# Genetic parameters for the size of udder cisterns in ewes diagnosed by ultrasonography among breeds: Improved Valachian, Tsigai, Lacaune and their crosses

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## SUMMARY

Udder cistern size in ewes was diagnosed by ultrasonography, using a 3.5MHz linear probe applied laterally *from side* or ventrally *from bottom*. The acquired ultrasound images were used to digitally determine the left and right cistern sizes. Both cisterns were scanned approximately 12 hours after the last milking. The area of the left (ALC1; 1933.35 mm$^2$) and right udder cisterns (ARC1; 1970.72 mm$^2$), were determined using *from side* scans of 378 ewes, obtained at different phases of the lactation cycle), for a total of 1198 measurements. The area of the left (ALC2; 2137.67 mm$^2$) and right udder cisterns (ARC2; 2171.12 mm$^2$) as determined using *from bottom* scans, from 265 ewes; for a total of 753 measurements. The sums of both cross-sectional areas detected by the method *from side* (SLRC1) was 3904.07 mm$^2$, and by the method *from bottom* (SLRC2) was 4308.77 mm$^2$. Primary data were processed using REML methodology and the multiple trait animal model, using the programs REMLF90 and VCE 4.0. In the models, *animal* was ascribed as a random additive genetic effect and *ewe* as a permanent effect. Control year (7 or 5 levels), lactation stage (4 levels), breed group (9 levels) and parity (3 levels) were all ascribed as fixed effects. We found higher values of heritability ($h^2$) for the parameters determined by the method *from bottom*. Heritability coefficients for ALC1 and ALC2 were 0.07 and 0.18 respectively, for ARC1 and ARC2 were 0.17 and 0.2 respectively, and for SLRC1 and SLRC2 were 0.12 and 0.17 respectively. Genetic correlations between ARC1 and ARC1 or ARC2 and ARC2 were high ($r_g$= 0.73 and 0.91). Similarly, the correlations between the size of left and/or right cistern and the total size of both cisterns were high using both ways of scanning ($r_g$= 0.90 to 0.98). In conclusion, measuring the size of the udder cisterns *from side* is recommended, although measurements *from bottom* show slightly higher heritability coefficients.

**Paramètres génétiques de la taille des citernes de lait chez les brebis diagnostiqués par échographie parmi les races: Improved Valachian, Tsigai, Lacaune et leurs croisements**

mots-clés aditionnelles

Brebis.
Corrélations génétiques et phénotypiques.
Des citernes de la mamelle.
Échographie.

## RÉSUMÉ

La taille de la citerne de lait a été diagnostiquée chez les brebis en utilisant l'échographie et la sonde linéaire de 3,5 MHz de deux façons: La méthode *de côté* et celle *de bas*. L'échographie a été faite à partir de chaque scan et ensuite la taille du réservoir à gauche et à droite a été mesurée en utilisant la technique numérique. Les deux citernes ont été scannées environ 12 heures après la dernière traite. L'espace gauche de la citerne (ALC1; 1.933.35 mm$^2$) et celui de droite (ARC1; 1.970.72 mm$^2$), détectés par la méthode *de côté*, ont été diagnostiqués à plusieurs reprises chez 378 brebis (pendant l'allaitement ainsi qu'entre lactations); un total de 1198 mesures ont été effectuées. L'espace gauche de la citerne (ALC2; 2.137.67 mm$^2$) et de droite (ARC2; 2.171.12 mm$^2$), détectés par la méthode *du bas*, ont également été diagnostiqués à plusieurs reprises, notamment chez 265 brebis; 753 mesures ont été réalisées au total. La somme des deux zones de section transversale détectées par la méthode *de côté* (SLRC1) était de 3904.07 mm$^2$, et celle détectée par la méthode *de fond* (SLRC2) était 4308.77 mm$^2$. Les données primaires ont été traitées en utilisant la méthodologie REML et le modèle animal trait multiple, en utilisant des programmes comme REMLF90 et VCE 4.0. En plus de l'effet génétique additif aléatoire des animaux et l'effet permanent des brebis, les modèles incluent l'année de contrôle comme facteur fixe (7 ou 5 niveaux), un stade de lactation (4 niveaux), un groupe de race (9 niveaux) et la parité (3 niveaux). Nous avons trouvé des valeurs plus élevées de $h^2$ pour les paramètres diagnostiqués par la méthode *du bas*. Le coefficient d'héritabilité pour ALC1 et ALC2 était de 0.07 et 0.18, respectivement; pour ARC1 et ARC2 de 0.17 et 0.2, resp.; et pour SLRC1 et SLRC2 de 0.12 et 0.17, respectivement. Les corrélations génétiques entre ARC1 et ARC1 ou ARC2 et ARC2 étaient élevées ($r_g$= 0.73 ou 0.910). De même, les corrélations entre la taille de la citerne gauche et/ou droite, et la taille totale des deux citernes étaient élevées avec les deux modes

