

## OCCURRENCE OF CHROMOSOME ABERRATIONS IN HETEROSEXUAL TWINS OF MERINO SHEEP

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### **Summary**

This study was aimed at the occurrence of chromosome aberrations in heterosexual Merino sheep twins. The occurrence of chromosome aberrations in a healthy ram lamb and his sister have been compared. The sister showed the signs of malformation. The clinical finding revealed slight shift of vulva and small ear lobes. We have recorded 1 % and 4 % occurrence of centric fusions (CF) in ram lamb and in ewe lamb, respectively. There was the high percentage of associations of acrocentric chromosomes as well - i.e. as much as 23 % in ram lamb and 12 % in ewe lamb.

**Key words:** sheep, chromosome aberrations, heterosexual twins

### **Introduction**

The degree of inter-sexuality in animals affected with developmental abnormalities may be considerably altered in female reproductive organs only slightly reduced (Szatkowska et al., 1993), next to female gonades that are situated in rudimentary scrotum (Jonsson, Gustavsson, 1969; Dain, Tucker, 1970). The literature concerning the extent of masculinization in freemartins presents either bases or the aspects relative to the hermafroditism (Szatkowska et al., 1998). Freemartinism proves to be the common name for developmental abnormalities of reproductive organs in twins of different sex to be mostly sterile. Freemartinism is not a hereditary abnormality; its occurrence, however, is hereditary conditioned by the fact that it may originate merely with heterosexual twins and the pre-disposition for hatching of twins is usually hereditary conditioned (Vademecum of Veterinary Medicine, 1991).

Sheep karyotype ( $2n=54$ ) consists of three pairs of long metacentrics and 23 pairs of telocentric autosomes. Chromosome X is big and acrocentric, chromosome Y is small (Long, 1990). In view to the fact that the occurrence of phenotypically different animal - i.e. Merino ewe lamb coming from heterosexual twins was observed we have done the cytogenetic analysis for we have supposed that this may be the case of freemartinism with the possible occurrence of mosaic (XX/XY).

### **Materials and methods**

We have observed the cytogenetic picture of an ewe (mother) and her progeny (twins) of the ewe lamb having anatomical abnormalities (small ear lobes and shifted position of vulva) as well as Merino ram lamb. Heparinised blood (100 IU/ml) taken from *vena jugularis* was used for analysis. Lymphocytes obtained from peripheral blood were treated according to Moorhead et al. (1960). 0.5 ml of heparinised blood was added into the medium supplemented with phytohaemagglutinin. In addition, antibiotics – i.e. penicillin (100 IU/ml) and streptomycin (100 µg/ml) were added. Lymphocytes were cultured for 72 hrs at 37.5 °C. Two hours prior to the end of cultivation, colchicine (10 µg/ml) was added. The preparations were stained with 10 % Giemsa-Romanowski solution in the phosphate buffer ( $2.5 \cdot 10^{-2}$  mol.l<sup>-1</sup>) pH 7.0. To determine structure aberrations, we have used the Atlas of Chromosome Aberrations by Klen and Srb (1982) as well as the studies of Savage (1975) and Carran and Natarajan (1988).

### **Results**

The results of conventional chromosome analysis are presented in Table 1. Three Merino sheep were included in the study - i.e. mother and heterosexual twins. The ewe was not possible to be assessed due to the mitotic index. This is why it was possible to compare only the progeny namely phenotypically normal ram lamb and ewe lamb that was slightly malformed in the zone of vulva (slight shift) and in the size of ear lobes (small). Cytogenetically, the occurrence of CF was recorded in ram lamb and in ewe lamb (1 % and 4 %, respectively). We have also recorded rather high occurrence of the associations of acrocentric chromosomes in ram lamb and in ewe lamb (23 % and 12 %, respectively). In addition, chromatide breaks as well as gaps were recorded.

### **Discussion**

The results presented show an interesting occurrence of centric fusions and also high occurrence of associations of acrocentric chromosomes that may represent a "pre-degree," with the originating of centric fusions. Originating of these fusions is connected with the alteration of chromosome material in such a manner that the part of the little arms of a chromosome will be translocated and connected with either the arms or the centromer (CF) of another chromosome. The carrier, however, may pass so abnormally altered chromosomes to its embryonal cells where after the fertilization with a

cell with normal chromosomes the defect is not compensated and a part of chromosome is either missing or additional in the progeny of carriers (Kučerová et al., 1981).

Table 1 Chromosome aberrations in heterosexual sheep twins.

Breed: Merino	Ram lamb	Ewe lamb
Number of assessed	100 mitoses	50 mitoses
Hyposomie	11 (%)	4 (8 %)
Polypliodie	1 (%)	0
Chromatid breaks	4 (%)	1 (2 %)
Chromatid gaps	2 (%)	0
centromeric fusion	1 (%)	2 (4 %)
Association	23 (%)	6 (12 %)
2 Associations	1 (%)	0
Centromere separation of metacentric	14 (%)	3 (6 %)
Centromere separation	3 (%)	0
Fragment	0 (%)	1 (2 %)
Double minute	0 (%)	1 (2 %)

The occurrence of a mosaic (XX/XY) testifying for freemartinism was not recorded. This indicated to the fact that in sheep only 5 to 10 % of twins are being born with vascular anastomoses. On the other hand, the chimerism of red blood cells represents merely 5 % of this amount. In cattle, however, it is occurring almost regularly in heterosexual twins (as much as in 92 %) (Vademecum of Veterinary Medicine, 1991).

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### ELECTROPHORETIC CHARACTERISTIC OF PLASMA PROTEINASE INHIBITORS IN IMPROVED VALACHIAN SHEEP

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### Summary

$\alpha_1$ -proteinase inhibitor in blood plasma of Improved Valachian sheep has been studied by isoelectric focusing in a mixture of ampholytes pH 4.2 – 4.9; pH 4.5 – 5.4, and pH 3.5 – 9.3. Altogether, 16 – 17 fractions of proteinase inhibitors – i.e. trypsin were recorded in the blood plasma of breeding rams. Strong activity inhibitors in rams ranged within isoelectric points pH 3.75 – 6.0. Fractions with lower inhibitory activity were in the zone of pH 3.5 - 3.75. The lowest activity fractions were detected within pH 6.55 - 6.85 and in alkaline zone of pH 7.35 – 8.15. In ewes, 4 majority fractions were detected within pH 3.75 – 6.0 as well. In alkaline zone of pH 7.35 – 8.15 no proteinase trypsin inhibitors were detected. Altogether 12 to 13 fractions were detected in ewes. Both in males and females the esterolytic inhibition against chymotrypsin was determined. Fractions of P.I. trypsin following the charge electrophoresis covered the zone from post- $\gamma$ -globulin up to the prealbumin one.