reproduction efficiency. Thus, a comprehensive understanding of the factors influencing livestock reproductive traits is essential for enhancing these traits (Ajafar et al., 2022; Al-Jaryan et al., 2023). Sheep litter size has been reported to be influenced by their live body weight. Twin ewes have a higher twinning ratio when their live body weight increases. The litter size of ewes can generally be predicted by their live weights, with heavier ewes bearing more lambs than ewes bearing a single lamb (Al-Thuwain and Al-Hadi, 2022). Furthermore, sheep reproductive traits are a complex combination of endocrine signals exchanged among the pituitary gland, ovary, and adipose tissues. The adipose tissue and its secreted adipokines play a crucial

role in all aspects of reproduction (Al-Thuwaini, 2022). Adipokines regulate numerous physiological activities, serving as energy sensors and communicating body energy levels to hypothalamic neurons. For instance, ovarian functions are influenced by adipokines, impacting puberty, estrus behavior, follicular development, and ovulation, as well as the formation and functioning of the corpus luteum in livestock (Mishra et al., 2016).

Leptin is one of these adipokines that acts centrally in the hypothalamus to promote the release of gonadotropins, which are essential for initiating and maintaining the reproductive cycle, by increasing neuronal activity and secretion of gonadotropin-releasing hormones (GnRH) (Hausman et al., 2012; Kuźnicka et al., 2020). Neuropeptide Y, proopiomelanocortin, and kisspeptin are the pathways that interact with leptin to affect GnRH. Moreover, leptin affects follicular and luteal steroidogenesis in the ovary by altering the pituitary gland's sensitivity to GnRH (Hausman et al., 2012). A study conducted in animals found that administering leptin stimulates the production of gonadotropins, specifically follicle-stimulating hormone (FSH) and luteinizing hormone (LH), and restores fertility (Priyadarshini et al., 2015). Additionally, leptin impacts the maturation of oocytes, the rupture of follicles, and the development of the corpus luteum (Kuźnicka et al., 2020). In sheep, leptin and leptin receptor proteins regulate oocyte maturation and embryo development across all stages of ovarian follicle development. Furthermore, sheep with high leptin levels exhibit improved antrum formation and oocyte production rates, and faster maturation of their oocytes (Ma et al., 2022). The results of this study revealed that ewes with single births had the lowest leptin concentrations, while ewes with twin births had the highest concentrations. This may be due to the inverse relationship between leptin and estradiol levels. Leptin may suppress estradiol synthesis, thereby promoting the development of additional vesicles in sheep (Bobowiec et al., 2001). Additionally, the results showed a positive relationship between litter size and leptin levels. Interestingly, leptin concentration has a positive correlation with litter size ($P \leq 0.01$), as confirmed in the correlation studies by Kuźnicka et al. (2020). Another crucial adipokine is visfatin, which has been identified in the corpus luteum, ovarian follicles, cumulus cells, and oocytes of bovines, resulting in increased levels of progesterone and estradiol (Reverchon et al., 2016). Furthermore, visfatin stimulates the release of progesterone in the corpus luteum of water buffalo (Thakre et al., 2021). The hypothalamic-pituitary-gonadal axis (HPG), including the pituitary, could also be influenced by visfatin, in addition to its direct effect on the reproductive system. The HPG axis expresses visfatin in all of its structures (Szymanska et al., 2023a). Additionally, visfatin could influence LH and FSH secretion via the INSR, AKT/

PI3K, MAPK/ERK1/2, and AMPK signaling pathways in porcine anterior pituitary cells, thereby contributing to the physiological integration of reproduction and energy balance (Szymanska et al., 2023b). However, no publications have examined the effects of visfatin on litter size in Awassi ewes.

CONCLUSIONS AND RECOMMENDATIONS

There was an association between the litter size of Awassi ewes and adipose tissue biomarkers. Ewes with twin births had higher live body weights, leptin, and visfatin levels when compared to those with single births. Determining the association between litter size and adipose tissue biomarkers could improve various aspects of animal production and may provide further evidence that adipose tissue biomarkers are a key regulator of reproductive function.

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NOVELTY STATEMENT

This study investigated the relationship between the number of lambs born to ewes and biomarkers found in adipose tissue for the first time. The Awassi ewes with twins exhibited elevated levels of leptin and visfatin, making them potential candidates for further research in sheep breeding and reproduction.