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de scan ($r_g$= 0.90 à 0.98). En conclusion, la mesure de la taille des citernes de lait par la méthode *de côté* est plus recommandée, bien que les mesures avec la méthode *du bas* montrent des coefficients d'héritabilité légèrement plus élevés.

## INTRODUCTION

In large animal practice, a number of authors have studied the size of udder cisterns (Labussière *et al.*, 1981; Eyduran *et al.*, 2013; Kotb *et al.*, 2014; Makovický *et al.*, 2015a). The different morphological udder traits, milk yield, somatic cell score, udder health, fat or protein content and also their heritability in dairy ewes as well as their correlations with each other were studied (Rovai *et al.*, 2008; De la Fuente *et al.*, 2011; Legaz *et al.*, 2011; Pourlis, 2011; Gelasakis *et al.*, 2012; Makovický *et al.*, 2013, 2014a,b; Pérez-Cabal *et al.*, 2013; Prpić *et al.*, 2013; Ayadi *et al.*, 2014; Makovický *et al.*, 2015b,c; Sari *et al.*, 2015). Ultrasonography is a fast and accurate, noninvasive method for the investigation of mammary gland structures in animals (Fasulkov *et al.*, 2013; Kotb *et al.*, 2014; Fasulkov *et al.*, 2015; Hussein *et al.*, 2015). B-mode ultrasonography is a suitable method for portraying liquid cavities within all types of tissues, including the mammary gland cisternal cavities of dairy cows, goats and sheep (Bruckmaier and Blum, 1992). Many scientific papers describe how the internal structure of the mammary gland can be studied by means of ultrasonography, and several studies have reported about mammary gland ultrasonography in dairy sheep as an efficient method to evaluate the size and the productive capacity of sheep udders (Ruberte *et al.*, 1994; Nudda *et al.*, 2000; Olechnowicz and Jaśkowski, 2009; Fasulkov, 2012; Petridis *et al.*, 2014). Studies employing this method indicate that the mammary cistern size affects milk emission kinetics in dairy sheep, with this effect being greater than the amount of secretory tissue – cistern size affects milk secretion rate and milk emission kinetics during milking (Labussière *et al.*, 1981; Labussière, 1988; Nudda *et al.*, 2000). The method can also be used to estimate the distribution and movements of milk between the udder compartments and for non-invasive dynamic studies on cisternal milk (Caja *et al.*, 2004; Castillo *et al.*, 2008; Rovai *et al.*, 2008). This technique allows non-invasive investigation of the cistern and could be useful as a new approach to study udder changes to accommodate milk accumulation during different milking intervals and after milk letdown (Salama *et al.*, 2004). Ultrasonography has been used as a non-invasive method to study the internal structure of the mammary gland in dairy goats (Melo *et al.*, 2012; Díaz *et al.*, 2013; Alejandro *et al.*, 2014; Dar *et al.*, 2014; Fasulkov *et al.*, 2014a,b; Santos *et al.*, 2014, 2015), and cows (Bruckmaier *et al.*, 1994; Ayadi *et al.*, 2003; Bobić *et al.*, 2014; Esselburn *et al.*, 2015; Khoramian *et al.*, 2015) in order to measure the milk storage capacity within the udder. The aim of the present study was to estimate genetic parameters underlying selected udder measurements as detected by ultrasonography.

