The genetic improvement of the water buffalo (Bubalus bubalis): the contribution of the cytogenetics

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ABSTRACT
Although water buffalo is only 1/9 of the cattle population, it interests a larger human population, especially in south-eastern countries. The DNA-sequence of the entire genome has been performed for several domestic species but it is still lacking in river buffalo. However, high resolution Q-, G-, and R-banded standard karyotypes are available and cytogenetic maps at the low resolution density have been constructed for river buffalo. In addition, cytogenetic analyses on females with reproductive problems have established that about 20% of these animals are sterile for serious damages to internal sex adducts due to the presence of sex chromosome abnormalities. In this study I summarize the contribution of the cytogenetics in the genetic improvement of the water buffaloes concerning the following aspects: **(a)** chromosome characterization by high resolution chromosome banding; **(b)** chromosome evolution within Bovidae family by using both comparative chromosome banding and gene order (FISH-mapping technique) approaches; (c) clinical cytogenetics applied to reproduction; **(d)** molecular cytogenetics (the most advanced cytogenetic maps).

Chromosome characterization
Two main types of buffaloes are present in the world: the Asiatic water buffalo (Bubalus bubalis) and the African buffalo (Syncerus caffer). The water buffalo has two subspecies or types: the river and the swamp buffalo which differ in their chromosome number (2n=50 in river type and 2n=48 in swamp type). Crosses between these two species are possible and also the hybrids (2n=49) are fertile. The reason of this is due to a conservation of chromosome arms (and gene pool) between the two species. Indeed, the large swamp buffalo chromosome 1 originated by tandem fusion translocation between river buffalo chromosomes 5p and 9 (Di Berardino and Iannuzzi, 1981). During this chromosome rearrangement the nucleolus organizer regions (NOR) present in river buffalo 5p were lost, as well as large portions of the constitutive heterochromatin present in the centromeric regions of river buffalo chromosome 9 (Di Berardino and Iannuzzi, 1981). In particular, DNA SAT I was conserved and DNA SAT II was lost (Tanaka et al., 1999; Iannuzzi et al., 2009). Actually, a cross-breeding program has been performing in Philippines to improve swamp buffalo milk production by crossing river x swamp buffalo (the milk production increased three times in F1 and four time in F2 crossings, depending by the food availability). Having the two species a different chromosome number, the cyogenetic control could be another useful tool to check the various crosses. The African buffalos also have two subspecies: the Syncerus caffer caffer and the S. caffer nanus which differ in their diploid number being 2n=52 in S. caffer caffer and 2n=54 in Syncerus caffer nanus. Also these two subspecies are fertile since they conserved the same chromosome arms (and genes). The advent of chromosome banding techniques allowed to noticeably improve our knowledge on animal chromosomes permitting to construct banded karyotypes with high resolution banding patterns (450-500 band for haploid set) and, more importantly, standard banded karyotypes which have been the point of reference for clinical (identification of chromosome involved in chromosome abnormalities), evolutionary (relationships among related and unrelated species) and molecular (construction of detailed cytogenetic maps) studies. Figure 1 shows a bull RBG-banded karyotype arranged according to this standard at the 450 band level.

As shown in this figure, river buffalo has five biarmed autosomes, all remaining chromosomes being acrocentric, including both the X (the largest acrocentric) and Y (small acrocentric) chromosomes. The five biarmed river buffalo chromosomes (BBU1 to BBU5) correspond to five centric fusion translocations of cattle homologous chromosomes (and bovine syntenic groups) according to CSKBB (1994) and ISCNDB (2001): BBU1 (1;27-U10/U25), BBU2 (2;23-U17/U20), BBU3 (8;19-U18/U21), BBU4 (5;28-U3/U29), and BBU5 (16;29-U1/U7). The fusion of these biarmed pairs has been accompanied by a substantial loss of constitutive heterochromatin (HC). Indeed, very small C-bands are present in the centromeres of the biarmed pairs, compared to centromeres of all acrocentric chromosomes, including X, which shows the largest heterochromatin block (Figure 2). The Y chromosome shows variable C-banding patterns depending on the degree of chromosome denaturation. Indeed, the Y chromosome appears completely heterochromatic or with a strong C-band that is distally located (Figure 2). Thus, the C-banding technique (especially CBA-banding) distinguishes river buffalo sex chromosomes (especially the Y-chromosome) from the autosomes, permitting to easily reveal sex chromosome abnormalities (reviewed in Di Meo et al., 2008).

