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# Association between c.1189G > A single nucleotide polymorphism of *GDF9* gene and litter size in goats: A meta-analysis

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ABSTRACT

Litter size is one of the most important traits in goat production and breeding. The most common and presumed single nucleotide polymorphism (SNP) detected in the *Growth Differentiation Factor 9* gene is c.1189G > A (rs637044681, Ensembl) which results in an altered sequence of the encoded protein. In some studies, there was no effect of this SNP on litter size, while in other studies there was an effect. In the present study there was a meta-analysis conducted by pooling results from 11 eligible published studies to investigate effects of c.1189G > A polymorphism on litter size using four different genetic models including dominant (AA + AG compared with GG), recessive (AA compared with AG + GG), additive (AA compared with GG) and co-dominant (AA + GG compared with AG). Data were analyzed using fixed and random-effect models based on the I-squared value. Results indicate the c.1189G > A polymorphism is positively associated with litter size with use of the dominant model (SMD = 0.093, 95% CI = 0.028 to 0.158, *P*-Value = 0.005). There, however, was no effect of the c.1189G > A polymorphism using the recessive (SMD = 0.065, 95% CI = -0.164 to 0.295, *P*-Value = 0.577), additive (SMD = 0.172, 95% CI = -0.169 to 0.513, *P*-Value = 0.324) and co-dominant (SMD = -0.083, 95% CI = -0.200 to 0.034, *P*-Value = 0.164) genetic models. Results from use of the sensitivity analysis indicate the GG genotype affect litter size with use of the additive model ($P < 0.01$). The results from this meta-analysis indicate the GG genotype is associated with litter size in goats.

## 1. Introduction

The population of goats is increasing worldwide (FAO, 2016). An important reason of increased numbers of goats could be due to the fact that goats consume less water and plants that other species will not consume such as sheep and cows. Furthermore, goats can easily adapt to harsh and marginal regions of the world. One of the most considerable goals in animal breeding is to improve reproductive traits, because improvement of these traits leads to increased selection intensity and genetic progress of other traits, especially production traits.

It is clear that reproductive traits have a high economic value in animal breeding including goat farming. Considering the low heritability and late expression of these traits during an animal's life, using traditional methods of selective breeding leads to slow progress in improvement of the reproductive performance (Pardeshi et al., 2005). Using new selection methods such as marker assisted selection (MAS) to discover genes affecting reproductive performance could be an appropriate alternative method to overcome this problem in animal breeding (Williams, 2005). Results of recent studies indicate litter size can be regulated by a set of genes that are known as fecundity genes due to the association with fecundity. These genes each have a small effect on reproductive

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performance, but when considered together there can be considerable improvement in reproductive traits such as litter size (Shokrollahi and Morammazi, 2018).

One of the best known and important fecundity genes that has been widely studied in goats is *Growth Differentiation Factor 9* (*GDF9*) which belongs to *Transforming Growth Factor β* (*TGFβ*) superfamily. The protein for which this gene encodes has an important function during early folliculogenesis in female reproduction as a growth and differentiation factor secreted by oocytes in mammals (Elvin et al., 1999). Results of several studies indicate *GDF9* gene is involved in regulating granulosa cell enzymes which are essential for cumulus expansion and maintenance of an optimal oocyte microenvironment through an oocyte-somatic cell interaction and through synergistic actions along with *Bone Morphogenetic Protein 15* (*BMP15*) gene (Elvin et al., 1999; Juengel and McNatty, 2005). Hence, the combined actions of proteins encoded by these two genes is essential for normal ovulation, fertilization and female reproduction (Juengel and McNatty, 2005). The *GDF9* gene has a coding region of 4720 base pairs in length located on Chromosome 7 of goats and there are two exons. The *GDF9* gene has been studied and there are single nucleotide polymorphisms (SNPs) and associations of these SNPs with litter size in goats.

Several allelic variants of the *GDF9* gene have been reported in studies conducted on different breeds of goats. The most common SNP for which there are inconsistent thoughts as to whether the gene containing this SNP encodes for a protein important in goats fecundity is c.1189G > A located in Exon 2 of this gene. There have been several reports that there is an effect of the c.1189G > A polymorphism on litter size in goats (Dong and Du, 2011; An et al., 2012; Wang et al., 2013; Zhu et al., 2013; Zhao et al., 2016; Wang et al., 2019), whereas there are other reports where there was no association between this SNP and litter size (Feng et al., 2010; Dong and Du, 2011; Ren et al., 2012; Ahlawat et al., 2015, 2016; Shokrollahi and Morammazi, 2018). In some of these studies there was less than desirable statistical power to validate positive or negative effects of the c.1189G > A variant on litter size, as a consequence of small sample sizes. To address this problem, large sample sizes or meta-analyses are required to determine genetic effects of the c.1189G > A polymorphism on litter size in goats.

Meta-analyses are useful approaches for integration of results reported in different studies, and to provide a large data set to assess by pooling the results of these studies. Accordingly, by conducting meta-analyses there can be compensation for small sample sizes of individual studies by increasing the statistical power and precision of estimates of a SNP effect on a trait (Young, 2015). To our knowledge, no meta-analyses have been conducted on the association of SNPs with reproductive traits in small ruminants. A meta-analysis was conducted, therefore, by pooling results from all published studies in scientific journals and investigating the effect of the c.1189G > A polymorphism, the most common detected allelic variant of the *GDF9* gene, on litter size in goats.

## 2. Methods

### 2.1. Search strategy for identification of relevant studies

There was use of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist criteria to select eligible articles for this meta-analysis (Moher et al., 2009). All related journals and databases including PubMed, Science Direct, Wiley Online Library, Springer, Magiran (in Persian) and CNKI (in Chinese) were independently searched by two reviewers (P.M. and J.R.) to find relevant studies applying following combination of search terms: “*GDF9*”, “*GDF-9*”, “*Growth Differentiation Factor 9*”, “litter size”, “goat”, “prolificacy”, “SNP”, “polymorphism”, “fecundity” and “reproductive traits”. Furthermore, reference lists of the papers that were located were scrutinized to identify qualified studies that might have not been located in the journals and database search processes. All inconsistencies, regarding inclusion of results from manuscripts and exclusion of studies, were settled by the third investigator (M.R.).

