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The utility of chromosome microdissection in clinical cytogenetics: a new reciprocal translocation in sheep.

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1 **Abstract**

2 Local sheep breeders and scientists in Italy cooperate and conduct research on the genetic
3 improvement of autochthonous genetic types (AGTs) by various approaches, including a
4 cytogenetic breeding selection since 2011. The Laticauda sheep (*Ovis aries*, 2n = 54) breed is one
5 of the AGTs reared in the Campania region (southern Italy). Performing cytogenetic analyses, we
6 have detected and described a novel reciprocal translocation in a Laticauda sheep identified as
7 54,XX t(18; 23)(q14;q26). Our data support recurring appeals that suggest the regular performance
8 of cytogenetic analyses for monitoring genetic health of livestock species. In total, 5 cases of
9 reciprocal translocations in sheep are known, including the new case. None of them has any
10 phenotypic effect on the living offspring. However, affected animals are characterized by sterility or
11 have a low fertility which can have an effect on breeding success and on economical balance.
12 Presence and kind of the described novel chromosomal aberration were detected by performing
13 CBAbanding and FISH mapping with telomeric probes. RBAbanding allowed the karyotyping of
14 sheep chromosomes and the identification of aberrant chromosomes and regions involved in the
15 new reciprocal translocation. Whole chromosome painting (WCP) probes received from equivalent
16 chromosomes in cattle and the derivative sheep chromosome 18 confirmed the cytogenetic data.
17 This way, our study underlined both the importance of WCP probes by chromosome
18 microdissection and a new way to use WCP probes directly generated from derivative
19 chromosomes.

20

21 **Key Words**

22 Chromosome abnormality, Chromosome banding, Chromosome microdissection, FISH, Whole-
23 chromosome painting probes

24

1 **Introduction**

2 Bovids are the largest of 10 families within Cetartiodactyla, consisting of more than 140 extant
3 species and with the Caprinae subfamily including goats (*Capra hircus*, $2n = 60$, CHI), sheep (*Ovis*
4 *aries*, $2n = 54$, OAR), and relatives [Balmus et al., 2007]. Centric fusion translocations are a major
5 feature of karyotype evolution in bovids, where the fundamental number has almost always
6 remained constant at $FN = 60$ [reviewed in Iannuzzi et al., 2009]. They are the most frequent
7 chromosomal aberrations found in both humans [De Braekeleer and Dao, 1994] and domestic
8 animals [Bruere 1974; Gustavsson, 1980; Long, 1985; Ducos et al., 2008]. Actually, centric fusion
9 translocations are the most important and investigated autosomal abnormalities in sheep with 7
10 cases reported. On the other hand, there are only 4 cases of reciprocal translocations in sheep:
11 $t(1p;19q)$ [Glahn-Luft et al., 1978], $t(13q;19q)$ [Anamthawat-Jonsson et al., 1992], $t(2q;3q)$
12 [Popescu and Tixier, 1984; Popescu, 1990], and $t(4q;12q)$ [Iannuzzi et al., 2013]. In our opinion,
13 this low rate does not reflect reality mostly due to the difficulty to detect this type of abnormality
14 when chromosomes are conventionally stained without any type of banding. In addition, the
15 phenotypic effects of reciprocal translocations on the carriers depend on whether they are present in
16 a balanced or unbalanced form. In fact, the balanced forms are most frequent, because they do not
17 have any phenotypic effect on the carriers and are often associated with low fertility or sterility
18 [Gustavsson, 1980; Ducos et al., 2008].

19 Aims of the present study are: (a) the cytogenetic screening of a group of animals from the
20 Laticauda sheep breed, an autochthonous genetic types reared in the Campania region with <1,000
21 PT (total population) and considered an endangered breed; (b) a detailed description of a new case
22 of a reciprocal translocation identified in 1 of the investigated animals using various cytogenetic
23 techniques, including the use of whole-chromosome painting probes (WCPs); (c) the use, for the
24 first time, of derivative WCPs of the carrier on both OAR and BTA (*Bos taurus*) chromosomes.

