Archivos de Zootecnia
Journal website: https://www.uco.es/ucopress/az/index.php/az/
SHORT NOTE

Repeatability and effect of age on reproductive characteristics in Santa Ines rams

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Additional keywords

Reproductive potential.
Sheep.
Semen evaluation.

SUMMARY

Due to the importance of the early selection of Santa Ines rams in Brazil, we have estimated the repeatability and verified the effects of age on seminal characteristics, scrotal circumference and body weight in 51 Santa Ines rams aged 6 to 26 months during one year. Animals were divided by age: group 1 (6 to 12 months) and group 2 (12 to 26 months) and they were kept in semi-feedlots, receiving hay, concentrate and mineral supplement. Body weight, scrotal circumference and semen characteristics were evaluated once a month. The age groups did not influence sperm volume, sperm mass movements, sperm motility and sperm vigor. There were differences between age groups for sperm concentration, body weight and scrotal circumference which were higher in adult sheep (group 2). The major defects average also was different between groups, as the presence of sperm defects related inversely to age. Correlations between mass movement, motility and vigor were high and significant (P<0.01). The repeatability ranged from 0.39 (volume) to 0.09 (major defects), generally, low to those seminal characteristics. We conclude that multiple reviews of the seminal characteristics are necessaries to verify the reproductive efficiency of Santa Ines rams.

Repetibilidade e efeito da idade nas características reprodutivas em ovinos Santa Inês

Additional keywords

Avaliação seminal.
Ovino.
Potencial reprodutivo.

RESUMEN

Devido à importância da seleção precoce de carneiros da raça Santa Inês no Brasil, estimamos a repetibilidade e verificados os efeitos da idade sobre as características seminais, perímetro escrotal e peso corporal em 51 carneiros da raça Santa Inês com idades entre 6 a 26 meses de idade, durante um ano. Os animais foram divididos em grupos de idade: grupo 1 (6 a 12 meses) e grupo 2 (12 a 26 meses), mantidos em semi-confinamentos, recebendo feno, concentrado e um suplemento mineral diariamente. O peso corporal, circunferência escrotal e as características seminais foram avaliados uma vez por mês. Os grupos de idade não influenciaram o volume espermático, movimentos de massa, motilidade e vigor espermáticos. Houve diferenças entre os grupos etários para a concentração espermática, peso corporal e circunferência escrotal que foram maiores em ovinos adultos (grupo 2). A média dos defeitos espermáticos maiores também foi diferente entre os grupos, sendo a presença de espermatozoides anormais relacionada inversamente com a idade. Correlações entre movimento de massa, motilidade e vigor foram altas e significativas (P<0.01). A repetibilidade variou entre 0,39 (volume) a 0,09 (defeitos espermáticos maiores), em geral, baixa para essas características seminais. Conclui-se que são necessárias várias avaliações sobre as características seminais para verificar a eficiência reprodutiva de carneiros Santa Inês.

Information

Cronología del artículo.
Recibido/Received: 14.06.2016
Aceptado/Accepted: 10.10.2017
On-line: 15.01.2018
Correspondencia a los autores/Contact e-mail:
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INTRODUCTION

The sheep industry has increased in recent decades especially in developing countries, which are currently holders of the largest herds. Following this global trend, it is expected that the Brazilian herd is going to be five times larger in the next 20 years. In this context, there will be a widespread need to monitor the fertility of these animals, aiming more efficient multiplication of genotypes with high zootechnical value (Lima *et al.* 2010).

As reported by Santos (2003), Santa Ines sheep stands out among native and hair breeds raised in Brazil. Its high genetic diversity and lower reproductive seasonality are important features used in breeding programs. Therefore, it is very important the selection of rams, due to the greater potential to raise descendants (Salgueiro, 2000).

The sperm quality is one of the main characteristics to be evaluated when potential rams are chosen. However, conforming Emsen (2005), age is one of the factors that may influence the reproductive characteristics of the animal. The characteristics relating to reproduction can be considered of greater economic importance and should receive special attention from farmers (Dias *et al.* 2004). However, the direct selection

for these characteristics is often difficult to be implemented.