### 2.2. Inclusion and exclusion criteria

Studies were considered to be eligible for meta-analysis if the following criteria were met with there being: (1) a reporting about the c.1189G > A polymorphism; (2) a sample size reported for each genotype; (3) a report on the association between SNP of interest and litter size; (4) least square means reported for each genotype; and (5) a standard deviation or standard error reported for each lsmean. The exclusion criteria were as follows if there were: (1) results reported only in the form of an abstract; (2) insufficient data from the study that was reported; (3) duplications of results from the same study in different articles; and (4) multiple studies reported in a review paper and no original data reported.

### 2.3. Data extraction

Two researchers (P.M. and J.R.) independently extracted data from selected studies based on the specified inclusion and exclusion criteria to validate precision of extracted data. The extracted data included the first author’s name, year of publication, goat breed studied, sample size, least square mean and standard error/standard deviation for data from each genotype. Standard deviations were calculated from the standard errors of means with use of the sample size of genotypes using following equation:

$$SD = SE\sqrt{N}$$

where $SE$ is the standard error of mean for genotypes and $N$ is sample size of genotypes. When it was required, pooled least square means and standard deviations were computed using the approach described in Cochrane Handbook for Systematic Reviews of Interventions (Higgins and Green, 2011) as follows:

$$M_{pooled} = \frac{N_1M_1 + N_2M_2}{N_1 + N_2}$$

$$SD_{pooled} = \sqrt{\frac{(N_1 - 1)SD_1^2 + (N_2 - 1)SD_2^2 + \frac{N_1N_2}{N_1 + N_2}(M_1^2 + M_2^2 - 2M_1M_2)}{N_1 + N_2 - 1}}$$

where $N_1$ and $N_2$ are sample sizes, $M_1$ and $M_2$ are least square means and $SD_1$ and $SD_2$ are standard deviations reported for the first and second groups, respectively.

### 2.4. Statistical analysis

Data were analyzed using Review Manager v5.3 software. The Standardized Mean Difference (SMD) method was used to determine the effect size, because with all studies there was assessment of the same outcome, however, measurements did not always occur in the same way (Higgins and Green, 2011). The association between the c.1189G > A polymorphism and litter size in goats was investigated using dominant (AA + AG compared with GG), recessive (AA compared with AG + GG), additive (AA compared with GG) and co-dominant (AA + GG compared with AG) genetic models.

Heterogeneity of data among studies was evaluated using the Cochran's Q test considering $P < 0.05$ to be significant. Nevertheless, a non-significant value for Q does not necessarily indicate that the studies were conducted with the same animal population, because of small sample sizes for the comparisons and a small number of comparisons contributing to the meta-analysis (Vesterinen et al., 2014). To overcome this issue, the $I^2$ statistic was used to quantify heterogeneity of studies with a range from 0 to 100 percent ($I^2 > 50\%$ indicating moderate to high heterogeneity and was considered to be significant (Vesterinen et al., 2014). A fixed effects model was used to analyze data when there was no obvious heterogeneity among studies; otherwise, a random effects model was applied. A sensitivity analysis was applied by removing one study at a time to detect stability of overall results. Funnel plots and Egger's linear regression test were performed to detect publication bias among studies (Egger et al., 1997).

## 3. Results

### 3.1. Characteristics of included studies

The PRISMA flow diagram of studies inclusion and exclusion criteria is depicted in Fig. 1. A total of 45 articles were identified through searching different databases including PubMed, Science Direct, Wiley Online Library, Springer, CNKI (in Chinese) and

