Semen quality in sheep

*Y. Arboleda Marín, D. A. González Gaviria, L. K. Lopera Mesa, P. C. Lalinde Acevedo, A. F. Mejía, W. D. Cardona Maya and H. Cardona Cadavid**

Abstract

Reproductive evaluation, the selection of individual breeders, and reproductive biotechnologies are important tools in developing productive and reproductive rates. When choosing a male as a breeding future, determining its general health status and genotypic and phenotypic superiority are necessary. This study evaluated the conventional and functional seminal quality in ovine males. The semen of eleven ovines of different pure races was collected by electroejaculation. The following average values were observed for each conventional parameter: scrotal circumference (CE, 34.3 cm), seminal volume (vol, 1.63 mL), concentration (C, 768.4 x 10^6/mL), individual motility (MI, 80%), masal motility (MM, 4) and vigor (Vi, 3.7). On the other hand, functional analysis showed plasma membrane integrity (PMI) of 45.7%, mitochondrial membrane potential (MMP) of 38.5%, DNA fragmentation index (DFI) of 17.0%, lipoperoxidation of the membrane (LPO) of 32.7%, and the production of reactive oxygen species (ROS) of 28.56%. Additionally, a high and positive correlation was found for variables concerning seminal quality: Vi and MM, Vi and MI, MMP and MM, MMP and MI, PMI and MMP, as well as ROS and MMP. In contrast, two strongly negative correlations were obtained (C *vs.* Vol and LPO *vs.* CE). These are the first estimates of functional seminal quality evaluation of sheep in Colombia. In addition, this study provides a baseline for conventional and functional seminal parameters in the region.

Key words: *semen quality; flow cytometry; reproduction; sheep; semen*

Introduction

Ovine species in Colombia are kept by small producers as a source of food (meat and milk) of high nutritional value and income when marketing products such as skin, wool, and others (Barrios). To date, the census of sheep exploitation in the country is very low, since there are only two million animals, distributed mainly in six regions: La Guajira (42.4%), Magdalena (10.9%), Córdoba (7%), and Cesar (7.9%) and Boyacá (6.7%) (Instituto Colombianao Agropecuario, 2018). However, at present, the business of sheep farming is increasing due to the

Yurani ARBOLEDA MARIN, Daniela Alejandra GONZÁLEZ GAVIRIA, Leidy Katerine LOPERA MESA, Semillero de Investigación y Grupo de Estudio en Caprinos y Ovinos (GECO-GaMMA), Facultad de Ciencias Agrarias, Universidad de Antioquia. Medellín, Colombia; Paula Andrea LALINDE ACEVEDO, Walter D. CARDONA MAYA, Grupo Reproducción, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia; Andrés Felipe MEJIA, Empresa de Biotecnología de la Reproducción Ge3, Medellín, Colombia; Henry CARDONA CADAVID*, (Corresponding author, e-mail: henry.cardona@udea.edu.co), Grupo de Investigación en Genética, Mejoramiento y Modelación Animal (GaMMA), Universidad de Antioquia, Medellín, Colombia

increase in meat consumption and the profitability that entrepreneurs show in this activity, so much so that in recent years the development of productions and agribusiness in the sheep sector has promoted everything related to the import of animals, the incorporation of technology, the acquisition of equipment and implements and technical training. This has enabled the high demand for products to be partly met in Colombia and in some markets of neighbouring countries (Restrepo).

In any productive activity, including sheep production, the evaluation, and control of aspects such as management, feeding, facilities, production, and reproduction, are determining factors for establishing the viability of sheep. The area of animal reproduction has been extensively studied. However, in practice, there are still many shortcomings that hinder the fertility and fertilization process, both in ovine species and in other domestic species: inadequate nutritional and sanitary conditions at holdings, poorly trained workers, and failures in artificial reproduction processes (González-Stagnaro, 2011).

The female and the male are essential and indispensable in a production system; however, it is essential to state that the male accounts for more than 80% of the economic viability of a farm (Barrios, 2007). Consequently, strong excellent genotypic and phenotypic selection of males is essential; therefore, it is necessary that the holding knows the reproductive status of each animal. Reproductive examination of a male consists of a physical examination, in which scrotal circumference, body development, vision, good poise, body condition, and overall reproductive organ health are evaluated, in addition to a seminal quality test to know the male's fertilising capacity.

The techniques most used today for collecting semen in domestic animals, mainly ruminants (bovines, sheep, and goats), are the artificial vagina and electroejaculation (Morillo, Salazar, & Castillo, 2012; Yamasaki Maza et al., 2005). Semen can be evaluated by conventional methods in which the quality is defined according to macroscopic characteristics, usually flow cytometry (FC) (Izquierdo et al., 2016) to quantify functional seminal parameters that allow for better evaluation of the capacity for fertilisation (Laguado, 2007).

The use of FC in veterinary medicine has been increasing, though there is still much to evaluate (Córdova-Izquierdo, Saltijeral Oaxaca, Muñoz Mendoza, Córdova-Jiménez, & Córdova-Jiménez, 2006). An innovative idea has been to implement FC for sperm evaluation in male sheep. The objective of this study was to analyse the conventional and functional semen quality of sheep.