## MATERIAL AND METHODS

Nine different sheep genotypes were included in this seven year long experiment to determine the udder size traits of the ewes belonging to the following populations: Improved Valachian (IV), n= 219; Improved Valachian×East Friesian (25%), n= 63; Improved Valachian×East Friesian (50%), n= 84; Improved Valachian×East Friesian (75%), n= 80; Tsigai (T), n= 271; Tsigai×East Friesian (25%), n= 17; Tsigai×East Friesian (50%), n= 157; Tsigai×East Friesian (75%), n= 46; Lacaune (LC), n= 261. Three-breeding crosses with a 25%, 50% and 75% proportional genetic contribution of the specialized dairy breeds, Lacaune and East-Friesian (SDB) were significantly less than the assessed population (17 ewes, i.e. about 5% of the assessed population). For estimation of covariance components and genetic parameters used for determining the size of the udder cisterns of sheep, we employed measurement data, obtainedfrom a previously described experimental flock. Ultrasound images of the left and right udder cisterns were recorded by portable ultrasonography with a 3.5 MHz convex sector probe as previously described (Nudda *et al.*, 2000). The procedure uses contact gel and places the probe directly against the upper part of the median suspensory ligament in the inguinal abdominal fold. The operator performed an equal axis scan of the opposite side of the udder in order to obtain a sonographic image with the largest cistern size (*from side* method). The images were taken once for each half of the udder, 12 hours after the last milking. On the sonographic images, the length of the left (LLC1) and right (LRC1) cisterns and the width of the left (WLC1) and right (WRC1) cisterns (in millimetres) were measured from the cross sectional scans. By using digital technology the left (ALC1) and right (ARC1) cisterna areas (in mm$^2$) were measured, as well as the sum of the areas in both cisterns (SLRC1). For some control measurements, in addition to scanning the udder cisterns using the *from side* method, the sizes of the left and right udder cisterns were also investigated by scanning the entire ventral udder using the *from bottom* method. Udders were measured while immersed in water, with the probe held in the water against the udder wall as described (Bruckmaier *et al.*, 1997). Sonographic images obtained *from bottom* produced equal measurements for the udder cisterns as sonography *from side* (LLC2, LRC2, WLC2, WRC2, ALC2, ARC2, SLRC2).

Estimation of covariance components, followed by calculation of genetic parameters, was conducted using restricted maximum likelihood method (REML) and the multiple-trait animal model, using the REMLF90 and VCE 4.0 programs (Groeneveld and García-Cortés, 1998). The estimation of covariance was based upon a multiple trait animal model incorporating 7 traits. Genetic parameters were determined separately for length, width and area of the left and right cisterns surveyed using *from side* and *from bottom* methods. In the estimation of genetic parameters underlying udder cisterns size using the *from side* method and using untransformed data, 1023 measurements were carried out for the indicators LLC1, WLC1, LRC1, and WRC1 and 1198 measurements for ALC1, ARC1, and SLRC1. For estimating genetic parameters of udder cisterns size *from bottom*, 753 measurements were included for each character in 265 ewes, according to Serrano *et al.* (2002). In addition to genetic correlations, between-method correlation values were obtained using the Pearson phenotype correlation and calculated using the CORR procedure (SAS Institute, 2002-2008).

For estimation of covariance components and genetic parameters for all of the above parameters, the following model was used:

$$y_{ijklmno}=m+Y_i+LS_j+GEN_k+P_l+b*DIM_{ijklm}+a_m+tp_n+e_{ijklmno}$$

where:

$y_{ijklmno}$= is the vector of observations for the investigated characteristics (see above for details);

$Y_i$= year (fixed effect with 5 to 7 levels);

$LS_j$= lactation stage (fixed effect with 4 levels; from 40th to 99$^{th}$ lactation day, from 100$^{th}$ to 129$^{th}$ lactation day, from 130$^{th}$ to 159$^{th}$ lactation day and from 160$^{th}$ to 210$^{th}$ lactation day);

$GEN_k$= genotype (breed group, fixed effect with 9 levels; see above for characterization);

$P_l$= parity (fixed effect with 3 levels; first, second, third and further parity);

$a_m$= is the additive genetic effect of ewes;

$DIM_{ijklm}$= days in milk (covariate; 40 to 210 days in milk);

$tp_n$= the permanent environmental effect of ewes;

$e_{ijklmno}$= the random error.