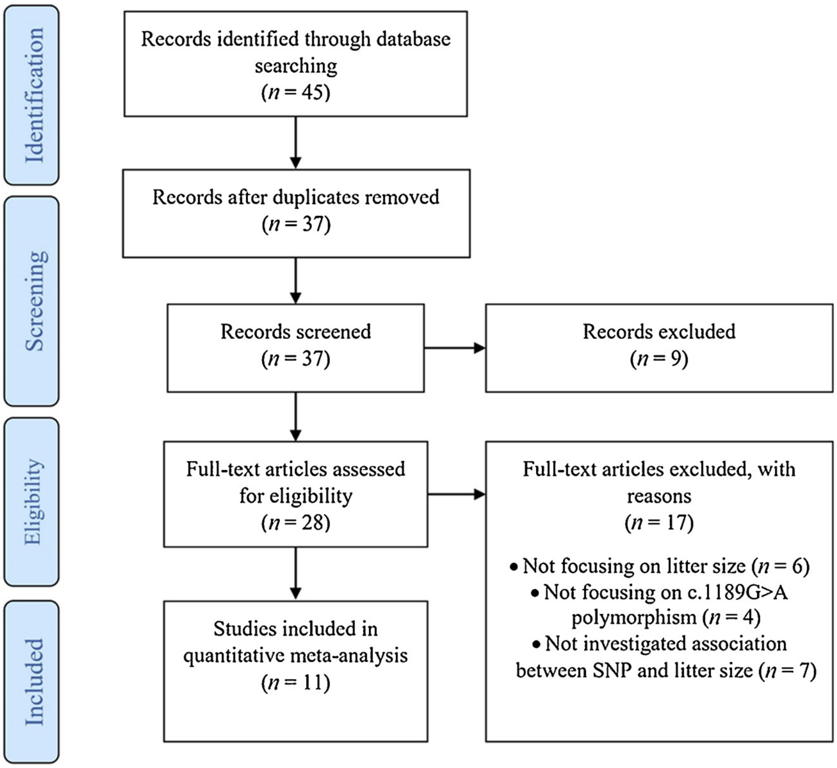


**Fig. 1.** The PRISMA flow diagram showing inclusion and exclusion criteria.

**Table 1**
Characteristics of studies included in this meta-analysis.

| First author | Year of publication | Breed | Genotypes | | | LSmeans[1] ± SE | | | Significant[2] |
|---|---|---|---|---|---|---|---|---|---|
| | | | AA | AG | GG | AA | AG | GG | |
| Ahlawat | 2015 | Black Bengal | 10 | 69 | 376 | 1.52 ± 0.21 | 1.59 ± 0.09 | 1.52 ± 0.05 | No |
| Ahlawat | 2016 | Barbari (Ba) | 0 | 10 | 39 | NA[3] | 1.80 ± 0.15 | 1.06 ± 0.07 | No |
| Ahlawat | 2016 | Beetal (Be) | 2 | 19 | 7 | 1.50 ± 0.33 | 1.68 ± 0.11 | 1.71 ± 0.17 | No |
| Ahlawat | 2016 | Black Bengal (BB) | 2 | 16 | 92 | 1.83 ± 0.33 | 1.56 ± 0.11 | 1.47 ± 0.05 | No |
| Ahlawat | 2016 | Ganjam (Ga) | 0 | 7 | 43 | NA | 1.00 ± 0.17 | 1.05 ± 0.07 | No |
| Ahlawat | 2016 | Osmanabadi (Os) | 0 | 13 | 28 | NA | 1.21 ± 0.13 | 1.14 ± 0.09 | No |
| Ahlawat | 2016 | Sangamneri (Sa) | 0 | 22 | 28 | NA | 1.30 ± 0.10 | 1.26 ± 0.09 | No |
| An | 2012 | Three Breeds | 362 | 168 | 95 | 1.82 ± 0.02 | 1.79 ± 0.03 | 1.47 ± 0.04 | Yes |
| Dong | 2011 | Jining Grey (JG) | 10 | 147 | 73 | 2.66 ± 0.18 | 2.77 ± 0.07 | 2.69 ± 0.06 | No |
| Dong | 2011 | Lubei White (LW) | 10 | 38 | 32 | 2.18 ± 0.14 | 2.60 ± 0.07 | 2.44 ± 0.06 | Yes |
| Dong | 2011 | Yimeng Black (YB) | 2 | 59 | 20 | 1.46 ± 0.09 | 1.11 ± 0.05 | 1.08 ± 0.03 | Yes |
| Feng | 2010 | Jining Grey | 3 | 55 | 120 | 2.20 ± 0.22 | 2.35 ± 0.17 | 2.43 ± 0.12 | No |
| Ren | 2012 | Chong-ming White | 0 | 17 | 21 | NA | 1.90 ± 0.77 | 2.12 ± 0.99 | No |
| Shokrollahi | 2018 | Markhoz | 100 | 25 | 39 | 1.21 ± 0.06 | 1.17 ± 0.07 | 1.27 ± 0.04 | No |
| Wang | 2013 | Henan Dairy | 2 | 54 | 112 | 1.67 ± 0.12 | 2.32 ± 0.17 | 1.91 ± 0.12 | Yes |
| Wang | 2018 | Cashmere | 451 | 679 | 381 | 1.63 ± 0.03 | 1.72 ± 0.02 | 1.65 ± 0.03 | Yes |
| Zhao | 2016 | Cashmere | 66 | 354 | 341 | 1.41 ± 0.04 | 1.49 ± 0.02 | 1.58 ± 0.02 | Yes |
| Zhu | 2013 | Big Foot Black | 33 | 17 | 46 | 2.96 ± 0.25 | 1.84 ± 0.42 | 1.66 ± 0.27 | Yes |
| Zhu | 2013 | Jintang | 44 | 21 | 16 | 2.77 ± 0.41 | 1.78 ± 0.28 | 1.58 ± 0.27 | Yes |

[1] Least Square Means reported for each genotype related to litter size in studies included in this meta-analysis.
[2] Statistically significant results reported for association between genotypes and litter size in each study.
[3] Not Available.

Magiran (in Persian). There were eight studies reported in difference articles removed for being duplicative. Among the 37 remaining articles, nine articles were in the form of abstract, thus these were rejected for inclusion in the meta-analysis. Remaining studies were screened to exclude irrelevant studies and 17 articles were rejected, because there was not investigation of the SNP and/or trait of interest and in some of these articles there was only a reporting of the c.1189G > A polymorphism frequencies and not investigation of an association with litter size. In conclusion, 11 studies involving 4796 goats were selected to be included in the meta-analysis, with nine of these articles being written in English and other two in Chinese. Among the studies that met the criteria for selection for inclusion in the meta-analysis, in three studies there were investigations of 11 different goat breeds and each breed was treated as a separate study in this meta-analysis. The characteristics for studies that were included in the meta-analysis are presented in Table 1.

### *3.2. Evaluation of heterogeneity among studies*

The results from using the Cochran's Q heterogeneity test and I-squared statistic ($I^2$) for all genetic models are presented in Table 2. The calculated $I^2$ for the recessive, additive and co-dominant models was greater than 50%, hence the random-effects model was used to investigate the association between the c.1189G > A polymorphism and litter size in goats. The $I^2$ that was derived with use of the dominant model was less than 50%, and for this reason, the fixed-effects model was applied to analyze data (Vesterinen et al., 2014).

