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Effect of inbreeding on body growth traits and sperm DNA fragmentation level in rams*

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On a small closed population of Mis sheep the relationship was studied of the influence of inbreeding on body weight growth from birth to the age of 18 months and sperm DNA fragmentation in rams. Two groups of male lambs were used. First was composed of outbred, while the second of inbred animals with inbreeding coefficient over 25%. Differences in body weight and daily gain related to the presence of inbreeding in the pedigree were not found significant (P>0.05). The mean value of sperm chromatin damage in rams of the outbred group varied from 1.93 to 12.37%, (mean = 7.32%) and in inbred group from 13.76 to 37.67% (mean = 25.23%). Significant difference was identified between the outbred and inbred rams in the mean percentage of sperm damaged (P<0.01).

KEY WORDS: body growth / DNA fragmentation / inbreeding / sperm DNA

It is well known that over several generations a small closed population is coming to an increase in homozygosity and number of side effects in the offspring [Petrovic 2000]. The unavoidable mating of related animals in a closed population leads to accumulation of inbreeding and decreased genetic diversity [Falconer and MacKay 1996]. This is particularly evident in sheep where the purebred animals are grown

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on a farm without mixing with other populations and where a small number of rams is used in each stage [Norberg and Sorensen 2007]. Many farmers keep flocks of 50-200 ewes and perform repairs by selecting animals from their own herd. Thus, after some years homozygosity increases and farmers, consciously or unconsciously, promote inbreeding, which results in the occurrence of inbreeding depression. Sheep quantitative traits have a genetic background, with various rate of manifestation on the phenotype level. In this direction are carried out various studies [García-Cortés *et al*. 2001, Ugarte 2007, Petrovic *et al*. 2011]. Many studies on domestic animals, as well as wildlife populations, show that inbreeding reduces fitness [Gomendio *et al.* 2000, Keller and Waller 2002]. According to the theory of genetics and modern scientific research, this would lead to inbreeding depression, which manifests through a reduction of vitality, body growth and reproductive capacity of the population [Petrovic 2000, Rzewuska *et al*. 2005, Norberg, and Sorensen 2007]. Inbreeding depression varies widely between species and populations [Gama and Smith 1993, Hedrick and Kalinowski 2000]. Understanding the full impact of these genetic factors and their basic mechanisms is important to reduce their adverse effects on animal populations. As stated by Lopez *et al*.[2010], inbreeding increases genome-wide homozygosity leading to the expression of deleterious recessive alleles, which results in high juvenile mortality, low female reproductive success and increased vulnerability to parasites. Inbreeding depression often affects male reproductive traits. According to Khalifa *et al*. [2008], the structural stability of paternal chromatin is of vital importance for the fertilization process and early embryonic development. Moreover, a relationship was reported between the level of inbreeding and sperm structure in rams [Margulis and Walsh 2002, Andrabi 2005, Zajitschek *et al.* 2009]. In humans, a positive relationship has been established between body weight and DNA sperm damage [Kort *et al*. 2006, Chavarro *et al.* 2010].

The aim of this study was to identify the relationship between inbreeding, growth rate, and sperm DNA fragmentation level in rams.

Material and methods

The present study was carried out on sheep of the Mis breed at the experimental farm of the Institute for Animal Husbandry, Belgrade, Serbia. Pedigree and performance data of a flock were kept on the farm since year 1991. For this study two groups of 20 ram lambs were used. The first group were outbred, and the second – inbred lambs with inbreeding coefficient over 25%. Lambs of both groups were reared from birth to the age of 18 months under the same maintenance, feeding and care conditions. During that time their following traits were measured and recorded: body weight at birth (BWb), on day 30 (BW 30), day 60 (BW 60), day 90 (BW 90) and month 6 (BW6), month 12 (BW12) and month 18 (BW18) of live. Moreover, the mean daily live weight gain was computed for the period from birth to day 30 (DG 30) and from day 31 to day 60 of life (DG 60).