## RESULTS AND DISCUSSION

**Tables I** and **II** show the basic statistical characteristics of the variation parameters which characterize the size of sheep udder cisterns (measured *from side* and *from bottom*). The area of left cistern (ALC) and right cistern (ARC) investigated by the method *from side* ranged from 133 mm$^2$ to 7560 mm$^2$, and from 10 mm$^2$ to 5799 mm$^2$, respectively. The sums of both cross-section areas (SLRC) ranged from 390 mm$^2$ up to 12900 mm$^2$ (mean= 3904.07 mm$^2$, v= 44.78%). The average area of the the left (ALC) and right cisterns (ARC) was 1933.35, and 1970.72 mm$^2$ respectively. The area of the left (ALC) and right cistern (ARC) investigated by the method *from bottom* ranged from 166 mm$^2$ to 6731 mm$^2$ and 178 mm$^2$ to 7832 mm$^2$ respectively. The sums of both cross-section areas (SLRC) investigated by the method *from bottom* ranged from 650 mm$^2$ up to 12646 mm$^2$ (mean= 4308.77 mm$^2$, v= 40.41%). **Tables III** and **IV** show the coefficients of heritability ($h^2$, on diagonal), genetic correlations (above diagonal) and phenotypic correlations (below diagonal) characterizing the size of the sheep udder cisterns measured *from side* and *from bottom* respectively. Heritability coefficients calculated using 7 characters ranged from 0.02 to 0.17 for the measurements *from side* and from 0.03 to 0.22 for measurements *from bottom*. The highest values for $h^2$ occurred when using cistern areas obtained using the *from side* method. The heritability coefficient of ARC1 was 0.17, and for SLRC1 was 0.12. The highest values were found in $h^2$ length of the cistern using the *from bottom* method. The heritability coefficient for LLC2 was 0.22 and for LRC2 was 0.19. However, heritability coefficients determined for areas of cisterns were only slightly lower: ALC2 $h^2$= 0.18, $h^2$= 0.12 ARC2 SLRC2 and $h^2$= 0.17. Relatively large differences in heritability coefficients between the right and left cisterns were found using the *from side* method. These

**Table I.** Basic statistical characteristics of the variation of selected parameters characterizing the udder cistern size of ewes (measured *from side*) (Caractéristiques statistiques de base de la variation des paramètres sélectionnés caractérisant la taille de la citerne de la mamelle de brebis (mesurées *de gauche*)).

| Measurement | N | Mean | SD | CV | Min. | Max. |
|---|---|---|---|---|---|---|
| Length of left cistern (mm) | 1023 | 69.14 | 16.31 | 23.59 | 17 | 133 |
| Width of left cistern (mm) | 1023 | 36.39 | 11.19 | 30.75 | 5 | 104 |
| Area of left cistern (mm$^2$) | 1198 | 1933.35 | 929.15 | 48.06 | 133 | 7560 |
| Length of right cistern (mm) | 1023 | 69.94 | 15.86 | 22.68 | 20 | 118 |
| Width of right cistern (mm) | 1023 | 37.68 | 10.74 | 28.50 | 10 | 84 |
| Area of right cistern (mm$^2$) | 1198 | 1970.72 | 927.95 | 47.09 | 10 | 5799 |
| Sums of both cross-section areas (mm$^2$) | 1198 | 3904.07 | 1748.46 | 44.78 | 390 | 12900 |

N= number of sets of measurements; SD= standard deviation; CV= coefficient of variability.