### *3.3. Meta-analysis of the relationship between the c.1189G > A polymorphism and litter size*

The meta-analysis results for association between the SNP and trait of interest using the four genetic models are summarized in Table 3 and Figs. 2 through 5 . There was no association between the c.1189G > A polymorphism and litter size using the recessive (SMD = 0.065, 95% CI = -0.164 to 0.295, *P*-Value = 0.577), additive (SMD = 0.172, 95% CI = -0.169 to 0.513, *P*-Value = 0.324) and co-dominant (SMD = -0.083, 95% CI = -0.200 to 0.034, *P*-Value = 0.164) genetic models. There, however, was an association ($P < 0.01$) between the c.1189G > A polymorphism and litter size using the dominant model (SMD = 0.093, 95% CI = 0.028 to

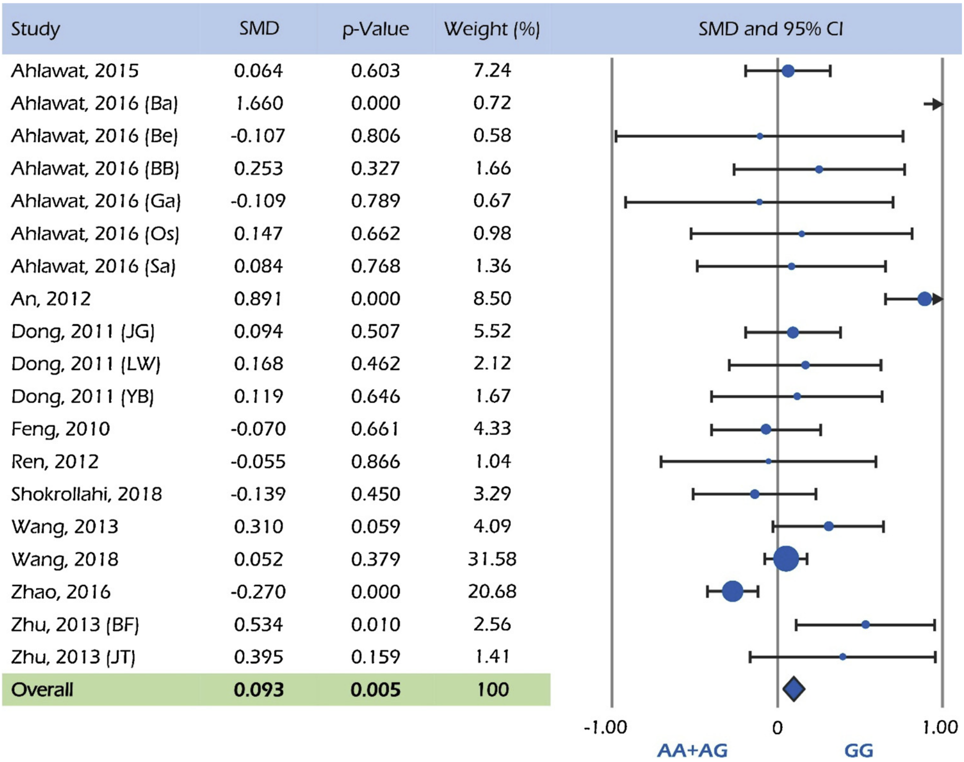
**Table 2**
Heterogeneity test results for applied genetic models.

| Comparison | Heterogeneity analysis | | | Model |
|---|---|---|---|---|
| | Q | *P*-Value | $I^2$ (%) | |
| Dominant (AA + AG compared with GG) | 22.45 | 0.072 | 19.82 | Fixed |
| Recessive (AA compared with AG + GG) | 56.62 | 0.000 | 76.04 | Random |
| Additive (AA compared with GG) | 96.83 | 0.000 | 86.56 | Random |
| Co-dominant (AA + GG compared with AG) | 40.27 | 0.002 | 55.30 | Random |

**Table 3**
Meta-analysis of the association between the c.1189G > A polymorphism and litter size.

| Comparison | No. breeds | SMD | 95% Confidence Interval<br>Lower Limit | <br>Upper Limit | *P*-Value |
|---|---|---|---|---|---|
| Dominant (AA + AG compared with GG) | 19 | 0.093 | 0.028 | 0.158 | 0.005 |
| Recessive (AA compared with AG + GG) | 14 | 0.065 | −0.164 | 0.295 | 0.577 |
| Additive (AA compared with GG) | 14 | 0.172 | −0.169 | 0.513 | 0.324 |
| Co-dominant (AA + GG compared with AG) | 19 | −0.083 | −0.200 | 0.034 | 0.164 |

SMD: Standardized Mean Difference.



| Study | SMD | p-Value | Weight (%) |
|---|---|---|---|
| Ahlawat, 2015 | 0.064 | 0.603 | 7.24 |
| Ahlawat, 2016 (Ba) | 1.660 | 0.000 | 0.72 |
| Ahlawat, 2016 (Be) | -0.107 | 0.806 | 0.58 |
| Ahlawat, 2016 (BB) | 0.253 | 0.327 | 1.66 |
| Ahlawat, 2016 (Ga) | -0.109 | 0.789 | 0.67 |
| Ahlawat, 2016 (Os) | 0.147 | 0.662 | 0.98 |
| Ahlawat, 2016 (Sa) | 0.084 | 0.768 | 1.36 |
| An, 2012 | 0.891 | 0.000 | 8.50 |
| Dong, 2011 (JG) | 0.094 | 0.507 | 5.52 |
| Dong, 2011 (LW) | 0.168 | 0.462 | 2.12 |
| Dong, 2011 (YB) | 0.119 | 0.646 | 1.67 |
| Feng, 2010 | -0.070 | 0.661 | 4.33 |
| Ren, 2012 | -0.055 | 0.866 | 1.04 |
| Shokrollahi, 2018 | -0.139 | 0.450 | 3.29 |
| Wang, 2013 | 0.310 | 0.059 | 4.09 |
| Wang, 2018 | 0.052 | 0.379 | 31.58 |
| Zhao, 2016 | -0.270 | 0.000 | 20.68 |
| Zhu, 2013 (BF) | 0.534 | 0.010 | 2.56 |
| Zhu, 2013 (JT) | 0.395 | 0.159 | 1.41 |
| **Overall** | **0.093** | **0.005** | **100** |

**Fig. 2.** Forest plot for association between c.1189G > A polymorphism and litter size using the dominant model for meta-analyses Size of blue circle represents weight of each study; black horizontal line is indicative of confidence interval for each study; Diamond located in the bottom of plot represents summary of results (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

0.158, *P*-Value = 0.005).

