

## Occurrence of freemartinism in Santa Ines sheep

[Ocorrência de freemartinismo em ovino Santa Inês]

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**ABSTRACT** - This study reports a case of freemartinism of the XX/XY chimera type in a Santa Ines sheep belonging to a property in northeastern Para, Brazil. Discrete masculinization and external genitalia presenting a pseudoprepuce with a hypoplastic penis were observed *in vivo*. Anatomopathological examination of the internal genitalia showed the absence of a cervix and bilateral presence of an ovary and testis. Variable degrees of epididymal development, accompanied by agenesis of the deferent ducts and other structures arising from the mesonephric ducts, were also observed. Follicular structures, a uterine tubular portion, with marked agenesis and aplasia of the uterine tubes and in some portions of the horns, as well as other parts of paramesonephric structures, were noted in the ovaries. Blood was collected for karyotype analysis and 10 metaphases obtained by short-term lymphocyte culture were examined. Two cell lines were identified: 54,XX (5 cells) and 54,XY (5 cells).

**Keywords:** Freemartin, XX/XY chimerism, sheep, Santa Ines

**RESUMO** - Relata-se um caso de freemartinismo do tipo quimera XX/XY em ovino da raça Santa Inês, proveniente de uma propriedade no Nordeste Paraense (Brasil). *In vivo* observou-se masculinização discreta, genitália externa apresentando um pseudo-prepúcio com pênis hipoplásico. No exame anatomopatológico verificou-se na genitália interna a ausência de cervix, ocorrência bilateral de ovário e testículo. Também se observou graus variáveis de desenvolvimento do epidídimo, com considerável agenésia dos ductos deferentes e outras estruturas provenientes dos ductos mesonéfricos. Nos ovários visibilizou-se estruturas foliculares, porção tubular uterina, com maior agenésia e aplasia das tubas uterinas e em algumas porções dos cornos, igualmente como outras partes das estruturas paramesonéfricas. A partir do sangue foi realizado a análise do cariótipo pela análise de dez metáfases, obtidas por cultura temporária de linfócitos, verificando a presença de duas linhagem celulares, sendo 54,XX (5 células) e outra 54,XY (5 células).

**Palavras-Chave:** Fremartin, quimerismo XX/XY, ovino, Santa Inês.

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## INTRODUCTION

An intersex is an animal that presents congenital anatomical variations that confuse the diagnosis of sex. This animal possesses the reproductive organs of both sexes, or may belong genetically to one sex and phenotypically to the other. The term intersex includes hermaphrodites, pseudo-hermaphrodites, freemartins, and other forms of sexual inversion (Hafez, 1995).

In sheep, the occurrence of an intersex corresponds in most cases to an XX/XY chimerism due to freemartinism (Grunert et al, 2005). A chimera is defined as an individual that possesses two or more genetically distinct cell populations originating from more than one zygote within a single individual. The most widely studied mechanism in sheep and goats is the fusion of placental blood vessels between twins of different sexes (Al-Ani et al., 1998; Capel and Coveney, 2004).

In sheep, freemartinism has traditionally been considered to be a rare anomaly, but recent studies suggest that this anomaly is more common than previously reported and its prevalence might be increasing (Marcum, 1974). The discovery of high fecundity genes and multiple ovulations have increased the risk of freemartinism in sheep when the number of lambs per litter is four or higher (Parkinson et al., 2001). Previous studies have reported an incidence of freemartinism in sheep of about 1% (Long, 1980). A similar prevalence (1.2%) has been detected in slaughtered animals<sup>10</sup> and in twin pregnancies of ewes in the field (Parkinson et al., 2001).

This study reports a case of freemartinism of the XX/XY chimera type in a Santa Ines sheep, pathology of rare occurrence in this species.

## MATERIALS AND METHODS

A Santa Ines sheep with an estimated age of one year and belonging to a farm located in the northeastern region of the State of Pará, Brazil, was sent for analysis to the Centro de Pesquisa em Caprinos e Ovinos do Estado do Pará (CPCOP), associated with the Sector of Veterinary Pathology, Universidade Federal Rural da Amazônia (UFRA). The farm had no records, identification or clinical data of the animal, and no information could be obtained about whether the animal originated from a twin pregnancy or about any other birth data.