25

26 **Materials and Methods**

1 *Animals Studied*

2 The cytogenetic screening analysis was carried out on 47 phenotypically normal young sheep from
3 the Laticauda breed (7 males and 40 females) reared in the Campania region. Several cytogenetic
4 analyses were performed to characterize their karyotypes. *Cell cultures, CBA, and RBA-banding*
5 These techniques followed the protocols reported in Iannuzzi and Di Bernardino [2008]. At least 100
6 cells were analyzed for each animal. R-banded karyotypes were prepared using the GENIKON
7 software and the latest international standard nomenclature for domestic sheep [Di Bernardino et al.,
8 2001].

9

10 *Generation of Whole-Chromosome Painting Probes*

11 WCPs for sheep chromosomes 18 and 23 were generated from the equivalent chromosomes 21 and
12 24 of *B. taurus* by laser microdissection (PALM ® MicroLaser system). For each chromosome
13 selected during microdissection, the centromere was not included to avoid aspecific signals. DOP-
14 PCR of microdissected chromosomes was performed without any pretreatment in a PTC-200
15 thermocycler (MJ Research) as described by Kubickova et al. [2002]. A volume of 2 µl of each
16 generated DOP-PCR mix from BTA chromosomes 21 and 24 was labeled with Biotin-16-dUTP
17 (Roche Diagnostics) and a direct fluorochrome (Orange-dUTP, Abbott) in 20 µl of a second DOP-
18 PCR reaction, respectively. On the other hand, the painting probe of der18 was generated following
19 the same procedure and then labeled with Orange-dUTP.

20

21 *Fluorescence in situ Hybridization (FISH)*

22 Three different FISH mapping analyses were conducted using a telomere PNA probe, WCPs of
23 cattle equivalent chromosomes 18 and 23, and WCP of der18.

24 *Telomere PNA Probe.* The telomere PNA probe, mapping on all telomeres, was hybridized on
25 metaphase cells using the telomere PNA FISH kit/FITC (Dako Cytomation).

1 *WCPs of Cattle Equivalent Chromosomes 18 and 23*. Hybridization was performed according to
2 Kubickova et al. [2002] with a slight modification. For dual-color painting, 10 µl of the
3 hybridization mixture containing 50% formamide, 2. SSC, 10% dextran sulphate, 5 µg of salmon
4 sperm DNA, 1.3 µg of bovine Hydrobloc DNA (Applied Genetics Laboratories), and 0.8 µl of each
5 labeled probe was denatured at 75 ° C for 10 min and pre-annealed at 37 ° C for 40 min. Slides
6 were denatured in 70% formamide, 2. SSC (pH 7.0) at 72 ° C for 2 min, dehydrated, and hybridized
7 overnight in a moist chamber at 37 ° C with the probes. After overnight hybridization, the slides
8 were washed in 0.7. SSC/0.3% igeal (pH 7.0–7.2) at 73 ° C. Hybridization sites of biotinylated
9 probe were visualized by indirect staining using fluorescein-avidin DCS A-2011 (Roche) and anti-
10 avidin antibody D BA-0300 (Roche) and finally counterstained with 4',6-diamidino-2-phenylindole
11 (DAPI) and mounted in Vectashield mounting medium (Vector laboratories). Probe signals on
12 metaphase spreads were visualized with an Olympus BX60 fluorescent microscope. Digital images
13 were captured using the ISIS 3 software (MetaSystems).

14 *WCP of der18* . In this case, FISH analysis was performed on chromosomes of 2 different species:
15 OAR and BTA metaphases. FISH treatment, visualization of fluorescence signals, and digital image
16 analyses followed the protocol mentioned above.