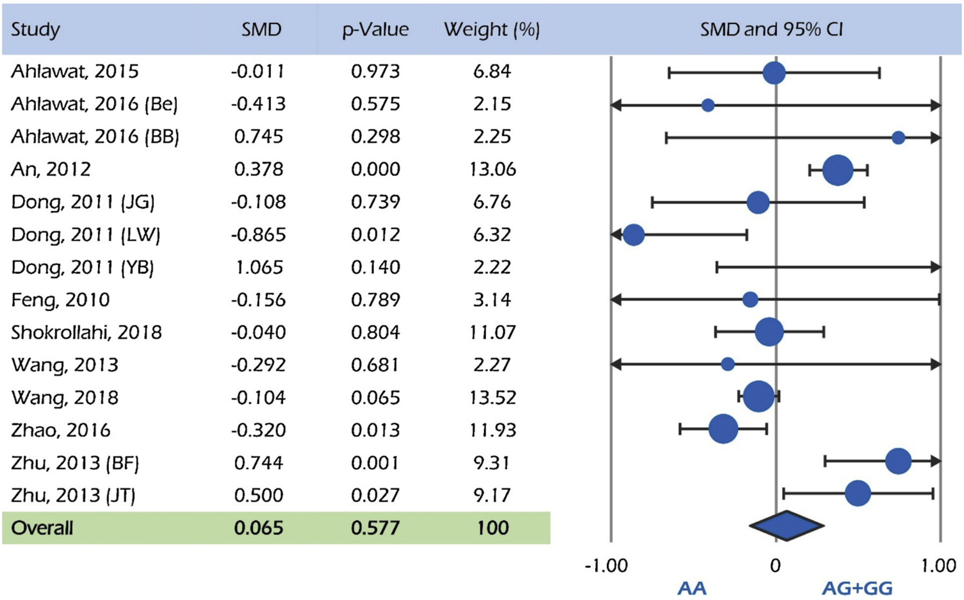
### 3.4. Sensitivity analysis and publication bias

The sensitivity analysis was conducted to assess the effects of each included study in this meta-analysis on the pooled results stability by removing one study at a time. There were not any differences in pooled SMDs before and after removing studies one by one with use of dominant, recessive and co-dominant models indicating there was stability of results with use of the three genetic models. There, however, was an inconsistency in results with use of the sensitivity analysis with a difference being detected for the effect size by not including the study performed by Zhao et al. (2011) for the Cashmere breed with use of the additive model (Fig. 6).

The funnel plots for studies included in the meta-analysis with use of all genetic models are depicted in Fig. 7. The shape of all plots indicate there was no publication bias with use of any of the four models utilized in the present study. Furthermore, the results with use of the Egger’s regression test for all four comparison models indicated there was no publication bias ($P < 0.10$).

## 4. Discussion

During recent years, the effect of single nucleotide polymorphisms in the G*DF9* gene on reproductive traits such as litter size has been widely studied in different breeds of goats. The *GDF9* gene consists of two Exons having 1362 base pairs in total length. The

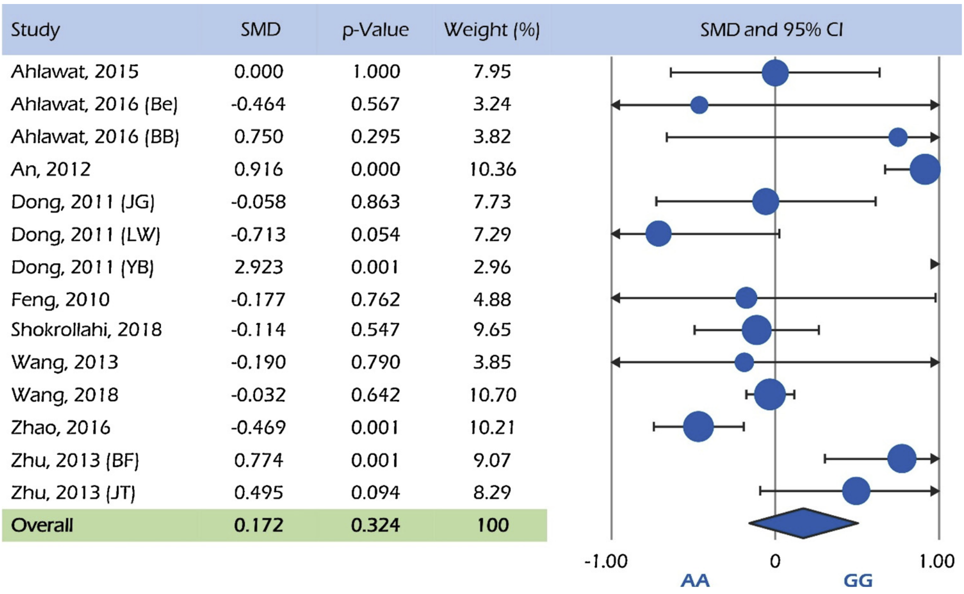


| Study | SMD | p-Value | Weight (%) |
|---|---|---|---|
| Ahlawat, 2015 | -0.011 | 0.973 | 6.84 |
| Ahlawat, 2016 (Be) | -0.413 | 0.575 | 2.15 |
| Ahlawat, 2016 (BB) | 0.745 | 0.298 | 2.25 |
| An, 2012 | 0.378 | 0.000 | 13.06 |
| Dong, 2011 (JG) | -0.108 | 0.739 | 6.76 |
| Dong, 2011 (LW) | -0.865 | 0.012 | 6.32 |
| Dong, 2011 (YB) | 1.065 | 0.140 | 2.22 |
| Feng, 2010 | -0.156 | 0.789 | 3.14 |
| Shokrollahi, 2018 | -0.040 | 0.804 | 11.07 |
| Wang, 2013 | -0.292 | 0.681 | 2.27 |
| Wang, 2018 | -0.104 | 0.065 | 13.52 |
| Zhao, 2016 | -0.320 | 0.013 | 11.93 |
| Zhu, 2013 (BF) | 0.744 | 0.001 | 9.31 |
| Zhu, 2013 (JT) | 0.500 | 0.027 | 9.17 |
| **Overall** | **0.065** | **0.577** | **100** |

**Fig. 3.** Forest plot for association between c.1189G > A polymorphism and litter size using the recessive model for the meta-analysis; For additional details refer to Fig. 2 caption.

