

UDC 636.32/.38:636.082.26:636.061

doi: 10.15389/agrobiol.2020.6.1139eng

doi: 10.15389/agrobiol.2020.6.1139rus

## IDENTIFICATION OF INTERSPECIFIC HYBRIDS ARGALI (*Ovis ammon*) AND DOMESTIC SHEEP (*Ovis aries*) OF DIFFERENT GENERATIONS BY EXTERIOR INDICATORS

B.S. IOLCHIEV ✉, N.A. VOLKOVA, V.A. BAGIROV, N.A. ZINOVIEVA

Ernst Federal Science Center for Animal Husbandry, 60, pos. Dubrovitsy, Podolsk District, Moscow Province, 142132 Russia, e-mail: baylar1@yandex.ru (✉ corresponding author), natavolkova@inbox.ru, vugarbagirov@mail.ru, n\_zinovieva@mail.ru

ORCID:

Iolchiev B.S. orcid.org/0000-0001-5386-726

Bagirov V.A. orcid.org/0000-0001-8385-2433

Volkova N.A. orcid.org/0000-0001-7191-3550

Zinovieva N.A. orcid.org/0000-0003-4017-6863

The authors declare no conflict of interests

Acknowledgements:

Supported financially by Russian Science Foundation, grant No. 18-16-00079 and the Ministry of Science and Higher Education of the Russian Federation, theme no. AAAA-A18-118021590132-9.

Received August 17, 2020

### Abstract

When creating new breeds and breeding forms, various breeding methods are used, including hybridization. For a long time in the Ernst Federal Science Center for Animal Husbandry, work is underway to use the genetic resources of wild species, in particular argali (*Ovis ammon*), to obtain interspecific hybrids with domestic sheep (*Ovis aries*) in the framework of creating new breeding forms and studying the biological characteristics of certain species of the genus *Ovis*. This raises the question of identifying the obtained interspecific hybrids. Along with conducting expensive studies on the genotyping of such animals, it is of interest to use informative phenotypic indicators characteristic of hybrid individuals. In this work, for the first time, comparative results of differentiation by exterior characteristics of interspecific hybrids of different generations from mating argali with sheep are presented. Romanov breed and original parental forms. The possibility of using exterior indicators for preliminary identification of hybrid individuals without expensive genomic studies was confirmed. Hybridization in the second and subsequent generations resulted in the splitting of hybrid individuals by genotype and phenotype. The work aimed at comparing morphometric parameters of the purebred Romanov sheep and their interspecific hybrids with argali and to reveal informative exterior indicators for identifying hybrid individuals. The lambs of Romanov breed ( $n = 20$ ) and the interspecific hybrids  $1/2$  Romanov sheep  $1/2$  argali (F1,  $n = 12$ ),  $3/8$  Romanov sheep  $5/8$  argali (F3,  $n = 17$ ), and  $7/16$  Romanov sheep  $9/16$  argali (F4,  $n = 18$ ) were reared from birth under the conditions of vivarium (the Ernst Federal Science Center for Animal Husbandry, 2019-2020). The following measurements were recorded: height in withers, height at the sacrum, back height, oblique body length, body length, chest width, sacrum width, chest depth, metacarpal girth. Linear measurements were taken at the age of 6, 42 days, and 3 months using a measuring tape, tape measure, and a measuring compass. The animals were weighed on an electronic balance. To assess the development of animals on the basis of weight and linear measurements, the body indices were calculated: long-legged index, elongation index, overgrowth index, breast index, bone index, body mass index. The SPSS v.23 software was used for statistical analysis. As a factor influencing the linear measurements of lambs, the breed of individuals was chosen. Hybrid animals at the age of 3 months in comparison with purebred individuals of the Romanov breed had higher indicators of the long-legged index, which is typical for argali. The advantage of F1, F3 and F4 hybrids over purebred animals for this indicator was 4, 8 and 2%, respectively. At the same time, hybrids F1, F3, and F4 had a more compressed rectangular body shape, therefore, they were inferior to purebred lambs in terms of elongation index, respectively, by 18, 22 and 18% ( $p < 0.05$ ). Differences were established between purebred and hybrid animals in linear and latitudinal measurements. The advantage of purebred sheep over F1, F3 and F4 hybrids was 18, 20 and 14 % in oblique body length, 35, 33 and 20% ( $p < 0.05$ ) in chest width, and 17, 19 and 7 % ( $p < 0.05$ ) in chest depth. Hierarchical classification of hybrids and purebred animals according to the exterior characteristics, it showed that interspecific hybrids with  $1/2$  and  $5/8$  argali bloodlines were grouped in one cluster. Hybrids with  $9/16$  bloodiness according to the argali and purebred Romanov sheep formed separate clusters. The results obtained confirm that the exterior parameters can be used for preliminary identification of hybrid individuals, in some cases reducing the cost of