*In vivo* examination of the animal showed discrete masculinization and the external genitalia presented a pseudoprepuce with a hypoplastic penis (Figure 1).

The animal was sacrificed for anatomopathological analysis. Examination of the internal genitalia revealed the absence of a cervix and bilateral presence of female and male gonads (ovary and testis), but no ovotestis. Variable degrees of epididymal development were also observed, but were accompanied by marked agenesis of the deferent ducts and other structures arising from the mesonephric ducts. Follicular structures, a uterine tubular portion with greater agenesis and aplasia of the uterine tubes and in some portions of the horns, as well as other parts of paramesonephric structures, were noted in the ovaries (Figure 2).

Blood was collected from the external jugular vein into Vacutainer tubes containing EDTA for cytogenetic study. Karyotype analysis was performed using the conventional Giemsa stain for chromosome staining. Analysis of 10 metaphases obtained by short-term lymphocyte culture showed the presence of two cell lines: 54, XX (5 cells) and 54, XY (5 cells).

The study was approved by the Ethics and Biosafety Committee of Instituto da Saúde e Produção Animal (ISPA), UFRA, and the animal experiments were conducted according to ethical guidelines.

## RESULTS AND DISCUSSION

An intersex with an XX/XY karyotype may be the result of mosaicism due to failure of cell division of the zygote, or of chimerism. The latter may occur when a polar body is accidentally fertilized by a Y chromosome spermatozoon and the corresponding oocyte is fertilized by an X spermatozoon, with the occurrence of a mixture of genetic material of two diploid cells resulting in an individual with two cell populations (Cribiu and Chaffaux, 1990). However, chimerism may also result from freemartinism (Freeman, 2007; Brace et al., 2008). Although no history of the animal was available, the latter hypothesis is more likely in the present case since the phenomenon is more common among sheep.

The fusion of blood vessels permits the passage of substances or hormones that inhibit the growth and differentiation of Müllerian ducts (Müllerian-inhibiting factor, MIF), as well as the passage of testis differentiation factor, in addition to the exchange of cells during the embryonic period, XX/XY

chromosome chimerism (Padula, 2005). However, the masculine phenotype is not due to MIF or testosterone produced by the testis of the developing male embryo (Capel and Coveney, 2004), but rather to the expression of the SRY gene on chromosome Y, which also controls MIF (McElreavey et al., 1993; Clarkson and Harley, 2002).

The reproductive system of female freemartins is characterized by masculinization and the presence of a pseudoprepuce and hypertrophy of the clitoris and, in cases of maximum masculinization, by the presence of a hypoplastic penis as observed in the present case. The internal genitalia of freemartins are characterized by hypoplasia of the uterine body or horn, detection of tubular segments, traces of Wolffian and Müllerian ducts, and rudimentary gonads (ovaries and testes) as observed in the animal studied (Szatkowska, 1995; Szatkowska and Switonski, 1996). However, these findings are in contrast to those reported by Smith et al., who described testis-like gonads, a high degree of masculinization, and no ovaries (Smith et al, 2003).

Genetic breeding has led to an increasing selection of sheep with a tendency toward twin births, a fact resulting in the occurrence of freemartins (Parkinson et al., 2001). However, there are no reports of the occurrence of this phenomenon in Santa Ines sheep or any other sheep species reared in northern Brazil or other regions of the country.

## CONCLUSIONS

The lack of anamnesis data impaired the diagnostic conclusion, but the *in vivo* findings, morphological evaluation of the internal organs and karyotype analysis, in comparison to literature data, led to the diagnosis of freemartinism of the chimera type.

## ETHICS COMMITTEE AND BIOSECURITY

The study was approved by the Ethics Committee and the Biosafety Institute for Animal Health and Production (ISPA), the Federal Rural University of Amazonia (UFRA) and animal studies, in this work were conducted in accordance with ethical standards.

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