Genetic improvement programs are intended to change the inheritance of some characteristics in populations by increasing desirable genes or frequency of desirable genotypes in order to increase the productivity of livestock. Therefore, it is necessary the knowledge of genetic parameters such as repeatability and genetic correlations, to guide the choice of selection methods (Siqueira *et al*., 2013).

As reported by Abreu *et al.* (2001) the repeatability and the correlations among semen characteristics can be an extremely important element to take decisions regarding the disposal of rams due to inadequate production of quality semen. There are few studies about repeatability of reproductive characteristics in rams. Due to the importance of the early selection of Santa Ines rams, this study aimed to determine the effects of age on semen characteristics, scrotal circumference and body weight, and also calculate the repeatability values and correlations of these traits.

MATERIAL AND METHODS

Experimental animals and location

This research was carried out in the north of Rio de Janeiro, Brazil. In this region the animals do not show reproductive seasonality. A total number of 51 fertile Santa Ines rams 6 to 26 months old were selected to be used in this study. The selection was based on the entry in puberty, when they were able to perform complete mating, having sperm motility above 10% and concentration of at least 50×10^6 spermatozoa/mL (Wheaton and Godfrey, 2003). Animals were divided into two age groups - In Group 1 (G1) there was 32 animals ranging from 6 to 12 months; and In group 2 (G2) there were 19 animals ranging from 13 to 26 months. Assessments were performed monthly, during a year, in each group.

Table I. Effect of age groups of Santa Ines rams on physical characteristics of semen, body weight, scrotal circumference and sperm defects (Efeito do grupo de idade de ovinos Santa Inês nas características seminais, circunferência escrotal e defeitos espermáticos).

	Age groups	
Characteristics	G1 (6-12 months)	G2 (13-26 months)
VOL (mL)	1.14 ± 0.55[a]	1.31 ± 0.56[a]
MM (1-5)	4.37 ± 1.03[a]	4.24 ± 0.96[a]
MOT (%)	80.73 ± 13.76[a]	78.20 ± 11.50[a]
VIG (1-5)	4.27 ± 0.98[a]	4.11 ± 0.77[a]
CONC (109 sperm/mL)	2.90 ± 1.09[b]	3.53 ± 1.16[a]
MAD (%)	6.99 ± 8.29[a]	4.07 ± 5.96[b]
MID (%)	20.57 ± 13.24[a]	17.05 ± 11.25[a]
TOD (%)	27.82 ± 16.25[a]	21.12 ± 13.29[b]
BW (Kg)	77.46 ± 26.67[b]	96.64 ± 26.82[a]
SC (cm)	30.96 ± 2.65[b]	33.34 ± 3.23[a]

[a,b]Means followed by different letters in the same row are statistically different from each other ($p<0.05$) by the SNK test. VOL= Ejaculate volume; MM= sperm mass movement; MOT= sperm motility; VIG= sperm vigor; CONC= sperm sperm concentration; MAD= major sperm defects; MID minor sperm defects; TOD= total sperm defects; BW= body weight; SC= scrotal circumference.

Reproductive evaluation

Firstly, the consistency, symmetry and movement of the testis into scrotum were observed, and then body weight (BW) and scrotal circumference (SC) were recorded with the aid of a mechanical scale and a metal tape.

Afterwards, semen was collected using preheated artificial vagina (42-45ºC). Immediately after semen collection, the following characteristics were evaluated, according to Colegio Brasileiro de Reprodução Animal (2013): ejaculate volume (VOL), which is made immediately after collection, directly from a graduated tube and it's expressed in milliliters; sperm aspect (ASP), which can be in aqueous form (1) serous form (2), milky form (3) and creamy form (4); sperm progressive motility (MOT) that is a subjective evaluation of a semen aliquot placed between slide and cover slip preheated to 37°C and viewed in light field optical microscopy with 100x magnification showing the percentage of sperm with straight progressive movements; sperm vigor (VIG) that expresses the intensity of sperm movement on a scale of 1 to 5; sperm mass movement (MM), which is determined by evaluating a drop of semen placed on a preheated blade 37ºC and viewed in light field optical microscopy with 100x magnification, expressed on a scale from 0 to 5; and sperm concentration (CONC) (number of sperm/mL) that is obtained by counting the number of spermatozoa diluted in citrate formaldehyde solution at a proportion of 1: 400 in a Neubauer chamber.