expensive genomic studies.

Keywords: interspecific hybrids, *Ovis ammon*, argali, *Ovis aries*, Romanov breed, exterior

Various methods of genotypic and phenotypic analysis are used to analyze and assess the biological diversity of populations, as well as to identify breeds [1, 2]. Solving the problem of preserving the biodiversity of wild animals and the gene pool of farm animals requires an integrated approach using modern and classical methods [3].

Genetic variability makes it possible to expand the range of breeds through their introduction into a new natural and climatic zone and serves as the basis for the creation of new breeds, including those adapted to local conditions [4, 5].

To describe the breed characteristics of sheep, morphometric methods are used [6-8]. Morphological parameters, along with genetic ones, are used to differentiate populations and breeds [9, 10]. Currently, the characterization of the genetic variability of farm animals, including sheep, is carried out on the basis of the analysis of microsatellite markers [11].

Morphometric studies are important for characterizing the exterior, identifying differences between breeds [12], and assessing the physique of animals and economically useful traits [13-15]. Based on morphometric data, indices are calculated by which the body type can be determined [16]. Morphometric parameters are also used for indirect estimation of live weight and direction of animal productivity [17], selection and breeding by conformation [18]. The study of morphometric parameters over a long period allows one to characterize the structure of the breed and population, as well as the direction of selection for a certain period of time [19].

A number of studies have shown a close correlation relationship between some linear measurements of the exterior with live weight in sheep [20-22]. The live weight indicator depends on numerous factors, including breed, age, housing conditions, and feeding [23-26]. The number of lambs in a litter is negatively correlated with their live weight and affects preservation before weaning [27].

In this work, for the first time, the results of differentiation according to the exterior characteristics of interspecific hybrids of different generations from the mating of argali with sheep of the Romanov breed and the original parental forms are presented. The possibility of using exterior indicators for preliminary identification of hybrid individuals without costly genomic studies has been confirmed. During hybridization in the second and subsequent generations, the hybrids were split according to genotype and phenotype.

The aim of the work was a comparative characteristic of morphometric indicators of purebred sheep of the Romanov breed and their interspecific hybrids with argali to identify informative exterior indicators that identify hybrid individuals.

**Methods.** The studies were performed on purebred Romanov lambs ( $n = 20$ ) and interspecific hybrids  $1/2$  Romanov sheep  $1/2$  argali ( $F_1$ ,  $n = 12$ ),  $3/8$  Romanov sheep  $5/8$  argali ( $F_3$ ,  $n = 17$ ),  $7/16$  Romanov sheep  $9/16$  argali ( $F_4$ ,  $n = 18$ ) (Ernst Federal Science Center for Animal Husbandry, 2019-2020). The sheep were kept in stalls and pastures. From May to October, the animals were released to artificial and natural pastures using an electric shepherd. In winter, the sheep were kept in shelters under a canopy on deep bedding. The winter daily ration included hay (2.0 kg), concentrates (0.35 kg), haylage (2.5 kg), table salt (15 g), in summer, the ration was pasture grass and table salt (15 g). During the lambing period, ewes with lambs were kept in groups depending on the age of the lambs (no more than 15 queens in a group with lambs up to 1 week of age). Then the ewe with the grown lamb was placed in a separate cage for 5-6 days. In those cases when the

ewe did not accept her lamb well, the lamb did not recognize the mother or was very weak, they were kept in an individual cage for a longer time. At the same time, the feeding of the lamb was closely monitored and, if necessary, it was put under the ewe every 2-3 hours.

