DETECTION AND PLACING OF CHROMOSOMAL ABERRATIONS IN SHEEP (*OVIS ARIES*)

DETECCIÓN Y LOCALIZACIÓN DE ALTERACIONES CROMOSÓMICAS EN LA OVEJA (*OVIS ARIES*)

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Palabras clave adicionales

Citogenética. Ovino. Alteraciones cromosómicas. Programa de análisis de imagen por ordenador.

SUMMARY

Cytogenetics studies of 94 rams belonging to Rasa Aragonesa sheep breed have been carried out.

Karyotypes obtained by high resolution RBG and GTG banding methods have been applied to place each chromosome pair into an adequate identification neccesary in gene mapping.

Image Analyser Computer Program (BIOCOM) has been used to obtain the relative lenghts of sheep chromosomes and make photometry studies in chromosomes.

These techniques, both high resolution banding and image analysis, have served to place a deletion in chromosome number 9 (9q11:13,12-) and to detect, in two different rams, one fragile *locus* in the proximal region of the p arm belonging to the chromosome number 1 (1p-).

The results of these studies and the efficacy of the methods mean a very important advace in the Chromosomal Studies of sheep, especially in the field of detecting and locating chromosomal aberrations.

RESUMEN

Se han desarrollado estudios citogenéticos en 94 moruecos de la raza Rasa Aragonesa.

Con objeto de facilitar las tareas de cartografía génica, se han aplicado procedimientos de bandeo de alta resolución RBG y GTG, que permiten la identificación de cada par cromosómico.

El Programa de Análisis de Imagen por ordenador (BIOCOM) se ha utilizado para obtener las longitudes relativas de los cromosomas ovinos y realizar estudios de fotometría en cromosomas.

Las técnicas de bandeo cromosómico de alta resolución y de análisis de imagen han servido para determinar la posición de una deleción en el cromosoma 9 (9q11:13,12-) y para detectar (en dos moruecos diferentes) un *locus* frágil en la región proximal del brazo p del cromosoma 1 (1p-).

Los resultados de estos estudios y la eficacia de los métodos suponen un importante avance en los estudios cromosómicos de la oveja, especialmente en el campo de la detección y localización de alteraciones cromosómicas.

INTRODUCTION

All along the history of Cytogenetics,

a series of chromosomal aberrations has been found. In the beginning, they were natural-type aberrations but, at the moment, they are produced artificially in order to reveal fragile *loci*.

As a rule, the chromosomal aberrations have been classified into two groups: numerical and structural aberrations. This second group is composed of two chromatid affected aberrations (chromosomal aberrations) and only one chromatid affected aberrations (chromatidal aberrations).

While the first ones can be evidenced in the animal phenotype, in the most of the cases can appear like a fall in fertility, embryonic and neonatal mortality (miscarriages), freaks and anatomical or physiological malformations (Hare and Singh, 1979), the structural aberrations are much more unpredictable: aberrations like centric fusion 1/29, studied by Gustavsson in cattle, have produced losses valorised in 20 millions of Swedish crowns per year (Gustavsson, 1979). Nevertheless, a lot of structural aberrations are hardly detectable in the animal physiognomy.

The importance of these aberrations, in spite of the fact that it looks like evident, is, at the moment, a point for discussion in a lot of colloquiums about animal citogenetics. In one hand, chromosomal aberrations are transmissible to the progeny, with all the injuries that this supposes. In the other hand, they are source of variability or, in other words, they can take in the Evolutionary Process (Pearce *et al.*, 1994)

MATERIAL AND METHODS

Along the realisation of this cyto-genetic studies of the aragonese ovine population belonging to Rasa Aragonesa sheep-breed, 94 rams dedicated to reproduction have been investigated.

The cellular material used has been composed by leukocytes from peripheral blood, cultured in RPMI with calf serum at 10 p. cent, during 48 hours at 37º C. Lecitin from Phytolacca americana (Pokeweed) was also added, to obtain a good mitotic number.

The cellular cycle synchronisation was produced by two different methods:

- Adding to the cultures Democolchicine one hour before the harvest.
- Using the BRdU method described by RÞnne (1989), and Democolchicine one hour before the harvest.

As soon as the cellular material has been taken out by the hypotonic shock, the chromosomes were extended and stained with Giemsa stain.