17

18 **Results**

19 The 47 animals investigated presented normal karyotypes ($2n = 54$) except for a 1-year-old female
20 which showed an abnormal acrocentric chromosome, shorter than the shortest normal acrocentric
21 chromosome, in each metaphase analyzed (fig. 1). In order to distinguish between derivate
22 chromosomes (der) and fragments, it was necessary to show the presence of the centromeres and
23 telomeres on both aberrant chromosomes by CBA-banding and telomere PNA FISH analysis,
24 respectively. Indeed, after CBAbanding, all chromosomes (in particular the acrocentric autosomes)
25 showed a distinct and entire centromere (heterochromatin block) in all studied metaphases including
26 the small der (fig. 2 A). On the other hand, FISH with a telomeric probe revealed the presence of

1 telomeres in all chromosomes, including the small der (fig. 2 B). The RBA-banding analysis (fig.
2 3 A, B) revealed the presence of a reciprocal translocation between chromosomes identified as
3 OAR18 and 23, and the 2 ders were classified as der18 and der23, considering the corresponding
4 centromere. Figure 3 C shows an ideogrammatic representation of the possible rearrangements of
5 this new reciprocal translocation. The confirmation of both chromosomes and their regions involved
6 was validated by FISH mapping analysis using 2 specific WCPs. The probe for the chromosome
7 OAR18 mapped on both OAR18 and the distal part of der23; the probe for the chromosome OAR23
8 mapped on OAR23, the proximal part of der23, and the telomeric part of der18 (fig. 4). It was not
9 possible to detect a green signal on der18, because the region of chromosome 18 involved was
10 probably not included in the painting probe. For this reason, the WCP of der18 has been assigned to
11 both OAR and BTA metaphases. While centromeric signals were detected in all acrocentric
12 autosomes of the sheep carrier (fig. 5 A), 2 sub-centromeric signals on chromosomes 21 and 2
13 distal signals on chromosomes 24 were identified in cattle metaphases (fig. 5 B, C). Eventually,
14 according to the results of cytogenetic analysis, the chromosomal translocation was then classified
15 as t(18q;23q)(q14;q26).

16

17 **Discussion**

18 Cytogenetic analysis still represents one of the most important tools for the genetic improvement of
19 livestock and, as a consequence, an economical profit for breeders. Although the staining patterns
20 obtained by banding procedures are sometimes ambiguous, the analysis of banded karyotypes still
21 remains the best way to evaluate chromosome aberrations which can be further confirmed by FISH
22 analysis with specific markers or WCPs. So far, centric fusions in sheep are more frequent than
23 reciprocal translocations, while in human newborns the frequency of these anomalies is quite
24 similar. In cattle, only 16% of all reciprocal translocations can be detected by analyzing the
25 conventional karyotype, and, consequently, the real frequency could be 5 times higher than de novo

1 Robertsonian translocations [De Lorenzi et al., 2012]. It is important to remember that
2 chromosomal abnormalities are one cause of economic loss in the animal production.
3 This is due to the production of unbalanced gametes which are known to give rise to
4 chromosomally unbalanced embryos destined to die in early embryonic life or to be aborted
5 [reviewed in Ducos et al., 2008]. The detection and clarification of chromosome abnormalities by
6 WCPs generated by chromosome microdissection as described above also underlines the general
7 importance and usability of such chromosome probes for cytogenetic analyses of livestock species.
8 All WCPs generated and used in this study propose new ways for their application and,
9 consequently, new perspectives. For the first time, we produced a WCP of der18 by chromosome
10 microdissection in order to study its rearrangements on sheep and cattle chromosomes. The first
11 result was a centromeric signal on the majority of sheep chromosomes (acrocentric autosomes)
12 probably due to the presence of the centromeric sequence material within the WCP of der18. It is
13 known that the majority of mammalian chromosomes have satellite DNA (tandemly repeated DNA)
14 located at or very close to the centromeres.
15 In fact, the amplification of such regions, especially with DOP-PCR, will result in nonspecific
16 signals after hybridization. Therefore, the same WCP of der18 was hybridized on cattle metaphase
17 chromosomes in order to study the comparative rearrangements, and this time we obtained an
18 unexpected result. In fact, we obtained hybridization signals only on BTA21 and BTA24
19 (homoeologous to OAR18 and OAR23) (fig. 5 B, C) probably due to such difference in centromere
20 repetitive sequences between the cattle and the sheep [Chaves et al., 2000]. Although centromeric
21 sequences are made up of repetitive sequences, this study has very important consequences as it
22 shows how they can be different within Bovids.