The morphometry of animals was carried out according to the following measurements: height in withers (HW, cm), height at the sacrum (HS, cm), back height (BH, cm), oblique body length (OBL, cm), body length (BL, cm), chest width (CW, cm), sacrum width (SW, cm), chest depth (CD, cm), metacarpal girth (MG, cm). Linear measurements were taken at the age of 6, 42 days, and 3 months using a measuring tape, tape measure, and a measuring compass. The animals were weighed on an electronic balance. To assess the development of animals on the basis of weight and linear measurements, the body indices were calculated: leg length index, elongation index, overgrowth index, breast index, bone index, and body mass index.

The SPSS v.23 software (IBM, USA) was used for statistical analysis. The obtained data were processed by means of an analysis of variance. As a factor influencing the linear measurements of lambs, the breed of individuals was chosen. The arithmetic mean values ( $M$ ) and standard errors of the means ( $\pm$ SEM) were calculated. To identify the statistical significance of differences in mean values, Student's  $t$ -test was used. Paired comparisons of each indicator were made depending on the factors taken into account. Physique indices were used for the hierarchical classification of the studied groups. Based on the data obtained, a dendrogram was constructed using the method of intergroup communication and Euclidean distances.

**Results.** Using analysis of variance of morphometric data, a statistically significant effect of the breed and type of animals on the height in withers, height and width at the sacrum, back height, metacarpal girth, body weight ( $p < 0.001$ ), overgrowth ( $p < 0.01$ ), elongation indices and body weight ( $p < 0.05$ ) were established (Table 1).

**1. Results of one-way analysis of variance of the influence of genotype on morphometric parameters in lambs of the Romanov breed (*Ovis aries*) and hybrids of different bloodlines from the mating of Romanov sheep with argali (*O. ammon*) (Ernst Federal Science Center for Animal Husbandry, 2019-2020)**

Dependent variable	df	F	p
Height in withers, cm	3	5.27	0.006
Height at the sacrum, cm	3	5.66	0.004
Back height, cm	3	6.64	0.002
Width at the sacrum, cm	3	9.53	0.000
Metacarpal girth, cm	3	7.11	0.001
Oblique body length, cm	3	9.88	0.000
Chest depth, cm	3	3.58	0.030
Body weight, kg	3	4.00	0.020
Elongation index	3	3.18	0.030
Overgrowth index	3	5.60	0.003
Body mass index	3	2.77	0.050

Note. df — degree of freedom, F — F-test, p — significance level.

At the age of 6 days, hybrid animals were predominantly inferior to their purebred peers in terms of live weight and a number of linear measurements (Table 2). Significant differences were established between purebred animals of the Romanov breed and interspecific  $F_1$  hybrids with  $1/2$  argali blood lines. The advantage of purebred animals over  $F_1$  hybrids in terms of body weight, HW, HS, BH, OBL, MG was 25, 8, 10, 10, 9, and 18%, respectively ( $p < 0.05$ ). Differences in these indicators between purebred animals and hybrids of later generations  $F_3$  and  $F_4$  leveled off with an increase in bloodiness for the Romanov breed. The hybrids

exceeded their purebred counterparts in body length and sacrum width. The advantage of F<sub>1</sub>, F<sub>3</sub>, and F<sub>4</sub> hybrids over purebred animals in BL was 12, 17 and 16% ( $p < 0.01$ ), in SW 35, 50, and 60% ( $p < 0.01$ ).