The wet chamber technique was used for morphological evaluation of sperm, which a considerable amount of the ejaculate is deposited in a tube containing 1 mL citrate formaldehyde solution. In the laboratory, the contents of the tube should be carefully homogenized and a drop of this should be deposited on a clean and dry slide. A coverslip should be placed on the drop and a paper towel should be lightly pressed to remove excess contents. Then, the evaluation was performed under phase contrast microscope by counting the normal spermatozoa and sperm defects under 1000X magnification. Finally, 200 sperms were analyzed and the results were expressed in percentage of minor sperm defects (MID), major sperm defects (MAD) and total sperm defects (TOD) (Blom, 1973).

Number of measurements

The minimum number of measurements needed to predict the actual value of each characteristic, based on a preset coefficient of determination (R^2) of 0.80, was calculated by the following expression (Cruz *et al*., 1997): $Nm = R^2 (1- t) / (1-R^2) t$

Table II. Pearson's correlation coefficient of physical characteristics of semen, body weight, scrotal circumference and sperm defects of Santa Ines sheep (Coeficiente de correlação de Pearson entre as características físicas do sêmen, peso corporal, circunferência escrotal e defeitos espermáticos de ovinos Santa Inês).

	VOL	MM	MOT	VIG	CONC	MAD	MID	TOD	BW	SC
VOL	-									
MM	-0.02	-								
MOT	0.16	0.72**	-							
VIG	0.08	0.74**	0.80**	-						
CONC	0.22*	0.13	0.13	0.13	-					
MAD	-0.05	-0.13	-0.21*	-0.16	-0.12	-				
MID	-0.35**	0.20*	0.11	0.20*	-0.22*	0.07	-			
TOD	-0.31**	0.10	-0.01	0.09	-0.24*	0.56**	0.86**	-		
BW	0.06	-0.16	-0.09	-0.06	0.06	0.11	-0.13	-0.05	-	
SC	0.03	-0.21*	-0.15	-0.12	0.18	0.03	0.06	0.07	0.08	-

*P<0.05; **P<0.01. VOL= Ejaculate volume; MM= sperm mass movement; MOT= sperm motility; VIG= sperm vigor; CONC= sperm sperm concentration; MAD= major sperm defects; MID minor sperm defects; TOD= total sperm defects; BW= body weight; SC= scrotal circumference.

Where: Nm = number of measurements; R^2 = coefficient of determination; t = repeatability coefficient.

Statistical analysis

Data were statistically analyzed using the MIXED PROC of SAS (2009) and subjected to analysis of variance to compare the effects of age. Differences among means were compared by SNK test. Simple correlations were calculated (PROC CORR). Repeatability was estimated using the procedure of the: ratio-of-variances estimation, which requires that variance components be estimated for observations both within- and among-individuals. Repeatability (t) then can be estimated as: $t = \sigma^2a / \sigma^2a + \sigma^2w$. Where σ^2_a indicates variance among individuals, σ^2_w indicates variance within individuals (Falconer *et al.*, 1996). The denominator in this ratio is an estimate of the phenotypic variance (σ^2_P). To compute repeatability from this equation, variance components were estimated from a mixed model using the REML procedure. Analyses were carried out utilizing PROC MIXED and PROC VARCOMP of SAS (2009).

Table III. Repeatability estimates (t) and number of measurements required (Nm) for semen traits and scrotal circumference of Santa Ines sheep, obtained in univariate models (Repetibilidade (t) estimada e Número de mensurações requeridas (Nm) para as caracteristicas seminais e circunferencia escrotal de ovinos Santa Inês, obtidos em modelos univariados).