The chromosomes treated with BRdU were utilised to obtain RBG bands (Rønne 1989, modified by S. Llambi). The GTG bands were obtained by Lin *et al.* (1977) method.

The images of the chromosomes can be produced through the Image Analyser BIOCOM. This analyser, amplified and *developed* the images from the negative photographic films and, it was also, utilised to know the measures of the chromosomes and to establish a colorimetric range through the different density of the stain upon the chromosomes.

RESULTS

Three different types of chromosomal aberrations could be detected in four different rams (around 4,25 p. cent).

The aberration in the 9th pair consists

in a subcentromeric proximal deletion (9q(11:13,12-)). It can be revealed through Giemsa stain and after that a G-banding was realised in order to determine the chromosomal pair which belongs to and to locate the fine situation along the chromosome. This aberration appeared in a percentage of 95 p. cent over 50 metaphases in the same ram.

The **figure 1** belongs to one metaphase stained with Giemsa. The altered chromosome can be observed in this amplification marked with one arrow. The **figure 2** shows one G-banded metaphase, in which is possible to appreciate the deletion in the subcentromeric region of the 9 chromosome between 11 and 13 band (marked with one arrow).

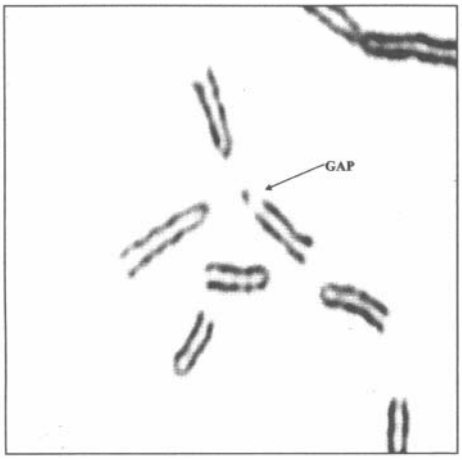

Figure 1. *Image of a ovine metaphase. The gap is showed by an arrow. The image was obtained by the image analyser BIOCOM and printed by a color bubble jet printer.* (Imagen de una metafase ovina. El desgarro es mostrado por una flecha. La imagen fue obtenida mediante el analizador de imágenes BIOCOM e impresa a color mediante impresora de chorro de tinta).

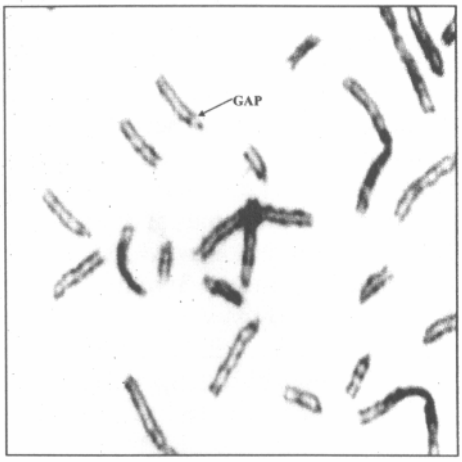

Figure 2. *The same* gap *in a G-banded metaphase. The position of the* gap *was located in the proximal region of the chromosome number 9: 9 del(q11: 13,12-).* (El mismo desgarro en una metafase con bandas G. La posición del desgarro fue localizada en la región proximal del cromosoma número 9:9 del(q11:13,12-).

One chromosomal aberration common in three different rams was found in the proximal third of the small arm in the chromosome number 1 (1 p2?- in two of them and 1p(14:21, 15-) in the third). It shows a chromosomal deletion affecting the two arms. However this aberration appeared with a low frequency (around the 2 p. cent of the metaphases in the same ram).

Through chromosomal measuring methods (Relative Length to X chromosome and χ^2 test) was possible to determinate that the aberration, in all the rams, was situated in the same chromosome and as well, in the same relative position along the chromosome **(table I)**.