**2. Age dynamics of linear measurements and live weight of lambs of the Romanov breed (*Ovis aries*) (ROM) and hybrids of different bloodlines from the mating of Romanov sheep with argali (*O. ammon*) (ARG) ( $M \pm SEM$ , Ernst Federal Science Center for Animal Husbandry, 2019–2020)**

Parameters	Genotype			
	ROM	1/2 ROM	1/2 ARG	3/8 ROM 5/8 ARG
	6-d a y - o l d			
Height in withers, cm	38.25±1.56	35.33±0.84	37.88±0.85 <sup>c</sup>	39.14±0.72 <sup>c</sup>
Height at the sacrum, cm	37.75±0.53 <sup>c</sup>	34.12±0.83	37.72±0.83 <sup>c</sup>	38.23±0.71 <sup>c</sup>
Back height, cm	37.75±0.15 <sup>c</sup>	33.97±0.82	37.88±0.83 <sup>c</sup>	38.52±0.71 <sup>c</sup>
Oblique body length, cm	28.00±1.95	25.45±1.05	28.83±1.06	28.18±0.90 <sup>c</sup>
Body length, cm	28.00±1.49	31.45±0.80	32.72±0.81 <sup>a</sup>	32.30±0.69 <sup>a</sup>
Chest depth, cm	13.00±0.69	12.50±0.37	12.85±0.37	13.66±0.32
Chest width, cm	6.50±0.79	6.75±0.43	7.50±0.43	7.92±0.37
Sacrum width, cm	5.50±0.68	7.43±0.37 <sup>a</sup>	8.25±0.37 <sup>a</sup>	8.77±0.31 <sup>ac</sup>
Metacarpal girth, cm	5.75±0.14 <sup>abcd</sup>	4.70±0.07	5.05±0.07 <sup>c</sup>	5.05±0.06 <sup>c</sup>
Body weight, kg	3.95±0.37	2.98±0.20	3.69±0.20	4.05±0.17 <sup>c</sup>
	42-d a y - o l d			
Height in withers, cm	44.90±1.02	43.50±1.18	43.75±1.40	47.78±0.97 <sup>cd</sup>
Height at the sacrum, cm	44.55±0.77	42.93±0.88	44.33±1.05	47.15±0.73 <sup>acd</sup>
Back height, cm	45.07±0.90	43.00±1.02	44.91±1.22	48.30±0.85 <sup>5acd</sup>
Oblique body length, cm	38.72±0.70 <sup>abcd</sup>	31.87±0.79	30.83±0.95	33.32±0.66
Body length, cm	37.88±1.00	40.00±1.14	41.00±1.36	41.26±0.95 <sup>a</sup>
Chest depth, cm	19.03±2.47	15.62±2.83	15.33±3.37	17.80±2.35
Chest width, cm	13.22±2.91	8.56±3.32	8.83±3.96	10.56±2.76
Sacrum width, cm	10.35±0.55	9.62±0.63	10.75±0.75	11.07±0.52
Metacarpal girth, cm	5.50±0.25	5.18±0.29	5.33±0.35	5.67±0.24
Body weight, kg	9.06±0.51	7.82±0.58	7.58±0.69	9.04±0.48 <sup>cd</sup>

Note. Designation of the compared groups: a — Romanov breed; b — hybrids 7/16 ROM 9/16 ARG; c — hybrids 1/2 ROM 1/2 ARG; d — hybrids 3/8 ROM 5/8 ARG.

\* Differences between the compared groups are statistically significant at  $p < 0.05$ .

