DOI: 10.2478/v10083-012-0032-y

ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA

VOL. XXX (4) SECTIO EE 2012

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Application of bovine heterosome painting probes to identification sex chromosomes in goral *(Nemorhaedus caudatus)*

Wykorzystanie bydlęcych sond malujących heterosomy do identyfikacji chromosomów płci u gorala (*Nemorhaedus caudatus*)

Summary. The syntenic conservation nature of many chromosomes enables to use several molecular probes obtained from one species of animals to detect homologous DNA segments in other species. The aim of this study was identification of sex chromosomes in goral (*Nemorhaedus caudatus*), using bovine heterosomes painting probes in the FISH technique . The results obtained showed strong red fluorescence signals in small acrocentric heterosomes Y and distinct yellow-green signals in big acrocentric chromosomes X in all goral metaphase plates.

Key words: goral (*Nemorhaedus caudatus*), genetic conservatism, sex chromosomes, bovine heterosomes probes, FISH technique

INTRODUCTION

Phenomenon of genetic conservatism makes it possible to compare genomes of different species at the level of nucleotide sequences [Rejduch *et al*., 2004; Kozubska-Sobocińska *et al.*, 2007 a; 2009 a], chromosome banding patterns [Rubini *et al*. 1990, Bonnet *et al*. 2001, Chi *et al*. 2005; Kozubska-Sobocińska *et al*. 2006, 2007b, Oh *et al*. 2011] and groups of linked or syntenic genes that are often in the same relationships even in taxonomically distant species [Hayes 1995, Rejduch *et al*. 2010 a, 2010 b, Danielak-Czech *et al*. 2010].

Conservation nature of some chromosomes enables to use a number of molecular probes obtained by microdissection or chromosome sorting in one species of animals, for FISH chromosome painting in another species [Révay *et al.* 2000, 2002, Kozubska-Sobocińska *et al*. 2003, 2005, Huang *et al*. 2005, Kozubska-Sobocińska and Rejduch *2*008].

This study was designed to use two commercial bovine molecular probes (ID Labs), specific to the heterosomes to identify sex chromosomes in goral (*Nemorhaedus caudatus*) and establish genetic conservation of heterosome synteny groups in *Bovidae.*

MATERIAL AND METHODS

Metaphase chromosome spreads (50 cells) of two gorals with normal karyotype 56, XY were obtained from peripheral blood lymphocyte culture (pokeweed mitogen stimulated) according to the routine protocol.

In this paper we present identification of heterosomes by FISH technique with two commercial bovine probes (ID Labs): Bovine IDetect^{TM} Chr X Point Probe GREEN and Bovine IDetect^{TM} Chr Y Point Probe RED (Cambio Ltd., Cambridge, UK). Fluorescence *in situ* hybridization was performed according the manufacture's procedure. DAPI-banding was applied to precisely identify the chromosome subregions. Hybridization signals were observed under an OPTON-Axiophot fluorescent microscope using triple attenuation filters DAPI/FITC/Texas Red and the computer image analyse system LUCIA-FISH (Laboratory Imaging Ltd, Prague, Czech Republic).

RESULTS AND DISCUSSION

In the initial part of our experiment the commercial heterosomes bovine probes were tested by hybridization with metaphase chromosomes of bull (*Bos Taurus*) (Figure 1).

The results of cross-species hybridizations presented in Figure 2 show the distinct red fluorescence signal identifying small acrocentric chromosomes Y and yellow-green fluorescence signal corresponded to acrocentric X heterosomes in all goral metaphase plates.

The first comparative study in the *Bovidae* family showed band homology on the chromosomes of cattle, sheep, goats and water buffaloes [Evans *et al*. 1973].

Comparison of GTG-banded, haploid sets of sheep (2n = 54) and aoudad (*Ammotragus lervia*) chromosomes (2n = 58) revealed complete chromosome homology in the karyotypes of both species and indicated that centric fusions of autosomes led to evolutionary rearrangements [Słota *et al*. 2001].

The karyotype of goral (*Nemorhaedus caudatus*) was subsequently determined as consisting of 56 chromosomes including 54 acrocentric autosomes of similar size and shape, X chromosome – defined as submetacentric by Oh *et al*. [2011] or acrocentric by Huang *et al.* [2005] and acrocentric chromosome Y [Huang *et al*. 2005, Oh *et al*. 2011].