23

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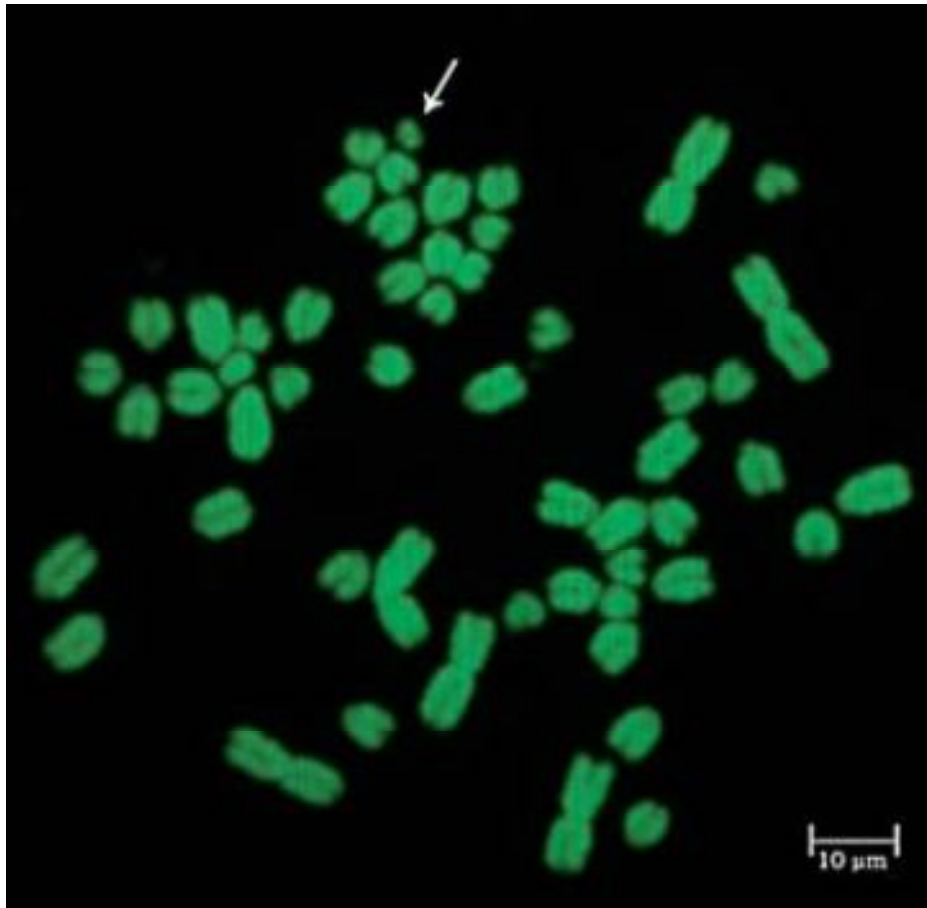
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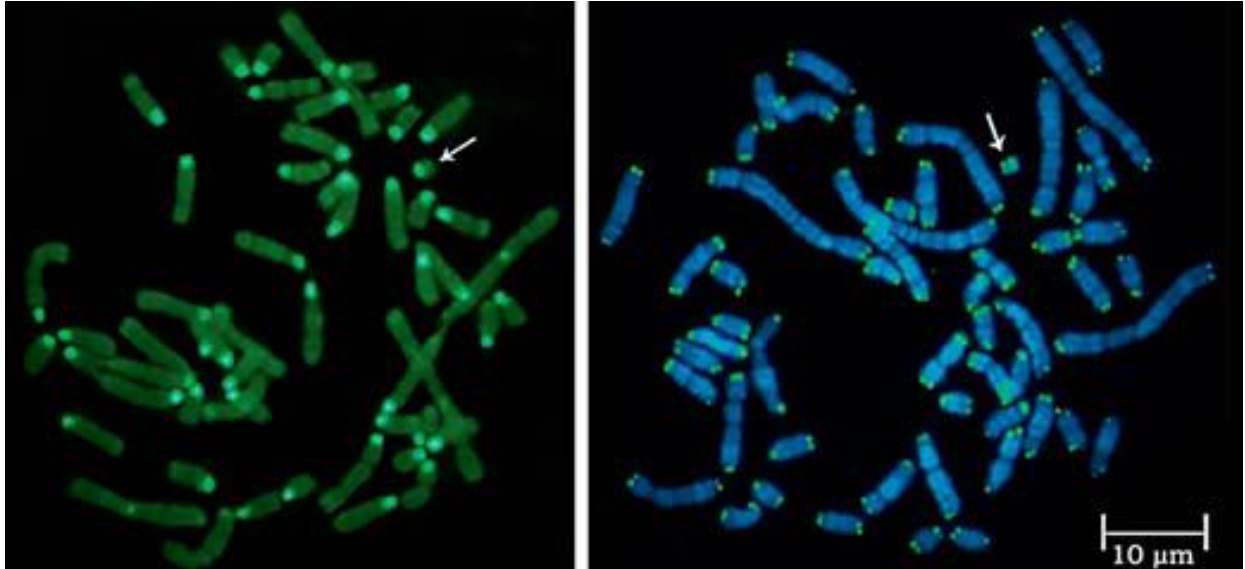
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1