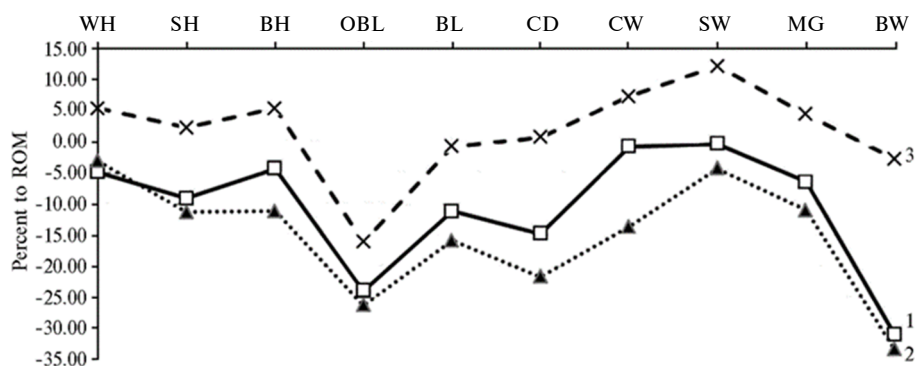
At the age of 42 days, the advantage of purebred animals over hybrids of all generations was retained only in terms of body weight, OBL, CW, and CD (see Table 2). The differences between purebred sheep and F<sub>1</sub>, F<sub>3</sub> and F<sub>4</sub> hybrids in body weight were 14, 16, and 0.2%, respectively ( $p < 0.05$ ), in OBL 18, 20, and 14% ( $p < 0.05$ ), in CW 35, 33, and 20% ( $p < 0.05$ ), and in CD 17, 19, and 7% ( $p < 0.05$ ). The hybrid animals, as at 6 days of age, overcome their purebred peers in body length. The excess in this indicator increased with an increase in argali bloodiness in F<sub>1</sub>, F<sub>3</sub>, and F<sub>4</sub> hybrids, amounting to 6, 8, and 9%, respectively ( $p < 0.05$ ).

The differences between purebred and hybrid animals in terms of BH, HW, and HS varied depending on the bloodiness of the hybrids in the original parental forms. If the F<sub>1</sub> and F<sub>3</sub> hybrids were inferior in these linear measurements to purebred animals, then the F<sub>4</sub> hybrids, on the contrary, surpassed them.

The variability of differences in linear measurements between purebred animals and hybrids of different generations was also noted at the age of 3 months (Fig. 1). Purebred animals excelled F<sub>1</sub> and F<sub>3</sub> hybrids in all measurements, but were inferior to F<sub>4</sub> individuals in these indicators, except for OBL and body weight.

Body indices were calculated to assess the development of purebred and hybrid animals. At the age of 6 days, the hybrid animals had higher indices of breast, body weight, and bone structure and were inferior in terms of elongation and overgrowth indices as compared to purebred peers (Table 3). The established advantage of purebred animals over F<sub>1</sub>, F<sub>3</sub>, and F<sub>4</sub> hybrids in the breast index, extension, and overgrowth indices remained at the age of 42 days and 3 months. The opposite tendency was observed in the indices of bone density and body

weight: the differences revealed at the age of 6 days were further leveled.



**Fig. 1. Exterior profile of interspecific 3-month-old hybrids of different generations from the mating of argali (*Ovis ammon*) (ARG) with Romanov sheep (*O. aries*) (ROM) as compared to peers of the Romanov breed: 1 –  $1/2$  ROM  $1/2$  ARG, 2 –  $3/8$  ROM  $5/8$  ARG, 3 –  $7/16$  ROM  $9/16$  ARG; WH – height in withers, cm; SH – height at the sacrum, cm; BH – back height, cm; OBL – oblique body length, cm; BL – body length, cm; CW – chest width, cm; SW – sacrum width, cm; CD – chest depth, cm; MG – metacarpal girth, cm; BW – body weight, kg (Ernst Federal Science Center for Animal Husbandry, 2019-2020).**