The **table I** shows the relative measurements (RLX) of all the acrocentric ovine chromosomes, measured upon the **figures 3** and **4**. Afterwards, the chromosomes were paired comparing the measures obtained for each chromosome with the theoretical measures expected for each autosomal pair. In this way, it can be verified that the chromosomes A3, A6, B4 and B6 belonged to the pair number 2 **(table II)** and the chromosomes A4, A5, B2 and B5 to the third ovine pair 3 (with a 95 p. cent of security). However, although the chromosomes A1, A2, B1 y B3, had the same relative length of the q arm (11.11) the chromosomes A2 and B3 (deleted chromosomes) owned a length of the p arm different from A1 and B1, about 3 in both chromosomes (3.33 and 2.7).

The deletion can be observed in two Giemsa-stained metaphases **(figures 3** and **4)**. In the **figures 5** and **6** is possible to observe the gap in the chromosome 1, next to others aberrations in only one RGB-banded metaphase.

One of the rams shows a series of aberrations, until four gaps in the same metaphase. The cells of this ram were cultured with BRdU/FRdU in order to get RGB-banded chromosomes. The results can be observed in the **figures 5** and **6**.

Table I. *RLX of the different acrocentric chromosomes from the figures 3 and 4.* (RLX de los diferentes cromosomas acrocéntricos de las figuras 3 y 4).

Chromosome	R.L.X .p	R.L.X.q	whole R.L.X
A1	10	11.11	21.11
A2	3.33	11.11	14.44
A3	7.77	9.44	17.21
A4	7.2	8.88	16.08
A5	6.66	7.77	14.43
A6	8.3	9.4	17.7
B1	9.4	11.11	20.51
B2	7.77	8.88	16.65
B3	2.7	11.11	13.81
B4	7.77	9.4	17.17
B5	6.11	7.77	14.88
B6	7.77	9.4	17.17

Table II. *Contingence table to realise the* χ^2. (Tabla de contingencia para la prueba χ^2).

Chromosome	Expected	Observed A	Observed B
2	18.004	17.43	17.17
3	16	15.255	15.765

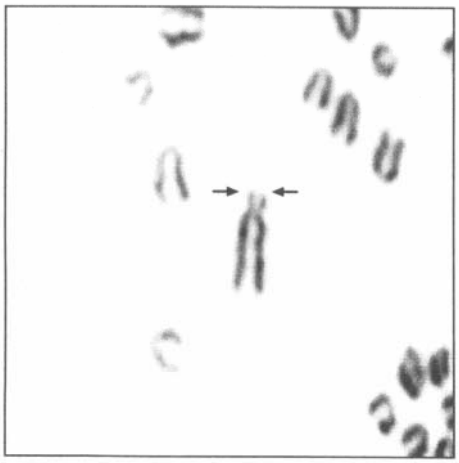

Figure 3. *Image of a ovine metaphase. The deletion is indicated by two arrows. This deletion in the p arm of the chromosome number 1 (1p2?-) was observed in this animal with a frequency of 2 p. cent over 30 observed metaphases.* (Imagen de una metafase ovina. La deleción es indicada por dos flechas. Esta deleción en el brazo p del cromosoma número 1 (1p2?-) fue observada en este animal con una frecuencia del 2 p. cien sobre treinta metafases observadas).

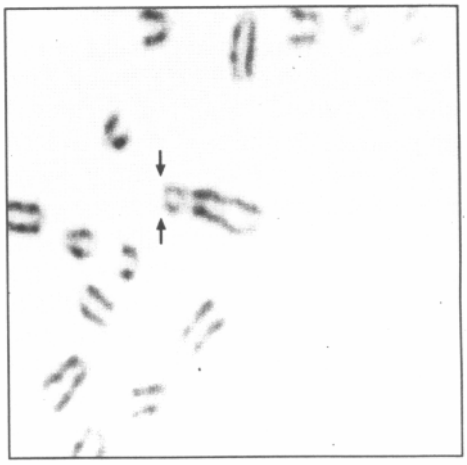

Figure 4. *The deletion appears in other metaphase from a different ram. The deletion-type and the affected chromosome were the same in both metaphases.* (La deleción aparece en otra metafase de un morueco diferente. El tipo de deleción y el cromosoma afectado fueron los mismos en ambas metafases).

The chromosome 6 owns two gaps: one in 6q(13;15,14-) and the second in 6q(21:23,22-), both in only one of its arms. The chromosome 1 reveals also, two gaps: the first, 1p(14:21,15-), affects to both arms and the other one, 1p(22:32,31-),appears in only one of its arms.