2 **Fig. 1.** Conventionally metaphase of the carrier showing the smallest translocated chromosome
3 (arrow).

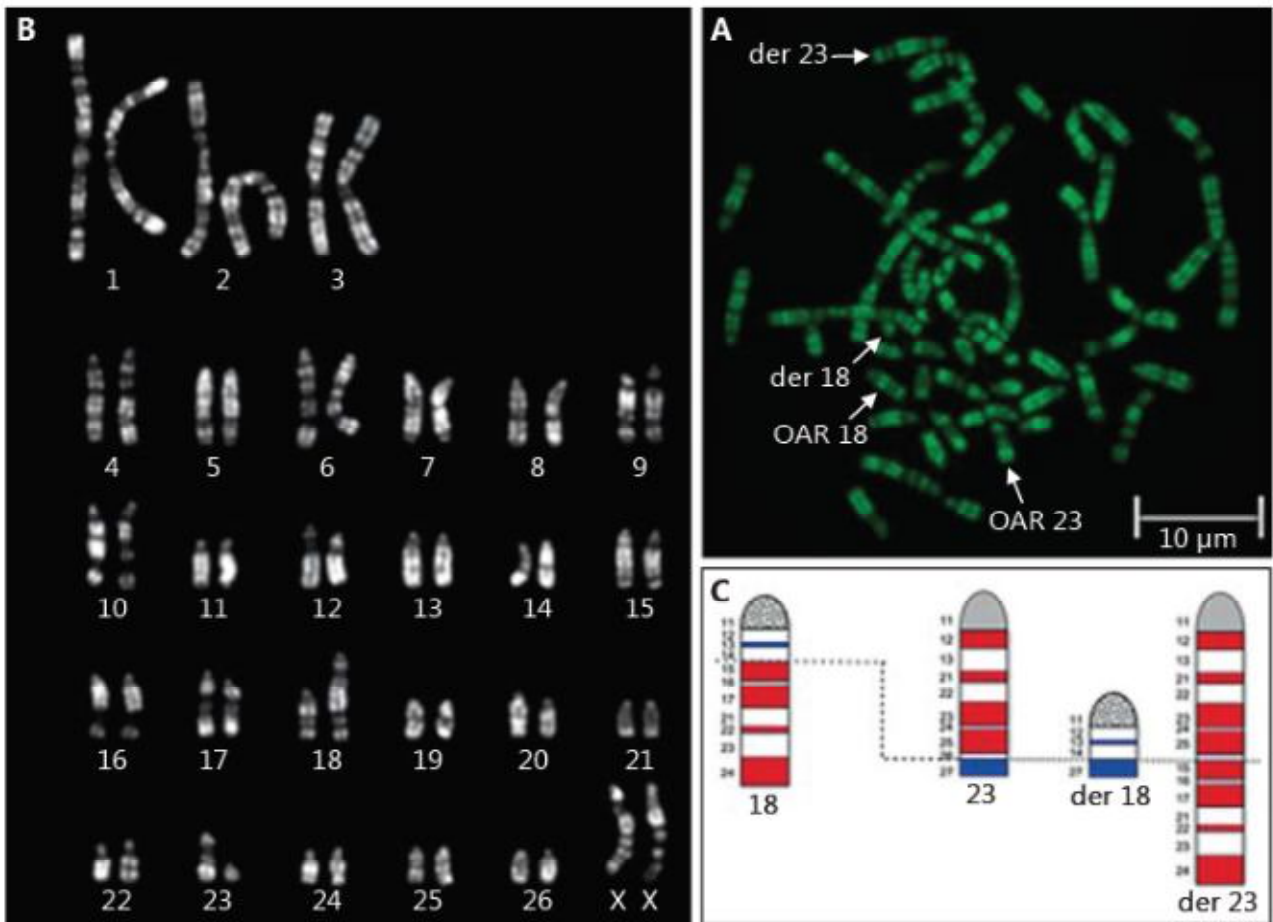
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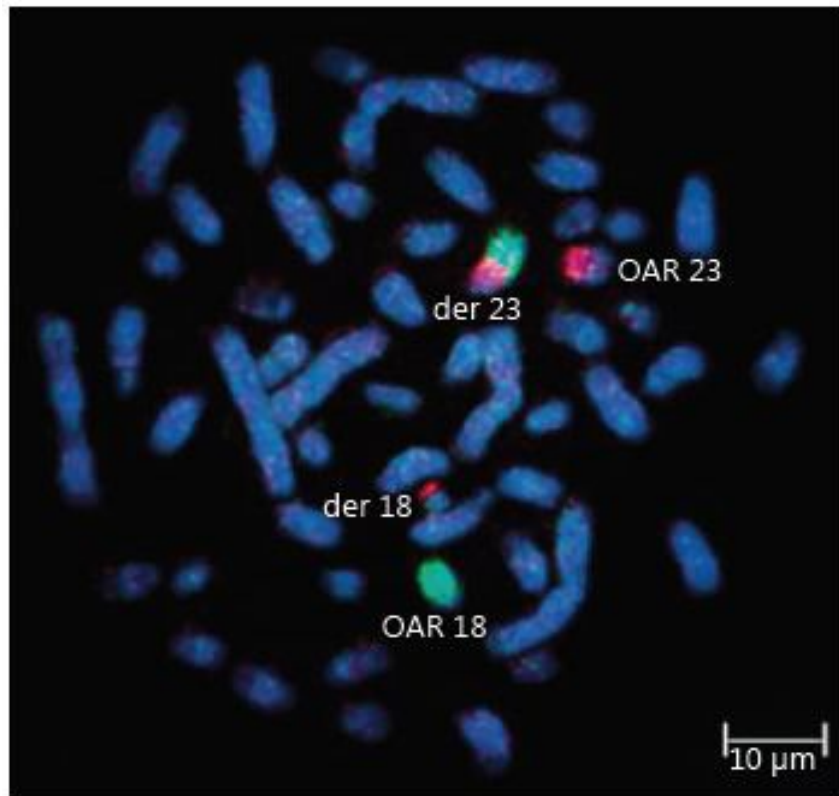
2 **Fig. 2.** **A** CBA-banding pattern on a metaphase plate of the carrier showing the presence of a
3 centromere in all chromosomes including the der (arrow). **B** Metaphase of the translocation carrying
4 sheep after FISH with the telomeric PNA probe. Telomeric signals are present in all chromosomes
5 including the der (arrow).