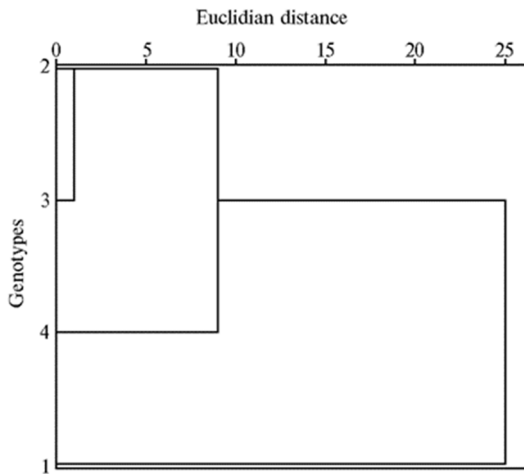
### 3. Age dynamics of body indices in lambs of the Romanov breed (*Ovis aries*) (ROM) and hybrids from the mating of argali (*Ovis ammon*) (ARG) with Romanov sheep ( $M \pm SEM$ , Ernst Federal Science Center for Animal Husbandry, 2019-2020)

Indices	Genotype			
	ROM	$\times$ ROM $1/2$ ARG	$3/8$ ROM $5/8$ ARG	$7/16$ ROM $9/16$ ARG
	6-d a y - o l d			
Chest index	51.85 $\pm$ 1.91	53.88 $\pm$ 2.44	58.48 $\pm$ 2.60 <sup>a</sup>	56.99 $\pm$ 1.99
Elongation index	80.34 $\pm$ 2.02 <sup>*cb</sup>	73.19 $\pm$ 2.57	75.74 $\pm$ 2.75	71.86 $\pm$ 2.10
Overgrowth index	100.63 $\pm$ 0.71 <sup>*cb</sup>	96.92 $\pm$ 0.91	99.29 $\pm$ 0.97	96.96 $\pm$ 0.74
Long-legged index	65.28 $\pm$ 0.88	64.27 $\pm$ 1.13	66.09 $\pm$ 1.21	64.99 $\pm$ 0.92
Bone index	10.99 $\pm$ 1.23	13.50 $\pm$ 1.57	13.30 $\pm$ 1.68	12.92 $\pm$ 1.28
Body mass index	7.77 $\pm$ 0.62	8.49 $\pm$ 0.80	9.52 $\pm$ 0.85	10.26 $\pm$ 0.65 <sup>*a</sup>
	42-d a y - o l d			
Chest index	58.90 $\pm$ 2.43	55.55 $\pm$ 3.05	58.40 $\pm$ 3.60	59.13 $\pm$ 2.33
Elongation index	87.21 $\pm$ 1.25 <sup>*cbd</sup>	74.05 $\pm$ 1.59	70.73 $\pm$ 1.85	71.08 $\pm$ 1.19
Overgrowth index	100.09 $\pm$ 1.05	99.10 $\pm$ 1.31	101.05 $\pm$ 1.56	99.45 $\pm$ 1.00
Long-legged index	65.01 $\pm$ 1.53	63.90 $\pm$ 1.92	65.95 $\pm$ 2.27	63.09 $\pm$ 1.47
Bone index	12.04 $\pm$ 0.37	11.98 $\pm$ 0.47	12.27 $\pm$ 0.56	11.85 $\pm$ 0.36
Body mass index	20.28 $\pm$ 1.17	18.29 $\pm$ 1.47	17.50 $\pm$ 1.74	19.64 $\pm$ 1.12
	3-m o n t h - o l d			
Chest index	53.87 $\pm$ 1.90	62.47 $\pm$ 2.70	58.82 $\pm$ 2.32	56.90 $\pm$ 2.33
Elongation index	93.46 $\pm$ 2.04	75.94 $\pm$ 2.88	72.00 $\pm$ 2.46	75.38 $\pm$ 2.49
Overgrowth index	102.11 $\pm$ 0.95	98.49 $\pm$ 1.34	94.00 $\pm$ 3.30	99.65 $\pm$ 1.16
Long-legged index	58.32 $\pm$ 1.24	62.20 $\pm$ 1.76	66.00 $\pm$ 1.95 <sup>*a</sup>	59.85 $\pm$ 1.52
Bone index	12.13 $\pm$ 0.20	11.82 $\pm$ 0.28	11.00 $\pm$ 0.69	11.87 $\pm$ 0.24
Body mass index	29.83 $\pm$ 2.49	24.80 $\pm$ 3.52	18.29 $\pm$ 8.66	30.98 $\pm$ 3.05