AUTHORS CONTRIBUTION

Tahreer M. AL-Thuwaini designed the idea and wrote the draft of the manuscript. Suroor H. Mohammed carried out the laboratory analysis. Both authors checked and approved the final version of the manuscript for publishing in the Journal of Animal and Health Production.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

REFERENCES

Ajafar MH, Kadhim AH, Al-Thuwaini TM (2022). The reproductive traits of sheep and their influencing factors. *Rev. Agric. Sci.*, 10: 82-89. https://doi.org/10.7831/ras.10.0_82
 Al-Jaryan IL, Al-Thuwaini TM, Merzah LH, Alkhammas AH (2023). Reproductive physiology and advanced technologies in sheep reproduction. *Rev. Agric. Sci.*, 11: 171-180. https://doi.org/10.7831/ras.11.0_171

Al-Jumaili WS, Kadhim AH, Al-Thuwaini TM (2023). Polymorphism of the ADIPOQ gene and its association with productive traits in Awassi Ewes. *Mol. Biol. Rep.*, 50(1): 913-917. <https://doi.org/10.1007/s11033-022-07975-0>
 Alkhammas AH, Al-Thuwaini TM (2023). Association of birth type and LHX4 gene polymorphism with reproductive hormones, growth hormone, and prolactin in Awassi ewes. *Mol. Biol. Rep.*, 50(4): 3951-3956. <https://doi.org/10.1007/s11033-023-08285-9>
 Al-Thuwaini TM (2022). Adiponectin and its physiological function in ruminant livestock. *Rev. Agric. Sci.*, 10: 115-122. https://doi.org/10.7831/ras.10.0_115
 Al-Thuwaini TM (2021). The relationship of hematological parameters with adaptation and reproduction in sheep; A review study. *Iraqi J. Vet. Sci.*, 35(3): 575-580. <https://doi.org/10.33899/ijvs.2020.127253.1490>
 Al-Thuwaini TM, Al-Hadi ABA (2022). Association of lamb sex with body measurements in single and twin on the Awassi ewes. *Adv. Anim. Vet. Sci.*, 10(8): 1849-1853. <https://doi.org/10.17582/journal.aavs/2022/10.8.1849.1853>
 Al-Thuwaini TM, Kareem ZA (2022). Novel missense variant L46Q of fatty acid synthase gene and fatty acids content in Awassi sheep. *Acta Sci. Anim. Sci.*, pp. 44. <https://doi.org/10.4025/actascianimsci.v44i1.56273>
 Bobowiec R, Kosior-Korzecka U, Martelli F (2001). Plasma leptin in sheep with single and double ovulation. *Medycyna Weterynaryjna*, 57(2): 122-126.
 Diot M, Reverchon M, Rame C, Froment P, Brillard JP, Brière S, Levêque G, Guillaume D, Dupont J (2015). Expression of adiponectin, chemerin and visfatin in plasma and different tissues during a laying season in Turkeys. *Reprod. Biol. Endocrinol.*, 13(1): 1-14. <https://doi.org/10.1186/s12958-015-0081-5>
 Estienne A, Reverchon M, Partyka A, Bourdon G, Grandhay J, Barbe A, Caldas-Silveira E, Rame C, Nizański W, Froment P, Dupont J (2020). Chemerin impairs *in vitro* testosterone production, sperm motility, and fertility in chicken: Possible involvement of its receptor CMKLR1. *Cells*, 9(7): 1599. <https://doi.org/10.3390/cells9071599>
 Fontanesi L (2016). Metabolomics and livestock genomics: Insights into a phenotyping frontier and its applications in animal breeding. *Anim. Front.*, 6(1): 73-79. <https://doi.org/10.2527/af.2016-0011>
 Goldansaz SA, Guo AC, Sajed T, Steele MA, Plastow GS, Wishart DS (2017). Livestock metabolomics and the livestock metabolome: A systematic review. *PLoS One*, 12(5): e0177675. <https://doi.org/10.1371/journal.pone.0177675>
 Goldansaz SA, Markus S, Plastow G, Wishart DS (2022). Predictive blood biomarkers of sheep pregnancy and litter size. *Sci. Rep.*, 12(1): 10307. <https://doi.org/10.1038/s41598-022-14141-w>
 Habtegiorgis K, Haile A, Getachew T, Jimma A, Gemiyo D (2022). Litter size, litter weight, and lamb survivability of Doyogena sheep managed under community-based breeding program in Ethiopia. *Heliyon*, 8(11). <https://doi.org/10.1016/j.heliyon.2022.e11576>
 Hausman GJ, Barb CR, Lents CA (2012). Leptin and reproductive function. *Biochimie*, 94(10): 2075-2081. <https://doi.org/10.1016/j.biochi.2012.02.022>
 Häussler S, Sadri H, Ghaffari MH, Sauerwein H (2022). Symposium review: Adipose tissue endocrinology in the periparturient period of dairy cows. *J. Dairy Sci.*, 105(4): 2075-2081.