Another Asiatic buffalo species which is in danger of extinction is Bubalus depressicornis (2n=48), which is found on the Sulawesi (Indonesia) island as two subspecies: Bubalus depressicornis depressicornis (Lowland Anoa) and Bubalus depressicornis quarlesi (Mountain Anoa). This species is the smallest buffalo in the world. Its karyotype is very similar to that of the river buffalo. Indeed, four of the six biarmed chromosome pairs in Anoa are centric fusion translocations of cattle homologs similar to what occurred in river buffalo (1;27, 2;23, 8;19, 5;28) whereas the other Anoa biarmed chromosomes are different combinations of cattle chromosomes (11;20 and 17;15) (Gallagher et al. 1999).

Chromosome evolution

Chromosome comparative banding studies among bovid species have revealed that autosome arms have been highly conserved. Indeed, while the diploid number varies between 30 to 60, the fundamental number (FN) (which takes in account the number of chromosome arms), varies only between 58 to 62, with three exceptions. This is due to the widely use of centric fusion translocations which reduced the autosome chromosome number but conserved the same FN. (reviewed in Iannuzzi et al., 2009). Chromosome banding comparison between the Asiatic and African buffaloes have revealed that no one biarmed chromosome pair is homologue (Iannuzzi and Di Meo, 1995; Gallagher and Womack 1992), thus explaining the impossibility of crosses between the two types of buffaloes and their affiliation in two different buffalo genera. Comparative chromosome banding studies were later confirmed by using comparative mapping studies (gene order), especially using the fluorescence in situ hybridization (FISH) technique and bovine (or ovine) BAC-clones, as probes. This new and much powerful tool not only confirmed the chromosome homologies but revealed, for the first time, a small autosome rearrangement which differentiated subfamily Bovinae (including cattle and water buffalo) from the remaining ones, including goat and sheep. Indeed, a pericentromeric region translocated from "bovinae" chromosome 9 to "caprinae" chromosome 14 (Iannuzzi et al, 2001a, 2009).

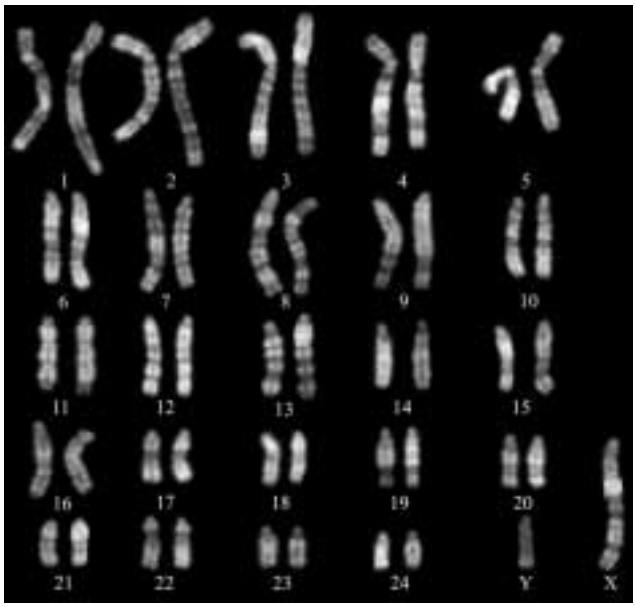

Figure 1: High resolution RBG-banded river buffalo karyotype in a bull (2n=50, XY) with normal karyotype and produced from a single cell according to the standard karyotype (CSKBB, 1994).

Comparison with human map confirmed that "Bovinae" subfamily is ancestral to the remaining bovid subfamilies (Iannuzzi et al., 2009).