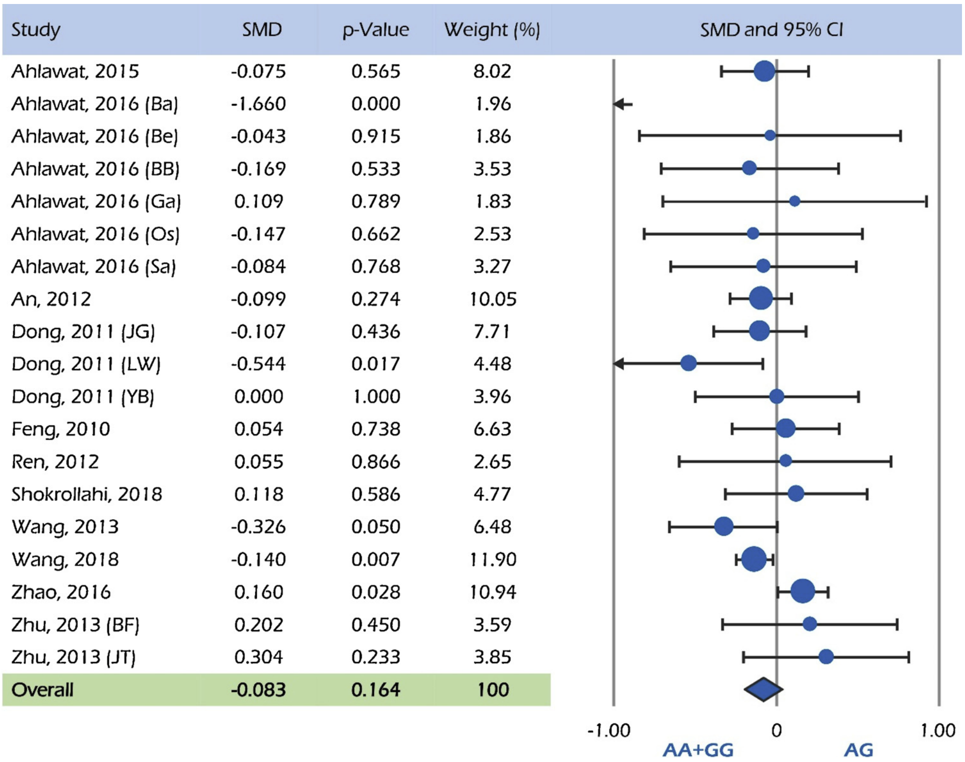
c.1189G > A polymorphism, also known as p.Val397Ile, is the most common polymorphism identified in different breeds of goats. This polymorphism is a missense SNP, therefore, resulting in changes in the amino acid sequence of the respective protein in which valine is replaced with an isoleucine at position 397 of the protein. The c.1189G > A polymorphism, therefore, may be a SNP that could be selected for improving the litter size in goats. From some studies, there have been reports that this SNP is associated with litter size, however, the results of other studies indicate there is no association of this SNP with litter size in goats. These inconsistent results indicate there may be a greater understanding of a SNP-litter size association if there was a meta-analysis conducted which could be a useful technique for determining whether the c.1189G > A polymorphism in the *GDF9* gene was associated with litter size in goats. To the best of our knowledge, this is the first study in which a meta-analysis was conducted using four different genetic models for investigating an association between the c.1189G > A polymorphism and litter size in goats.

The meta-analyses conducted using the recessive and co-dominant models indicated there was no association between the SNP of interest and litter size (Figs. 3 and 5). There, however, was a positive association of the c.1189G > A polymorphism with litter size