Material and methods

Study area and population

Semen samples were collected from 11 sheep pure bred rams: Dorper (n=4), Katahdin (n=4), and Santa Ines (n=3). The Ethics Committee approved this work for the Ethics Committee for Experimentation with Animals (CEEA) of the University of Antioquia in Act No. 109/2014.

Sample collection and evaluation

Each collection began with a physical examination of the whole animal, including the fore and hind limbs, physical examination of the foreskin, penis, scrotum, testicles, and epididymis, in addition to assessing body condition and the ruling out any vision problems. Finally, the scrotal circumference (SC) was measured.

The seminal sample was obtained by using the electroejaculation method (Yamasaki Maza et al., 2005). Once the sample was obtained, macroscopic evaluation was performed (volume,

appearance, colour, and presence of foreign elements), and then it was divided into two fractions: one for conventional field tests or microscopic evaluation (mass motility (MM, scale 1 to 5), percentage of individual motility (MI), vigor (Vi, scale 1 to 5), sperm concentration (C, 10^6/mL)) and the second was taken to the laboratory within 2 hours to quantify the mitochondrial membrane potential (MMP) using FC (Ospina Medina and Cardona Maya, 2015). This was performed using the dye 3,3′-dihexyloxacarbocyanine iodide (DIOC6) Mayorga-Torres et al., 2013; 2015; Mayorga Torres et al., 2015, B. J. Mayorga-Torres, Cardona-Maya, Cadavid, & Camargo, 2013, intracellular reactive oxygen species (ROS) (Mayorga Torres et al., 2013; 2015; Mayorga Torres et al., 2015), using 2′,7′-dichlorofluorescein diacetate (DCFH-DA) staining, plasma membrane integrity (PMI) Mayorga Torres et al., 2015; B. J. Mayorga-Torres et al., 2015; B. J. Mayorga-Torres et al., 2013 using SYBR 14, lipoperoxidation (LPO) Mayorga Torres et al., 2013; 2015; Mayorga Torres et al., 2015), using BODIPY C11 dye, and DNA fragmentation index (DFI) Mayorga-Torres et al., 2013; 2015), using acridine orange.

Statistical analysis

The characteristics analysed in this study were described using descriptive statistics (Table 1) and Pearson's correlation (Table 2), all variables were continuous. The statistical program R-Project was used through the RCORR procedure and the HMISC library.

Results

No males with physical problems or lesions were found in the physical evaluation. The evaluation of the reproductive system organs was within the normal parameters. It should also

Table 1. Descriptive statistics of continuous variables analysed by conventional method and flow cytometry

	N	Mean	Standard deviation	Minimum	Maximum
Scrotal circumference (cm)	11	34.3	2.5	30.0	38.0
Volume (mL)	11	1.6	1.2	0.25	4.5
Mass motility	11	4.0	0.6	2.5	4.5
Individual motility (%)	11	80.0	12.6	50.0	90.0
Vigor	11	3.8	0.5	2.5	4.5
Concentration (10^6/mL)	11	768.4	755.6	133.0	2810.0
pH	11	7.6	0.5	6.5	8.0
Age (months)	11	34.7	22.8	9.0	79.2
Mitochondrial membrane potential (%)	11	38.5	18.9	15.3	74.5
Plasma membrane integrity (%)	11	45.7	24.3	8.23	76.8
Lipoperoxidation (%)	11	32.7	35.7	1.31	95.5
Production of reactive oxygen species (%)	11	28.6	18.2	2.23	56.0
DNA fragmentation index (%)	11	17.0	6.5	13.0	36.0

Table 2. Correlation analysis

	Vol	MM	MI	Vi	C	MMP	PMI	LPO	ROS	DFI
Scrotal circumference (cm, CE)	-0.03	-0.25	-0.25	-0.26	-0.46	0.09	-0.14	-0.49*	0.30	-0.50
Volume (mL, Vol)		-0.31	-0.31	-0.41	-0.31*	0.51	0.33	-0.19	0.43	0.01
Mass motility (%, MM)			1.00	0.84**	0.47	0.57*	0.47	0.15	0.17	0.12
Individual motility (%, MI)				0.84**	0.47	0.57*	0.47	0.15	0.17	0.12
Vigor (Vi)					0.29	0.05	0.29	0.3	-0.09	0.03
Concentration (10^6/mL, C)						-0.05	0.49	0.37	-0.3	0.01
Mitochondrial membrane potential (%, MMP)							0.61**	-0.18	0.87**	-0.30
Plasma membrane integrity (%, PMI)								0.14	0.41	-0.29
Lipoperoxidation (%, LPO)									-0.22	-0.25
DNA fragmentation index (DFI)										0.25

ROS: Reactive oxygen species; *: $P<0.05$; **: $P<0.01$

be considered that the evaluated males belong to different breeds and different management systems.