6



1
 2 **Fig. 3.** RBA-stained metaphase plate showing both der18 and der23, OAR18, and OAR23 (**A**) and
 3 the corresponding karyotype (**B**). **C** Ideogrammatic representation of the OAR chromosomes
 4 involved in the reciprocal translocation $t(18q;23q)(q14;q26)$ with the breakpoints (dotted line).

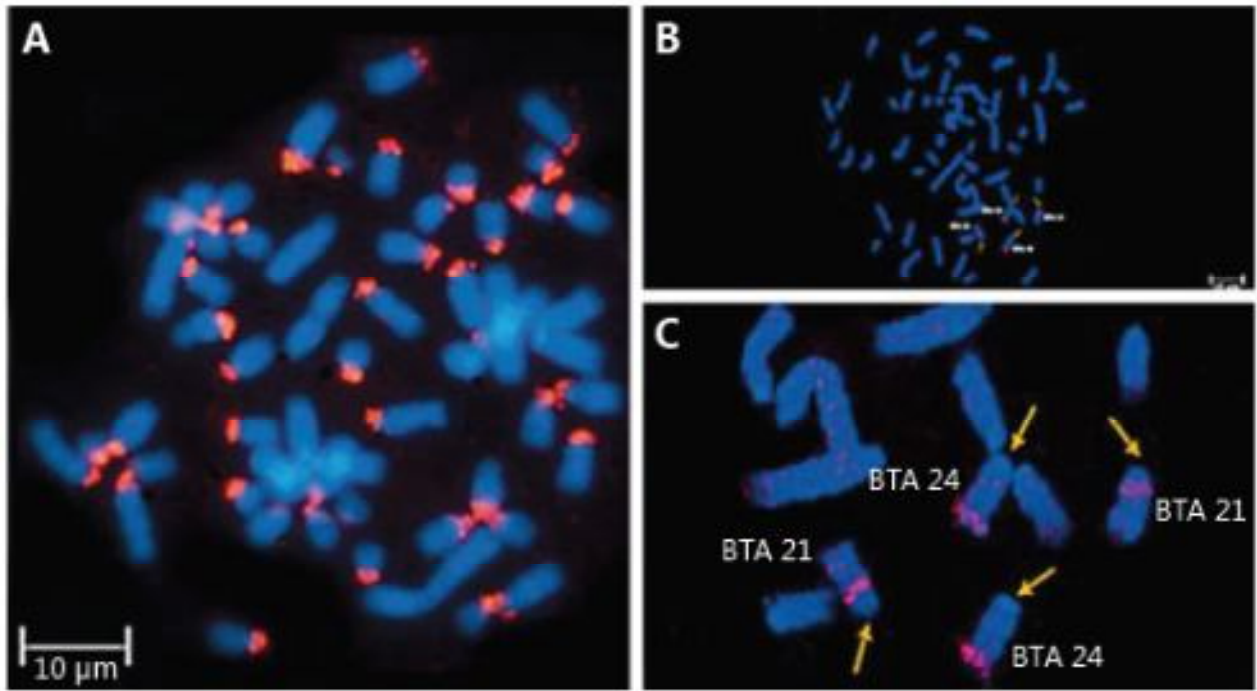
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1

2 **Fig. 4.** FISH on metaphase spreads of the carrier using cattle corresponding painting probes for
3 OAR chromosomes 18 and 23. The painting probe for OAR18 hybridizes on OAR18 and the
4 proximal part of der23 (green signals). The painting probe for OAR23 hybridizes on OAR23, the
5 distal part of der23, and the telomeric region of der18 (red signals).

6



1
2

3 **Fig. 5.** A FISH on normal OAR metaphase spreads using WCP of der18, labeled with spectrum
 4 orange, shows signals present on all acrocentric autosomes. **B** FISH on BTA metaphase spreads
 5 using the same WCP of der18 mentioned above. **C** Detail of the metaphase in **B** showing the red
 6 signals on homologous BTA chromosome 21 (pericentric region) and 24 (telomeric region). Arrows
 7 indicate the centromeres of BTA21 and 24.