DISCUSSION AND CONCLUSIONS

Because of the fault of a tracking in the ancestors and a testing of the progeny by this laboratory, is impossible to evaluate the problem and to know its extent.

However, the fact that the rams are dedicated to reproduction in Genetic Improvement Programs of the Rasa Aragonesa Sheep Breed Association (ANGRA), could guarantee the spread of these chromosomal aberrations to the progeny producing a fall in fertility and prolificity in the flocks where had been used the altered rams.

With rewards to the deletion in the chromosome 9, it is the first time that this aberration is detected in ovine. In the other hand, the deletion in the chromosome number 2 can be associated to the translocation 1/20 described by Glahn-Luft and Wassmuth (1980) and to the deletion studied by this laboratory in the same sheep breed (Arruga *et al.*, 1992). It can say that the ovine chromosome number 1 owns a fragile *locus* located at the distal subcentromeric region (regions 1-2) because of it appears like a deletion of a big fragment of the p arm, 1p2?-, like

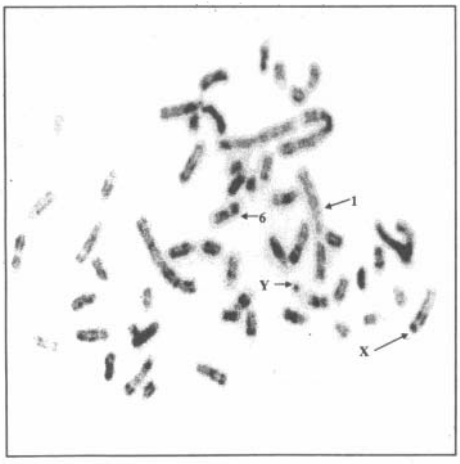

Figure 5. *Image of a ovine RBG-banded metaphase. The two sexual chromosomes and the altered chromosomes are marked with arrows.* (Imagen de una metafase ovina con bandas RBG. Los dos cromosomas sexuales y los cromosomas alterados están marcados con flechas).

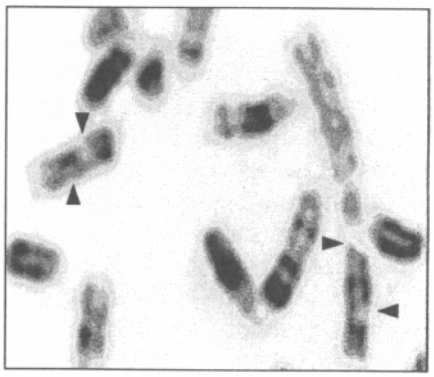

Figure 6. *This amplification of the image 5 shows the different aberrations in the chromosome 1: 1p(22:32,31-) and 1p (14: 21,15-) and in the chromosome 6: 6q(13: 15,14-) and 6q(21:23,22-).* (Esta amplificación de la figura 5 muestra las diferentes aberraciones en el cromosoma 1: 1p(22:32,31-) y 1p (14: 21,15-) y en el cromosoma 6: 6q(13:15,14-) y 6q(21:23,22-).

a gap, 1p(14:21,15-) or, as well, like a translocation tra 1p23-/20q24+.

The application of fluorocroms, like BRdU or FRdU, in order to obtain banded chromosomes is being employed in detection of fragile *loci* (López and Arruga, 1994) and in studies of sister chromatids interchange (Arruga *et al.*, 1987; Catalán *et al.*, 1995). In this case have served to reveal a series of spontaneous fragile *loci* at the treated chromosomes. Even though have appeared chromosomal aberrations, this don't mean that the animal has the alteration, but that they have been observed by the action of certain agents.

Authors like Stranzinger and Fechheimer (1988) had warned about the use of this products to produce banded chromosomes, because they could lead investigator into error, just by the action that these fluorocroms possess against the chromosomes. Other authors like Rønne *et al.* (1994), have established a series of relationships between the appearance of a fragile *locus* through artificial methods and the existence of an aberration in the same place.

In conclusion, the obtained percentage of chromosomal aberrations in the population of rams is relatively important, taken into account that the rams are being used in reproduction. In other way the existence of one fragile *locus* in the first ovine pair and one gap in the chromosome 9 could cause a lot of serious problems in reproduction.

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