**Table II.** Basic statistical characteristics of the variation of selected parameters characterizing the udder cistern size of ewes (measured *from bottom*) (Caractéristiques statistiques de base de la variation des paramètres sélectionnés caractérisant la taille de la citerne de la mamelle de brebis (mesurée *du bas*)).

| Measurement | N | Mean | SD | CV | Min. | Max. |
|---|---|---|---|---|---|---|
| Length of left cistern (mm) | 753 | 78.63 | 15.17 | 19.29 | 25 | 132 |
| Width of left cistern (mm) | 753 | 35.46 | 9.78 | 27.58 | 7 | 77 |
| Area of left cistern (mm$^2$) | 753 | 2137.67 | 919.56 | 43.02 | 166 | 6731 |
| Length of right cistern (mm) | 753 | 78.04 | 15.13 | 19.39 | 19 | 131 |
| Width of right cistern (mm) | 753 | 35.86 | 10.16 | 28.33 | 7 | 134 |
| Area of right cistern (mm$^2$) | 753 | 2171.12 | 940.10 | 43.30 | 178 | 7832 |
| Sums of both cross-section areas (mm$^2$) | 753 | 4308.77 | 1741.25 | 40.41 | 650 | 12646 |

N= number of sets of measurements; SD= standard deviation; CV= coefficient of variability.

**Table III.** Heritability coefficients (on diagonal), genetic (above diagonal) and phenotypic (below diagonal) correlations that characterize the size of sheep udder cisterns (measured *from side*) (Coefficients d'héritabilité (en diagonale), génétique (ci-dessus) de diagonale et phénotypique (ci-dessous) en diagonale corrélations qui caractérisent la taille des citernes moutons de la mamelle (mesurées *de gauche*)).

| Indicators | LLC1 | WLC1 | ALC1 | LRC1 | WRC1 | ARC1 | SLRC1 |
|---|---|---|---|---|---|---|---|
| LLC1-length of left cistern | 0.02 | -0.01 | 0.49 | 0.84 | 0.00 | 0.46 | 0.50 |
| WLC1-width of left cistern | 0.71 | 0.02 | 0.84 | -0.08 | 0.45 | 0.44 | 0.64 |
| ALC1-area of left cistern | 0.86 | 0.91 | 0.07 | 0.44 | 0.47 | 0.73 | 0.90 |
| LRC1-length of right cistern | 0.80 | 0.58 | 0.71 | 0.07 | 0.36 | 0.77 | 0.69 |
| WRC1-width of right cistern | 0.63 | 0.75 | 0.74 | 0.74 | 0.04 | 0.83 | 0.93 |
| ARC1-area of right cistern | 0.74 | 0.71 | 0.77 | 0.87 | 0.90 | 0.17 | 0.97 |
| SLRC1-sums of both cross-section areas | 0.84 | 0.86 | 0.94 | 0.83 | 0.87 | 0.94 | 0.12 |

**Table IV.** Heritability coefficients (on diagonal), genetic (above diagonal) and phenotypic (below diagonal) correlations that characterize the size of sheep udder cisterns (measured *from bottom*) (Coefficients d'héritabilité (en diagonale), génétique (ci-dessus) de diagonale et phénotypique (ci-dessous) en diagonale corrélations qui caractérisent la taille des citernes moutons de la mamelle (mesurées *du bas*)).

| Indicators | LLC1 | WLC1 | ALC1 | LRC1 | WRC1 | ARC1 | SLRC1 |
|---|---|---|---|---|---|---|---|
| LLC2-length of left cistern | 0.22 | 0.70 | 0.89 | 0.94 | 0.66 | 0.72 | 0.83 |
| WLC2-width of left cistern | 0.67 | 0.12 | 0.89 | 0.55 | 0.67 | 0.71 | 0.83 |
| ALC2-area of left cistern | 0.85 | 0.88 | 0.18 | 0.85 | 0.85 | 0.91 | 0.98 |
| LRC2-length of right cistern | 0.79 | 0.56 | 0.70 | 0.19 | 0.77 | 0.81 | 0.85 |
| WRC2-widthof right cistern | 0.62 | 0.61 | 0.68 | 0.71 | 0.03 | 0.97 | 0.92 |
| ARC2-area of right cistern | 0.73 | 0.61 | 0.75 | 0.86 | 0.87 | 0.12 | 0.97 |
| SLRC2-sums of both cross-section areas | 0.84 | 0.80 | 0.935 | 0.84 | 0.83 | 0.938 | 0.17 |