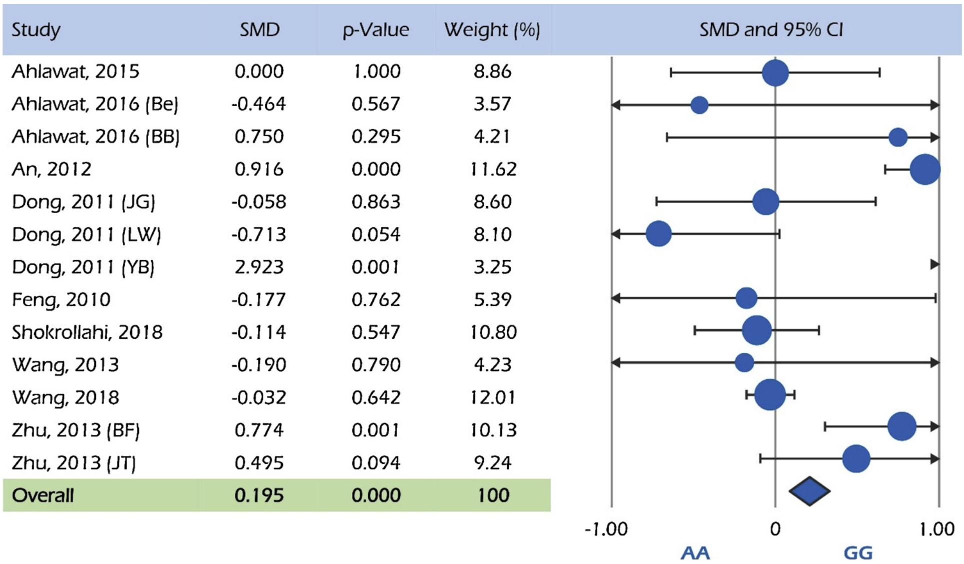
| Study | SMD | p-Value | Weight (%) |
|---|---|---|---|
| Ahlawat, 2015 | 0.000 | 1.000 | 7.95 |
| Ahlawat, 2016 (Be) | -0.464 | 0.567 | 3.24 |
| Ahlawat, 2016 (BB) | 0.750 | 0.295 | 3.82 |
| An, 2012 | 0.916 | 0.000 | 10.36 |
| Dong, 2011 (JG) | -0.058 | 0.863 | 7.73 |
| Dong, 2011 (LW) | -0.713 | 0.054 | 7.29 |
| Dong, 2011 (YB) | 2.923 | 0.001 | 2.96 |
| Feng, 2010 | -0.177 | 0.762 | 4.88 |
| Shokrollahi, 2018 | -0.114 | 0.547 | 9.65 |
| Wang, 2013 | -0.190 | 0.790 | 3.85 |
| Wang, 2018 | -0.032 | 0.642 | 10.70 |
| Zhao, 2016 | -0.469 | 0.001 | 10.21 |
| Zhu, 2013 (BF) | 0.774 | 0.001 | 9.07 |
| Zhu, 2013 (JT) | 0.495 | 0.094 | 8.29 |
| **Overall** | **0.172** | **0.324** | **100** |

**Fig. 4.** Forest plot for association between c.1189G > A polymorphism and litter size using the additive model for meta-analyses; For additional details refer to Fig. 2 caption.



| Study | SMD | p-Value | Weight (%) |
|---|---|---|---|
| Ahlawat, 2015 | -0.075 | 0.565 | 8.02 |
| Ahlawat, 2016 (Ba) | -1.660 | 0.000 | 1.96 |
| Ahlawat, 2016 (Be) | -0.043 | 0.915 | 1.86 |
| Ahlawat, 2016 (BB) | -0.169 | 0.533 | 3.53 |
| Ahlawat, 2016 (Ga) | 0.109 | 0.789 | 1.83 |
| Ahlawat, 2016 (Os) | -0.147 | 0.662 | 2.53 |
| Ahlawat, 2016 (Sa) | -0.084 | 0.768 | 3.27 |
| An, 2012 | -0.099 | 0.274 | 10.05 |
| Dong, 2011 (JG) | -0.107 | 0.436 | 7.71 |
| Dong, 2011 (LW) | -0.544 | 0.017 | 4.48 |
| Dong, 2011 (YB) | 0.000 | 1.000 | 3.96 |
| Feng, 2010 | 0.054 | 0.738 | 6.63 |
| Ren, 2012 | 0.055 | 0.866 | 2.65 |
| Shokrollahi, 2018 | 0.118 | 0.586 | 4.77 |
| Wang, 2013 | -0.326 | 0.050 | 6.48 |
| Wang, 2018 | -0.140 | 0.007 | 11.90 |
| Zhao, 2016 | 0.160 | 0.028 | 10.94 |
| Zhu, 2013 (BF) | 0.202 | 0.450 | 3.59 |
| Zhu, 2013 (JT) | 0.304 | 0.233 | 3.85 |
| **Overall** | **-0.083** | **0.164** | **100** |

**Fig. 5.** Forest plot for association between c.1189G > A polymorphism and litter size using the co-dominant model for meta-analyses; For additional details refer to Fig. 2 caption.



| Study | SMD | p-Value | Weight (%) |
|---|---|---|---|
| Ahlawat, 2015 | 0.000 | 1.000 | 8.86 |
| Ahlawat, 2016 (Be) | -0.464 | 0.567 | 3.57 |
| Ahlawat, 2016 (BB) | 0.750 | 0.295 | 4.21 |
| An, 2012 | 0.916 | 0.000 | 11.62 |
| Dong, 2011 (JG) | -0.058 | 0.863 | 8.60 |
| Dong, 2011 (LW) | -0.713 | 0.054 | 8.10 |
| Dong, 2011 (YB) | 2.923 | 0.001 | 3.25 |
| Feng, 2010 | -0.177 | 0.762 | 5.39 |
| Shokrollahi, 2018 | -0.114 | 0.547 | 10.80 |
| Wang, 2013 | -0.190 | 0.790 | 4.23 |
| Wang, 2018 | -0.032 | 0.642 | 12.01 |
| Zhu, 2013 (BF) | 0.774 | 0.001 | 10.13 |
| Zhu, 2013 (JT) | 0.495 | 0.094 | 9.24 |
| **Overall** | **0.195** | **0.000** | **100** |

**Fig. 6.** Forest plot for association between c.1189G > A polymorphism and litter size using the additive model for meta-analyses after sensitivity analysis; Compare with Fig. 4; For additional details refer to Fig. 2 caption.

using the dominant model. As depicted (by the positioning of the diamond to the right of the line of no effect) in Fig. 2, there was a difference on litter size between animals with the GG genotype and those with AA and/or AG genotypes ($P < 0.05$). Furthermore, for the GG genotype, there was not any difference as a result of the SNP from the AA genotype with use of the additive model for analyses (Fig. 4).