Characteristics	t	Nm
VOL	0.38	6
MM	0.11	32
MOT	0.12	29
VIG	0.12	29
CONC	0.21	15
MID	0.20	16
MAD	0.09	40
TOD	0.25	12
SC	0.37	6

VOL= Ejaculate volume; MM= sperm mass movement; MOT= sperm motility; VIG= sperm vigor; CONC= sperm sperm concentration; MAD= major sperm defects; MID minor sperm defects; TOD= total sperm defects; BW= body weight; SC= scrotal circumference.

RESULTS

The macroscopic aspect of semen was creamy, shade white to pale yellow and odor "sui generis". Means value and standard deviations of semen characteristics, body weight and scrotal circumference in Santa Ines rams are shown in **Table I**, according to age group.

According to the results of the analysis of variance, it was observed that there were differences (P<0.05) between age groups for sperm concentration, body weight, scrotal circumference, major sperm defects and total sperm defects. However there were no differences (P>0.05) between age groups for others physical characteristics of semen and minor sperm defects.

CONC was the only physical characteristic of semen that has differed between groups, showing the adult rams the highest average. The most obvious finding to emerge from the analysis was a trend of gradual increase in body weight and scrotal circumference while the animals grew older. Sperm defects were higher in young rams (G1), which had a decrease in adult rams (G2).

Moreover, there were no differences (P>0.05) between age groups for others physical characteristics of semen and minor sperm defects The overall averages of VOL, MM, MOT, VIG and CONC were 1.24 mL, 4.28, 78.96%, 4.15 and 3.27 x 10^9 sperm/mL, respectively.

Simple correlations between the characteristics are listed in **Table II**. The VOL had low correlation (P>0.05) with seminal characteristics (-0.02, 0.16, 0.08, 0.22, 0.05, -0.35 and -0.31 for MM, MOT, VIG, CONC,

MAD MID and TOD respectively), BW (0.06) and SC (0.03). Despite the low correlations of VOL with all traits, the correlation with MAD and TOD was significant (P<0.01), and inversely proportional, indicating that when the ejaculate volume increases, the amount of sperm defects decreases.

The correlations among the qualitative characteristics of semen (MM, MOT and VIG) were high (0.72, 0.74 and 0.80) and significant (P<0.01). The sperm defects showed low correlations with the physical characteristics of the semen. On the other hand, the correlation among MID, MAD and TOD was high and significant (P<0.01).

The repeatability of the seminal characteristics is observed in **Table III**. In general, seminal characteristics had low repeatability values (from 0.09 to 0.25). The number of measurements required to predict the actual value is large, 40 (MAD), 32 (MM) and 15 (CONC) for repeatability values 0.09, 0.11 and 0.25 respectively. Higher values for repeatability were showed by VOL (0.38) and for SC (0.37). The number of necessary measurements to predict the actual value would be 6 to 80% of reliability

DISCUSSION

The VOL averages for each group were considered normal for animals that did not have reproductive problems. Semen volume is relatively small in rams, ranging from 0.5 to 3 ml (CBRA, 2013).

The average of MM and VIG in both groups was within the recommended values (≥3 for both traits) for rams' semen collected by artificial vagina (CBRA, 2013). In general, semen mass motility up to 3 (on the scale of 0 to 5) is accepted as suitable for Artificial Insemination (AI) or cryopreservation (O'Hara *et al.* 2010). Regarding the MM in this trial, semen samples of both groups should be acceptable for AI, especially cryopreservation (Malejane *et al.* 2014).

The result showed for G2 CONC (3.63×10^9 sperm/mL) may be due to these rams had higher body weight and scrotal circumference, which may reflects better functions of testes in semen production (Mahmound, 2013). In agreement with Maia *et al.* (2011), sperm concentration ranges two to five billion sperm/mL, and in healthy and well-nourished animals, it is not hard to get ejaculated sperm with concentration above three billion. In a study conducted by Carvalho *et al.* (2002) in Rio de Janeiro, animals with 36 months had 5.17×10^9 sperm/mL. These findings may indicate the effect of age on CONC.