With over 18 months of age, from all 20 rams the semen samples were collected (electroejaculation) in order to investigate the structure of chromatin and DNA fragmentation. The samples were examined cytometrically (Guava Milipore – IMV flow cytometer, USA), and percentage of damaged chromatin (%DNA) was determined [Evenson *et al.* 2002]. Fresh semen was diluted to reach a final concentration of $2x10^6$ spermatozoa/ml, exposed to acid detergent solution and stained with acridine orange. In the sperm chromatin structure assay (SCSA) a flow cytometry method is based on the differences in the fluorescence of acridine orange after binding to double- and single-stranded DNA. The percentage of spermatozoa with high levels of red fluorescence (fragmented DNA) and high levels of green fluorescence (immature spermatozoa) were quantified. The DNA fragmentation index (DFI) was calculated as the ratio of red to total fluorescence.

Inbreeding coefficient for each animal was calculated using DFREML set of programmes [Meyer 1991]. Data of DFI and measuring the body growth were analysed using statistical software package SPSS 15.0 [2006].

Significance of differences between groups was evaluated using T-test procedure.

Results and discussion

Relations between inbreeding and growth traits of lambs are presented in Tables 1 and 2. Daily gain up to day 30 of life (DG30) in outbred rams occurred slightly lower than that found in inbreds and the difference of 31.2 g was not significant (P>0.05). In the second month, a similar trend was recorded. Specifically, daily gain for DG60 in outbred lambs was lower by 14.2 g, but the intergroup difference was still not significant (P>0.05). The BW90 indicates that the mean body weight of a single inbred ram at the age of 90 days was by 2.45 kg greater than of the outbred ram of the same age. However, the intergroup difference was not significant (P>0.05). At

Table 1. Means and standard errors for growth traits

Growth trait	Group	Mean	Standard error (±SE)
DG 30 (g)	outbreds	233.90	17.25
	inbreds	265.10	16.94
DG 60(g)	outbreds	269.40	18.06
	inbreds	283.60	11.69
BW 90 (kg)	outbreds	29.40	1.44
	inbreds	31.85	1.56
BW 6 (kg)	outbreds	50.10	1.38
	inbreds	52.80	1.77
BW 12 (kg)	outbreds	82.70	1.80
	inbreds	83.70	2.43
BW 18 (kg)	outbreds	85.45	1.74
	inbreds	86.80	2.32

Table 2. Paired samples for analysis of differences in growth traits

Growth trait	Group	Paired differences		T
		Mean	SE Mean	
DG30	outbreds-inbreds	-31.20000	21.02316	1.484
DG60	outbreds-inbreds	-14.20000	14.96054	0.949
BW90	outbreds-inbreds	-2.45000	1.59243	1.539
BW6	outbreds-inbreds	-2.70000	2.14502	1.259
BW12	outbreds-inbreds	-1.00000	2.17945	0.459
BW18	outbreds-inbreds	-1.35000	1.91057	0.707

the age of 6 months, the mean body weight of the outbred rams was again lower, the difference of 2.7 kg being significant (P>0.05). At the age of 12 months the mean body weight of the outbred ram was by 1,0 kg higher than that of the inbred ram. Mean body weight at the age of 12 months shows that difference of 1.0 kg in favour of outbred group was not significant (P>0.05). This trend continued until the end of the study which terminated at the rams' age of 18 months, when the intergroup difference of 1.35 kg also occurred not significant (P>0.05).

There are various reports about the effects of inbreeding on body growth of lambs. Norberg and Sorensen [2007], studied inbreeding trend and inbreeding depression in the Danish populations of Texel, Shropshire, and Oxford Down sheep. Inbreeding depression for birth weight, daily gain over first 2 months of life and litter size was estimated for all 3 breeds. All traits showed depression due to inbreeding of the animal itself. For most combinations of trait and breed, there was also a significant reduction of the phenotype due to inbreeding in the dam. The value of inbreeding depression was 1.2 to 2.6% of the mean, resulting in an increase in the inbreeding coefficient of the individual of 0.10, and estimates were similar for similar increases in maternal inbreeding. Carolino *et al*. [2004] investigated the effect of inbreeding and inbreeding depression in a Churra Badana sheep flock. Ceyhan *et al.* [2011] concluded that inbreeding had no effect on lamb's weaning weight and survival rate. Boujenane and Chami [1997] found non-significant effects of lamb inbreeding and significant negative effects of inbreeding of the dam in the Sardi breed. Significant effects of inbreeding of lambs in Indian breed of sheep Muzaffarnagari, were reported by Mandal *et al.* [2002]. Boujenene and Chami [1997] reported negative effect of inbreeding on body weight at birth and in early rearing periods in Beni Guil sheep. Negative effect of inbreeding was reported by Rzewuska *et al*.[2005], Analla *et al*. [1998, 1999] and Ercanbark and Knight [1991] in Rambouillet and Targhee sheep.