In studies on heterosomes conservation in *Bovidae* most interspecies hybridizations were based on bovine probes generally [Kozubska-Sobocińska *et al*. 2003, 2005, 2009b,

Kozubska-Sobocińska and Rejduch 2008]. The example of using a probe from *Bos indicus* (obtained from microdissected of Yp12 fragment) is identification of a complementary sequence in the X-Y bivalent at metaphase I in *Bos taurus* and performing comparative hybridization (using the Yq12.1-12.6 probe obtained from *Bos indicus*) of the appropriate segment on the q arm of the Y heterosome in *Bos taurus* [Goldammer *et al*. 1996]. A probe specific for the Yp12 fragment was also used to identify the Y chromosome in metaphase plates and spermatozoa [Révay *et al*. 2000].

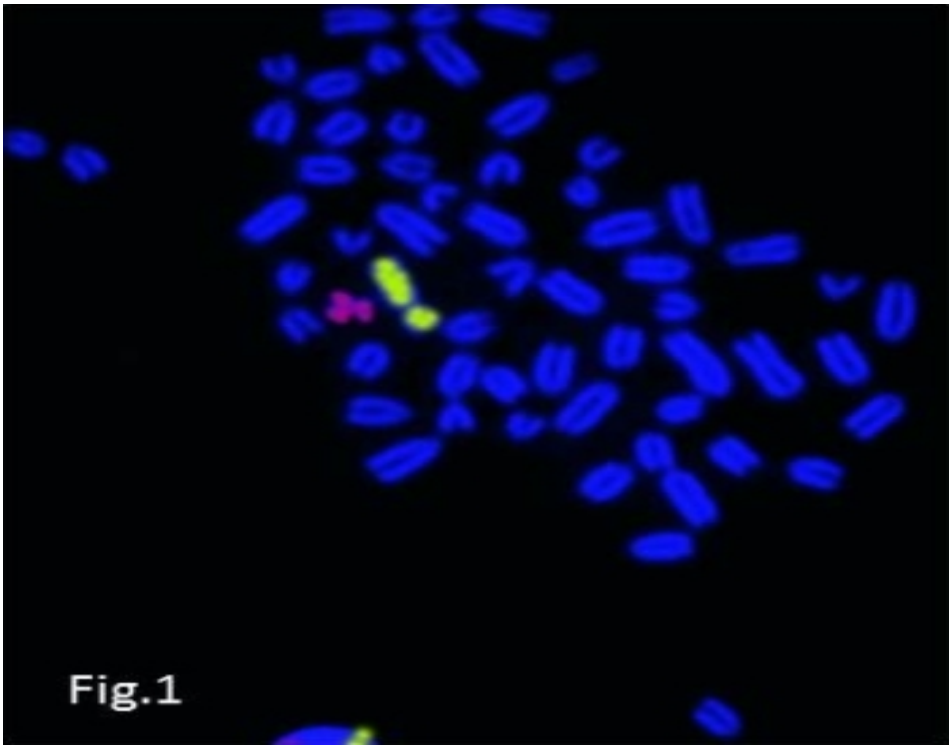

Fig. 1. Metaphase chromosomes of bull. FISH technique – red fluorescence signal labels small metacentric chromosome Y and yellow-green signal identifies submethacentric heterosom X
Rys. 1. Chromosomy metafazowe buhaja. Technika FISH – czerwony sygnał fluorescencyjny znakuje mały metacentryczny chromosom Y a żółto-zielony sygnał identyfikuje submetacentryczny heterosom X

Differently labelled bovine painting probes applied in FISH technique made it possible to determine the frequency of early-dissociation of sex bivalent in rams [Kozubska-Sobocińska *et al*. 2009b].

The high conservation of sex chromosomes in *Ruminantia* is evidenced by hybridization signals obtained by Révay *et al*. [2002] for bull spermatozoa, following the application of probes (using FISH) obtained by heterosome sorting of the yak (*Bos grunniens*).