- 3648-3669. <https://doi.org/10.3168/jds.2021-21220>
- Ibrahim AH (2021). Polymorphisms in hormone-sensitive lipase and leptin receptor genes and their association with growth traits in Barki lambs. *Vet. World*, 14(2): 515. <https://doi.org/10.14202/vetworld.2021.515-522>
- Kadhem AF, Al-Thuwaini TM (2022). Influence of litter size on the hematologic profile of Awassi ewes during gestation and lactation. *Vet. Integr. Sci.*, 20(3): 625-633. <https://doi.org/10.12982/VIS.2022.047>
- Kaminski T, Kiezun M, Zaobidna E, Dobrzyn K, Wasilewska B, Mlyczynska E, Rytelewska E, Kisielewska K, Gudelska M, Bors K, Smolinska N (2021). Plasma level and expression of visfatin in the porcine hypothalamus during the estrous cycle and early pregnancy. *Sci. Rep.*, 11(1): 8698. <https://doi.org/10.1038/s41598-021-88103-z>
- Kuźnicka E, Kunowska-Słószarz M, Gabryszuk M (2020). The ovulation rate, plasma leptin concentration, and litter size of a local ewe breed kept in a barn versus those kept under an overhead shelter. *Agriculture*, 10(9): 403. <https://doi.org/10.3390/agriculture10090403>
- Ma S, Ji X, Cang M, Wang J, Yu H, Liu Y, Zhang W, Wu Y, Zhao S, Cao G, Tong B (2022). Association analysis between novel variants in LEPR gene and litter size in Mongolia and Ujimqin sheep breeds. *Theriogenology*, 183: 79-89. <https://doi.org/10.1016/j.theriogenology.2022.02.014>
- McNamara JP, Huber K (2018). Metabolic and endocrine role of adipose tissue during lactation. *Ann. Rev. Anim. Biosci.*, 6: 177-195. <https://doi.org/10.1146/annurev-animal-030117-014720>
- Mishra SR, Bharati J, Bharti MK, Kar D, Sahoo PR (2016). Adipokines as metabolic modulators of ovarian functions in livestock: A mini-review. *J. Adv. Vet. Anim. Res.*, 3(3): 206-213. <https://doi.org/10.5455/javar.2016.c158>
- Mohammed MH, Al-Thuwaini TM, Al-Shuhaib MBS (2022). High association of a novel variant in the adiponectin gene with the litter size in Awassi ewes. *J. Saudi Soc. Agric. Sci.*, 21(5): 296-301. <https://doi.org/10.1016/j.jssas.2021.09.007>
- Priyadarshini L, Yadav AK, Singh HS, Mishra A, Jain AK, Ahirwar MK (2015). Role of leptin in physiology of animal reproduction. A review. *Agric. Rev.*, 36(3): 235-240. <https://doi.org/10.5958/0976-0741.2015.00027.6>
- Ptacek M, Duchacek J, Stádník L, Beran J, Stolec L (2014). Effects of ewes' live weight and backfat thickness at mating on fertility and production performance in Suffolk sheep and their crosses. *Bulgar. J. Agric. Sci.*, 20(5): 1261-1267.
- Reverchon M, Rame C, Bunel A, Chen W, Froment P, Dupont J (2016). Visfatin (NAMPT) improves *in vitro* IGF1-induced steroidogenesis and IGF1 receptor signaling through SIRT1 in bovine granulosa cells. *Biol. Reprod.*, 94(3): 54-61. <https://doi.org/10.1095/biolreprod.115.134650>
- Sarvinda DT, Bintara S, Budisatria IGS, Kustantinah K, Baliarti E (2022). The effect of litter size on ewe and their lamb performances under intensive management system. *Adv. Biol. Sci. Res.*, 18: 157-161. <https://doi.org/10.2991/absr.k.220207.033>
- Szymanska K, Zaobidna E, Rytelewska E, Mlyczynska E, Kurowska P, Dobrzyn K, Kiezun M, Kaminska B, Smolinska N, Rak A, Kaminski T (2023a). Visfatin in the porcine pituitary gland: Expression and regulation of secretion during the oestrous cycle and early pregnancy. *Sci. Rep.*, 13(1): 18253. <https://doi.org/10.1038/s41598-023-45255-4>
- Szymanska K, Rytelewska E, Zaobidna E, Kiezun M, Gudelska M, Kopij G, Dobrzyn K, Mlyczynska E, Kurowska P, Kaminska B, Kaminski T (2023b). The effect of visfatin on the functioning of the porcine pituitary gland: An *in vitro* study. *Cells*, 12(24): 2835. <https://doi.org/10.3390/cells12242835>
- Thakre A, Gupta M, Magar SP, Bhiram KB, Sardar VM, Korde JP, Bonde SW, Hyder I (2021). Transcriptional and translational abundance of visfatin (NAMPT) in buffalo ovary during estrous cycle and its *in vitro* effect on steroidogenesis. *Domest. Anim. Endocrinol.*, 75: 106583. <https://doi.org/10.1016/j.domaniend.2020.106583>
- Xu XC, Li BB, Wei X, Yang YX, Wang XL, Chen YL (2015). Differential expression of peroxisome proliferator-activated receptor γ , fatty acid synthase, and hormone-sensitive lipase in fat-tailed and thin-tailed sheep breeds. *Genet. Mol. Res.*, 14(4): 15624-15633. <https://doi.org/10.4238/2015.December.1.14>
- Yue Y, Cheng X, Zhao SG, Liu Z, Liu LS, Zhou R, Wu JP, Brown MA (2016). Effects of tail docking on the expression of genes related to lipid metabolism in Lanzhou fat-tailed sheep. *Genet. Mol. Res.*, 15(1). <https://doi.org/10.4238/gmr.15017323>