This inconsistency between results obtained by conducting the meta-analyses in the present study could be due to the different models applied to analyze data with use of the dominant and additive models for these analyses. Considering the large amount of

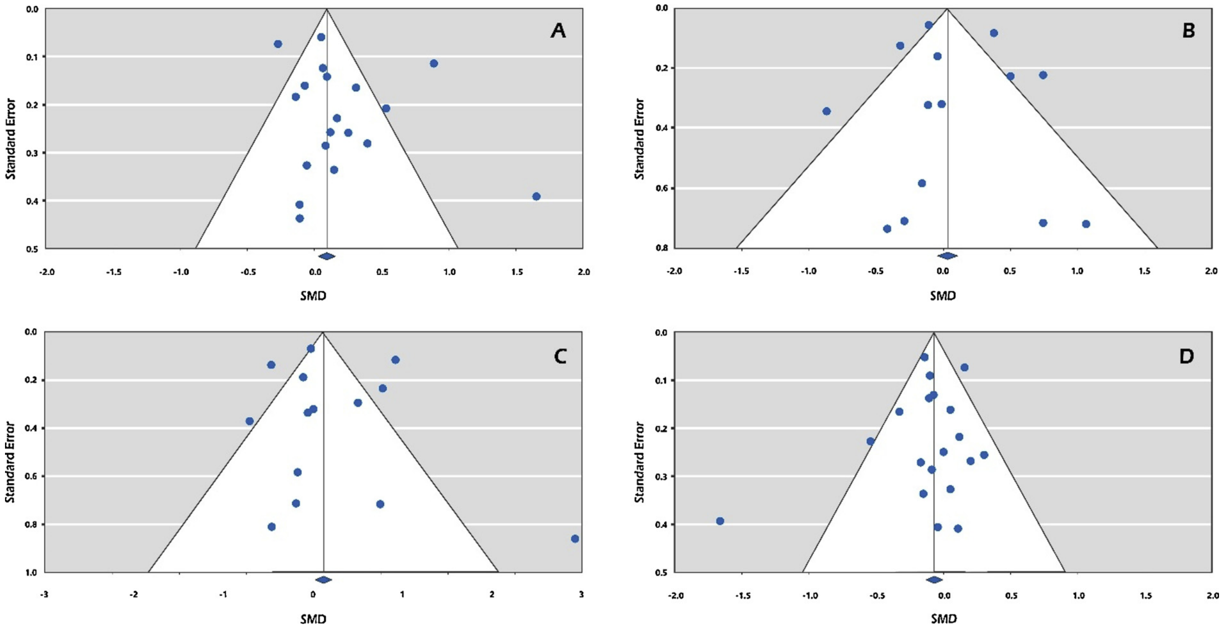


**Fig. 7.** Funnel plots for the publication bias using the dominant model (A); recessive model (B); additive model (C); and co-dominant model (D) for meta-analyses.

heterogeneity among studies with use of the additive model ($I^2 = 86.56\%$) for the meta-analyses, the random-effect model was used to analyze data. With the random-effect model, studies are weighted by the inverse of the sum of within and between study variances. In contrast, only the within study variance is used for weighting studies in analyses fitting the data with the fixed-effect model (Vesterinen et al., 2014). For this reason, there was no difference between AA and GG genotypes with use of the additive model for meta-analyses and this could be resulted from a between study variance, because using a random-effect model affects not only the confidence interval but also the estimate. When heterogeneity exists, therefore, there is the possibility for a different pooled estimate, a wider confidence interval and a larger *P*-Value (Higgins and Thompson, 2002), similar to what occurred with use of the additive model in the present meta-analysis.

To confirm the results obtained using the additive model, a meta-analysis was conducted using a fixed-effect model with the results indicating there was a difference between GG and AA genotypes ($P < 0.01$). The inconsistent meta-analysis results using the fixed and random effect models indicated that in the presence of heterogeneity, the random-effect model will weigh the studies comprising the meta-analysis more equally, resulting in smaller studies having a greater relative effect on the combined overall effect than with use of the fixed-effect model (Sterne et al., 2008). To identify the source of heterogeneity, the study conducted with the Cashmere breed of goats by Zhao et al. (2011) was not included in the meta-analysis and there was re-analysis of the data using the random-effect model. The results with the study of Zhao et al. (2011) excluded indicated the heterogeneity ($I^2 = 19.82\%$) was less and there were similar results with use of the fixed-effect model. Considering these findings, the G allele in the form of GG genotype may be positively associated with litter size in goats.

Generally, heterogeneity is a potential problem for interpreting results when conducting a meta-analysis. For livestock, pooling populations can be a source of heterogeneity in analyzing candidate gene effects, and there can be a separate analysis conducted with populations having different genetic backgrounds that can reduce the effects of population structure on heterogeneity (Hernández-Sánchez et al., 2003). In addition, some factors such as geographic conditions, herd management, nutrition, feeding and sampling difference could affect heterogeneity.

The meta-analysis conducted in the present study has some merit. First, there was inclusion of all studies published in different languages in the analyses. Second, there was a sensitivity analysis conducted by removing a single study at a time to validate the results obtained in the meta-analysis in the present study. Third and most importantly, four different genetic models were used to conduct the analyses including the dominant, recessive, additive and co-dominant models to comprehensively investigate associations between the c.1189G > A polymorphism and litter size in goats. Nevertheless, the meta-analysis conducted in the present study had several limitations. First, the number of studies were limited which may affect the validity of the results from this study. Second, the sample sizes in some studies included in the meta-analysis were not large enough for assessment of associations between the polymorphism and litter size in goats. Third, there was a moderate to high heterogeneity among studies when there was use of the recessive, additive and co-dominant genetic models for conducting the meta-analysis. Fourth, only the effect of genetic factors on litter size was studied; whereas, litter size is a trait that is affected by SNP-SNP and gene-gene interactions which were not considered with the meta-analysis in the present study. Considering these limitations, caution should be taken when interpreting these results and making conclusions.

In summary, with the meta-analysis conducted in the present study, there was a positive association between the c.1189G > A polymorphism and litter size in goats when there was use of the dominant model for these analyses. Furthermore, by conducting the sensitivity analysis by removing studies one at a time, results indicated the c.1189G > A polymorphism is positively associated with litter size when there was use of the additive model for the meta-analysis suggesting that with the GG genotype there is an increase in litter size of goats that have this SNP.

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