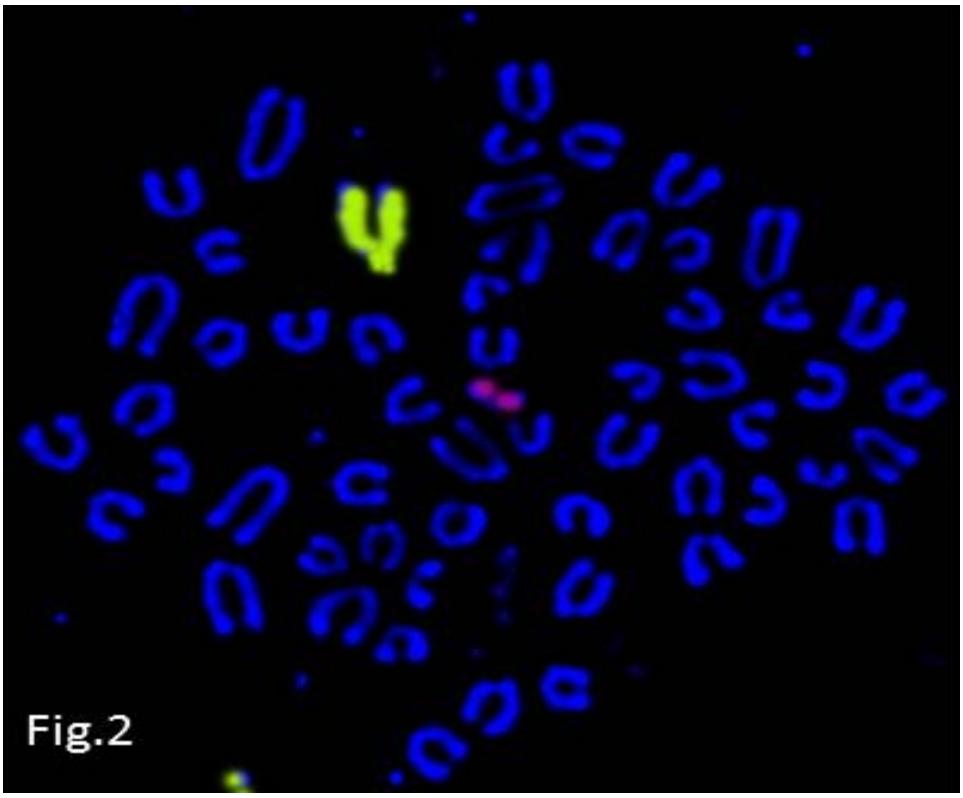

Fig. 2. Metaphase chromosomes of goral (*Nemorhaedus caudatus*) – 56,XY. The cross-species hybridizations with bovine probes specific to the heterosomes: red fluorescence signal labels small acrocentric chromosome Y and yellow-green signal identifies acrocentric X heterosome
Rys. 2. Chromosomy metafazowe gorala (*Nemorhaedus caudatus*) – 56,XY. Miedzydatunkowe hybrydyzacje z bydlęcymi sondami specyficznymi dla heterosomów: czerwony sygnał fluorescencyjny znakuje mały akrocentryczny chromosom Y a żółto-zielony sygnał idntyfikuje akrocentryczny hererosom X

The study presented in this paper confirmed usefullness of hetrosomes-specific bovine molecular probes for identification of sex chromosomes in goral (*Nemorhaedus caudatus*).

CONCLUSIONS

The experiments carried out revealed genetic conservation of heterosome synteny groups in *Bovidae,* which enable to apply of bovine heterosomes probes in cytogenetic diagnostics.

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Streszczenie. Synteniczno-konserwatywny charakter wielu chromosomów umożliwia wykorzystanie licznych sond molekularnych, otrzymanych dla jednego gatunku zwierząt, do detekcji homologicznych fragmentów DNA innych gatunków. Celem tych badań była identyfikacja chromosomów płci gorala *(Nemorhaedus caudatus),* przy zastosowaniu w technice FISH bydlęcych sond malujących heterosomy. Uzyskane wyniki ujawniły mocne czerwone sygnały fluorescencyjne na małych akrocentrycznych heterosomach Y i wyraźne żółto-zielone sygnały w dużych akrocentrycznych chromosomach X we wszystkich płytkach metafazowych gorala.

Słowa kluczowe: goral (*Nemorhaedus caudatus*), konserwatyzm genetyczny, chromosomy płci, bydlęce sondy malujące heterosomy, technika FISH