differences could theoretically arise from differential preferences for the right or left sides of the udder during suckling of lambs – especially when rearing a single lamb where half the udder would be stimulated to relatively greater milk production. Measurements *from side* showed lower heritability coefficients for values of the left cistern, while *from bottom* showed lower heritability coefficients for values of the right cistern. This fact highlights differences in scanning each half of the udder. As regards the genetic correlations, in most cases, especially when measured *from side*, udder cisterns area depended more on the width of the cistern (rg= 0.83 to 0.97) than its length (rg= 0.49 to 0.89). Cistern width is strongly correlated with the width of the udder.

Correlation between the length and width of cisterns was very different depending on the method of measurements, and this was probably related to the fact that the shape of cisterns is highly variable, depending on the size and shape of the udder, teats status, abundance and distribution of secretory tissue inside the udder and other factors. Correlations between the right and left area of cisterns were higher when measured by the *from bottom* method (rg= 0.91) than by the *from side* method (rg= 0.73). The amount of this correlation reflects the fact that most ewes have udders roughly symmetrical, but there are ewes with an unbalanced udder, called *outweighed*, with different large cisterns. Rate representation of these ewes with unbalanced udders in the evaluated group of animals greatly affects the correlation coefficient between the area of the right and left cisterns. Correlations between the area of cisterns and their sum were relatively high, the highest values were found between ARC1 and SLRC1 (rg= 0.97), due to the fact that in this case the first monitored character is part of the second. The highest phenotypic correlations were found between SLRC1 and ALC1 (0.94), while other phenotype correlations between the monitored indicators are considered to be sufficiently high for effective selection.

Calculated coefficients of heritability and the genetic and phenotypic correlations characterizing the size of the udder cisterns of ewes are the basis for deciding on the possibility of using ultrasound technology to facilitate selective breeding for improved functional and morphological characteristics of the udder. The main reason for consideration of the new selection criteria is that milking machines are widely promoted at dairy sheep farms, in which issues related to udder morphology and milking ability of ewes have a significant role. For measurements *from side* it is important to follow the same placement of the probe and the same procedure to scan the left and right side of the udder. In keeping these principles, one can expect to reduce the variability of measurements due to measurement error and consequently increase the coefficients of heritability for these characters. Bruckmaier *et al.* (1994) reported a correlation between scan area and cisternal milk (r= 0.80) at a 10-h milking interval in dairy cows. Caja *et al.* (1999) found a high interdependence of the width and section area of gland cisterns with milk production of ewes, amounting to rp= 0.81 and rp= 0.90, respectively. Phenotypic correlations between milk yield and the cisternal depth and width were only 0.34 and 0.38, respectively. Ślósarz *et al.* (2002) measured the section area of the gland cistern of the udder in sheep and determined the level of correlation with their milk yields at rp= 0.74. Wójtowski *et al.* (2002) reported in

Polish White Improved goats a higher level of interdependence between milk production and the area of the udder gland cistern rp= 0.86. The size of mammary cisterns in terms of milk storage may be an important factor in determining reduced yield associated with extended milking intervals in dairy species (Ayadi *et al.*, 2003). Castillo *et al.* (2008) reported that Manchega and Lacaune ewes presented the greatest correlations between cisternal area and cisternal milk at the 8-h interval (Manchega, r= 0.70; Lacaune, r= 0.56). Similar results were reported by Salama *et al.* (2004) in dairy goats (r= 0.72). Nudda *et al.* (2000) reported a correlation (r= 0.82) in large-cisterned Sarda dairy ewes.

## CONCLUSION

Heritability coefficients show a relatively low value for the size of sheep udder cisterns, but it is still usable for efficient selection. Due to the complexity of the preparation of the measurements (particularly time and labour intensity), the authors recommend the implementation of measuring the udder cisterns *from side*, even though measurements *from bottom* show slightly higher heritability coefficients. If rapid measurement is needed, the linear dimension of the width of cisterns is recommended.

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