Assessing individually the age groups, it was observed a highly decrease in percentage of MAD (G1 - 6.99%; G2 - 4.07%), suggesting that as animals' age increases, there is a reduction in MAD. These results agreed with Pacheco *et al.* (2009). The presence of a large number of abnormal sperm not only indicates genital disease processes, but is also associated with decreased fertility (Saacke, 2008).

The mean of TOD in G1 (27.82%) was far above the recommended by CBRA (2013) that is < 20%. This result can be explained by the fact that most these animals had not achieved sexual maturity. Souza (2003) reports that puberty occurred at 6 months old, however sexual maturity was only achieved at 10 months. According to Maia *et al.* (2011), puberty is more related to body weight than the age of the animals. With the beginning of spermatogenesis, there is a gradual improvement in semen quality, and sexual maturity is reached quickly.

The means of SC obtained in this study for G1 (30.96 cm) and G2 (33.34 cm) correspond to those described in literature, ranging from 28.93 cm (Pacheco *et al.* 2009) to 34.00 cm (Carvalho *et al.* 2002; Maia *et al.* 2011). Both groups were within the established standard for pubescent rams of at least 30 cm of SC, and this value is widely used by several organizations and groups involved in genetic improvement of sheep worldwide (CBRA, 2013). The greatest interest in this feature due to its importance in male selection processes with higher reproductive potential, which is a practical and effective measure able to predict, objectively, the production and sperm quality (Pacheco, 2008). Furthermore, it is easy for measurement, showing high correlation with body weight and seminal characteristics (Silva *et al.*, 2002).

Similar correlations among VOL, BW and SC were observed in a study carried by Souza *et al.* (2007) with Santa Ines sheep. The high and significant correlations among the qualitative characteristics are expected in rams, since they have expressive MM (Souza *et al.*, 2007). Thus, MM increases as the speed of sperm (VIG) increases, and consequently, the same is observed in MOT, because a higher number of sperm in straight forward movement.

There was a low correlation between BW and SC and of these characteristics with all the features evaluated in this experiment. Similar results were observed by Bertipaglia *et al.* (2012), explaining that the low genetic correlation between scrotal circumference and these traits, a fact resulting in low phenotypic correlation.

The repeatability coefficient is widely used in animal breeding as far as the upper limit of heritability and prediction genotype value from successive measurements on the animal. The repeatability of seminal characteristics suggested a great environmental influence and, therefore, should be assessed by several times and at different times of the animal's life.

VOL and SC had higher repeatability and few number of measurements required to predict these characteristics, suggesting that the greater the repeatability of a characteristic, the greater the confidence to have a few records of this characteristic. Silva *et al.* (2002) consider the scrotal circumference easily to measure, which has high repeatability, so it is more reliable, indicating its potential for ram selection.

Few studies over repeatability of reproductive characteristics in rams are found in literature, showing medium to low repeatability as the presented work. Langford *et al.* (1989), estimating the repeatability of SC and seminal characteristics of rams, found the follow-

ing values: SC (0.85), VOL (0.62) CONC (0.35), MOT (0.26). David *et al.* (2007) estimated the repeatability of Lacaune males sheep and they found 0.46 (VOL), 0.52 (CONC) and 0.38 (MOT) in adults (≥2 years) and 0.35 (VOL), 0.51 (CONC) and 0.30 (MOT) in young sheep (≤ 1 year).

CONCLUSIONS

In the selection of potentially fertile Santa Ines rams kept in semi-feedlots, it should make multiple evaluations of semen characteristics, since these have low repeatability. It is suggested to evaluate males at different times of year to investigate their reproductive efficiency before being used as breeding stock.

ACKOWLEGMENTS

We thank the group of PROVIN (Extension Project in Production and Sheep Selection) and the ovine breeders for providing the animals and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and FAPERJ (Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro) to contribute with financial support.

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