Correlations between all analysed variables (Table 2) by conventional methods and FC were determined, with their respective confidence levels. A strongly positive correlation was obtained for essential variables in determining semen quality, such as Vi and MM, Vi and MI, MMP and MM, MMP and MI, PMI and MMP, and ROS and MMP, while strongly negative correlations were found for C and Vol, and LPO and CE, Table 2.

The values analysed by FC showed that an average of 38.5% of the cells evaluated present high MMP: 28.56% ROS production, 17% DNA fragmentation, 32.7% OLP, an average of 32.7% was obtained, and 45.7% of the analysed cells have intact MI.

Discussion

The average semen volume was 1.63 mL, which is higher than that reported by Avellaneda et al. (2006) (0.6 mL) who also obtained the semen sample by electroejaculation, was higher than reported by Córdoba-Izquierdo et al. (2006) using an artificial vagina for natural mounting (0.57 mL), and similar to (Cabrera and Pantoja, 2008) obtained by artificial vagina (1.7 mL). It should be noted that the breeds used in the different studies cited are different from those examined in this study. Like sperm concentration, this characteristic presented a wide range of variation (0.25 to 4.5 mL and 133 to 2810 x 10^6/mL, respectively), possibly due to the heterogeneity presented in the study population regarding bred.

The average scrotal circumference was 34.3 cm, which is larger than the report by (Avellaneda et al., 2006) (22.6 cm), despite a similar average age of males in both studies (31.25 *vs*. 34.7 months).

The sperm concentration in the present study was 768.4 x 10^6/mL, which was lower than that reported by (Avellaneda et al., 2006) (1285.7 x 10^6/mL) and (Cabrera and Pantoja, 2008) (2735 x 10^6/mL), but higher than that reported by (Córdova-Izquierdo et al., 2006) (206.4 x 10^6/mL using an artificial vagina) and when obtaining the semen sample directly from the vagina after a natural mount (176.02 x 10^6/mL) in the same study.

Regarding individual motility, this was 80% on average, which was higher than the report by (Avellaneda et al., 2006) (58.87%) and (Córdova-Izquierdo et al., 2006) (64.5% using artificial vagina) and when obtaining the semen sample directly from the vagina after natural mating (60.4%) in the same study. Mass motility was 4.0 on average, similar to the report by (Cabrera and Pantoja, 2008).

Concerning the functional parameters, in humans as the most widely studied group, the reference limit for MMP has been estimated at 60% (Mayorga-Torres et al., 2013; 2017), Ospina Medina & Cardona Maya, 2015), suggesting that it is low in the present study. It should be noted that MMP is a predictive characteristic in fertilisation and it is highly correlated with some of the most predictive conventional characteristics of semen quality, such as mass and individual motility (Table 2).

The production of a high ROS average was 28; this value is considered optimal in humans (Mayorga-Torres et al., 2013; 2017). Likewise, ROS production is related to DFI, although a low negative correlation was observed in the present study (Table 2).

The results obtained for DFI show an average of 17.0% of DNA fragmentation, a high figure compared to that reported as optimal in humans (<15%) (Mayorga-

Torres et al., 2013; 2017), though this can be considered to still represent semen of acceptable quality and may indicate a high percentage of sperm viability. Regarding the LPO analysis, an average of 32.7% of cells with lipid oxidation was obtained; this is a functional parameter of interest because it is theoretically related to motility, although a low positive correlation is not significant in this study (Table 2). The PIM analysis shows that on average, 45.7% of the analysed cells have an intact MI which, as a parameter correlated with sperm viability, should be higher (> 50%) (Mayorga-Torres et al., 2013; 2017), as reported as optimal in humans.

Of the five variables analysed in FC, only one, the production is ROS, and it was within the values reported as optimal for humans. Still, this cannot assure that the evaluated males have low fertility since they are different species, and it would therefore be necessary to determine the optimal values in specific ovine species.

In conclusion, the values obtained in the conventional evaluation may vary according to the method used to obtain the semen sample and the breed of the males. Therefore, more studies are needed to estimate the minimum acceptable values to conclude that a male has good fertility.

Given that this study has a number of uncontrolled variables, several of the results may not be significant; therefore, a larger study with controlled variables such as breed, environment, age, time of feedings, and several feedings should be considered to relate these variables with fertility.

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Kvaliteta sjemena u ovaca

Yurani ARBOLEDA MARIN, Daniela Alejandra GONZÁLEZ GAVIRIA, Leidy Katerine LOPERA MESA, Semillero de Investigación y Grupo de Estudio en Caprinos y Ovinos (GECO-GaMMA), Facultad de Ciencias Agrarias, Universidad de Antioquia. Medellín, Colombia; Paula Andrea LALINDE ACEVEDO, Walter D. CARDONA MAYA, Grupo Reproducción, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia; Andrés Felipe MEJIA, Empresa de Biotecnología de la Reproducción Ge3, Medellín, Colombia; Henry CARDONA CADAVID, Grupo de Investigación en Genética, Mejoramiento y Modelación Animal (GaMMA), Universidad de Antioquia, Medellín, Colombia

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