Note. Designation of the compared groups: a – Romanov breed; b – hybrids  $7/16$  ROM  $9/16$  ARG; c – hybrids  $1/2$  ROM  $1/2$  ARG; d – hybrids  $3/8$  ROM  $5/8$  ARG.  
\* Differences between the compared groups are statistically significant at  $p < 0.05$ .

It should also be noted that hybrid animals, in comparison with purebred individuals, were characterized by a higher leg length index, which is typical for argali. The advantage of F<sub>1</sub>, F<sub>3</sub>, and F<sub>4</sub> hybrids over purebred animals at the age of 3 months for this indicator reached 6, 12 ( $p < 0.05$ ), and 3%, respectively.

A hierarchical classification was carried out on the basis of the conformation characteristics of purebred Romanov breed sheep and their interspecific hybrids with argali of different generations. It was found that interspecific hybrids with  $1/2$  and  $5/8$  argali bloodlines were grouped into one cluster. Hybrids with  $9/16$  bloodiness and purebred animals of the Romanov breed formed separate clusters (Fig. 2).



**Fig. 2. Hierarchical analysis of interspecific hybrids of different generations from the mating of argali (*Ovis ammon*) (ARG) with sheep of the Romanov breed (*O. aries*) (ROM) and of the Romanov lambs: 1 – ROM, 2 –  $\frac{3}{8}$  ROM  $\frac{5}{8}$  ARG, 3 –  $\frac{1}{2}$  ROM  $\frac{1}{2}$  ARG, 4 –  $\frac{7}{16}$  ROM  $\frac{9}{16}$  ARG (Ernst Federal Science Center for Animal Husbandry, 2019-2020).**

The research shows that the genotype is one of the main biotic factors affecting the conformation of sheep. The effect of this factor on body weight and linear measurements is statistically significant at  $p < 0.001$ . Particular attention is paid to morphometric indicators when studying the phenotype of animals, which is a combination of genotype-specific properties inherent in an individual at a certain stage of development. Morphological differences based on the general body type, morphometric parameters, or unusual anatomical forms are used to identify, compare, and classify species and groups, identify unidentified taxa, unknown hybrids, and identify mutations that lead to changes [28].

A morphological description is the most important component of the characteristics of the breed and breeding form [29] and can be used for their identification and classification [30].

For a long time, the main feature of the classification of breeds of farm animals, especially sheep, was the color of wool [31]. Eastham et al. [32] used morphometric indicators in their studies to identify several species of falcons and their hybrids. Hybrids and original species were grouped into separate clusters. In this study, the hybrid animals were also distinguished by their physique indices into separate clusters, the distances between which were determined by the genotype of the hybrids.

Our findings correspond to suggestions of using exterior indicators to identify breeds and genotypes during breeding [6-8].

Thus, the interspecific hybrids of the argali with the sheep of the Romanov breed inherited some of the conformational features of the argali in terms of conformation and differ from the original parent breed. Compared to purebred animals, they are characterized by a more compact constitution, have a more compressed rectangular body shape. This is expressed in a decrease in hybrid animals in comparison with purebred peers in the indices of the oblique body length and the elongation index, depending on bloodiness, by 14-20 and 18-22%, respectively ( $p < 0.05$ ). Our findings confirm that exterior indicators can be used for preliminary identification of hybrid individuals, reducing in some cases the cost of expensive genomic studies.

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