It can be concluded that many authors found reduction in birth weight, daily gain or body weight of inbred sheep. In this study such effect was not found. From the literature cited, we also know that some authors found no negative effects of inbreeding on growth of lambs which are in accordance with our results.

Inbreeding effect on sperm chromatin is shown in Table 3. The value of sperm chromatin damage of rams in the outbred group ranged from 1.93% to 12.37%.

The mean value of the damage amounted to 7.32%. In the group of inbred rams, the interval of damage ranged from 13.76% to 37.67% (mean for the whole group =25.23%). There was a highly significant difference in the percentage of the mean damage of sperm between the inbred and outbred group of rams ($P<0.01$).

In addition, if we recall that the chromatin damage in rams above 25% is a very serious problem, the significance of this research becomes even important. Male

Table 3. Means and standard errors for sperm DNA fragmentation level

Status of chromatin (DNA)	Group	Mean	Standard error (±SE)
Undamaged (%)	outbreds	92.67	8.09
	inbreds	74.77	2.19
Damaged (%)	outbreds	7.33	1.22
	inbreds	25.23	1.89

fertility declines when DNA Fragmentation Index (DFI) values exceed 10-20% in bulls, and 8% in boars [Evenson and Wixon 2006]. The magnitude of the level of sperm DNA damage found in the endangered species (gazelles) with high levels of inbreeding is thus enormous when compared to outbred populations [Lopez *et al.* 2010]. Findings of the authors mentioned suggest that the link between inbreeding and semen quality is mediated by the effects of inbreeding upon sperm DNA damage.

Such high levels of sperm DNA fragmentation are thus likely to have a considerable impact upon male fertility. On the other hand, males with high levels of sperm DNA damage may fertilize under optimum conditions (for example, given enough time and repeated sexual access to females) and in the absence of competition from other males [Ballachey *et al.* 1988, Evenson *et al*. 1994], as is the case in captive breeding programmers. In these cases, the damage in sperm DNA may result in deleterious effects upon offspring [Aitken *et al.* 2004, Lewis and Aitken 2005] . Many studies suggest that inbreeding in sheep and other populations has an impact on the vitality of the offspring [Overall *et al.* 2005].

When it comes to rams with damaged sperm various studies have been carried out. Most research concerns the link between the degree of damage and the important properties of sperm, as well as links to fertility rams. As for the relation between the level of damage in the sperm chromatin and inbreeding, in the literature no data are available. However, our findings are consistent with the already cited study on the population of gazelles [Lopez *et al.* 2010] where the negative effects can be seen of inbreeding on sperm chromatin. Some future research in rams will deny or confirm our results.

In addition, it is interesting to detect the relationship between body growth and sperm DNA damage. No relevant information on that matter is available for the population of sheep, but Chavarro *et al*. [2010] report that sperm with high DNA damage were significantly more numerous in obese than in normal weight men. Kort *et al*.[2006] found that overweight and obese men showed a significantly higher

percentage of sperm with DNA damage when compared to normal weight men. Although the above cited results could not be literally compared to the present study, they can be taken as evidence of a link between sperm DNA damage and body weight. But it is interesting that despite the fact that body weight differences between groups in our research were not significant (P>0.05), however, animals of inbreeds group-with greater DNA damage, during the whole period of growth had a higher gain and higher body weight. Therefore, our research can be understood as a contribution to a deeper understanding of the unity of the animal organism and its genetic correlation with all the characteristics of the body, including growth and reproduction.

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