In contrast to the high autosome banding similarity, sex chromosomes evolved by more complex chromosome rearrangements. Indeed, size and shape varies among bovid species. However, three main X-chromosomes can be found in bovids: the submetacentric cattle type, the acrocentric eland (or river buffalo) type, and the acrocentric sheep (or goat) type with small and visible p-arms. Chromosome banding comparisons demonstrated that large portions of these chromosomes are conserved (Iannuzzi and Di Meo, 1995), with the presence of large blocks of constitutive hetorochromatin (HC) in BBU-X (Figure 2) and their absence in both BTA-X and OAR/CHI-X (Iannuzzi and Di Meo, 1995). Detailed comparative cytogenetic maps representing the order of loci along sex chromosomes of cattle, river buffalo and sheep/goat X-chromosomes revealed complex chromosome rearrangements that occurred during evolution of the karyotypes of these species (Robinson et al. 1998: Piumi et al. 1998; Iannuzzi et al. 2000a). In particular, BTA-X and BBU-X share the same gene order but a different centromere position. Hence, a centromere transposition (or centromere repositioning) with loss of constitutive heterochromatin (HC) differentiates BTA-X from BBU-X (Iannuzzi et al. 2000a). When comparing "bovine" X (BTA-X and BBU-X) with "caprine" X (OAR-X and CHI-X), at least four chromosome transpositions including a centromere repositioning were found (Iannuzzi et al. 2000a, 2009).

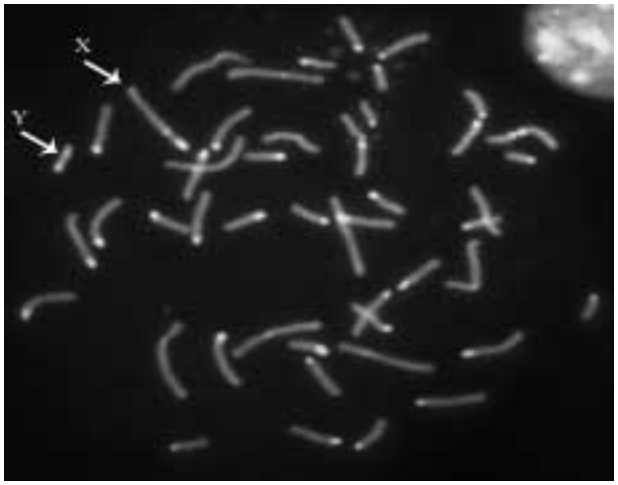

Figure 2: Reverse CBA-banded male river buffalo metaphase obtained from a female found with the mosaicism XX/XY (freemartin). Note the strong C-bands in all acrocentric chromosomes (including the X chromosome which shows the largest centromeric HC-block with a clear, additional proximal positive C-band) and the very small C-bands in biarmed chromosomes. The Y chromosome appears to be C-band positive only in the distal (almost telomeric) region, whereas the centromere is C-band negative.

A similar evolution has been observed in the Y-chromosomes of bovids. Indeed, comparative FISH-mapping analyses performed among the Y-chromosomes of cattle (Bos taurus), zebu (Bos indicus, BIN), river buffalo and sheep/goat revealed complex chromosome rearrangements. In particular, BTA-Y (submetacentric) and BIN-Y (acrocentric with small and visible p-arms) differ in a centromere transposition (or repositioning) or pericentric inversion yet they retain the same gene order along the distal regions (Di Meo et al. 2005). BTA-Y and BBU-Y differ in a pericentric inversion with loss (from BBU-Y to BTA-Y) or gain (from BTA-Y to BBU-Y) of HC, BBU-Y being larger than BTA-Y. OAR-Y/CHI-Y (very small metacentrics) differ from BBU-Y in a pericentric inversion and greater loss of HC and from BTA-Y and BIN-Y in a centromere transposition with loss of HC (Di Meo et al. 2005).

The nucleolus organizer regions (NORs) are all located at the telomeres of five (cattle, sheep, goat, swamp buffalo) and six (river buffalo) autosomes of domestic bovids. Considering the high degree of autosome corservation among bovids (with only centromeric regions affected by chromosome rearrangements), we expected to find the same nucleolus organizer chromosomes (NOCs) in bovids but, due to simple NOR-translocations, only some NORs were conserved to homologous chromosomes or chromosome arms (Iannuzzi et al. 1996; Gallagher et al. 1999). In particular, only two homologous NOCs were common to river buffalo and cattle (BBU6 and BBU24, homologous to BTA3 and BTA25, respectively), as well as to river buffalo and goat/sheep (BBU4p and BBU6, homologous to CHI3 and CHI28, respectively, and to OAR1p and OAR25, respectively). These four bovids share only one NOC (BBU6, BTA3, CHI3, and OAR1p), while goat and sheep share the same NOC, confirming their evolutionary proximity (Iannuzzi et al., 2009).

Clinical cytogenetics applied to reproduction

While autosome numerical aberrations are rare (the phenotype is abnormal and the breeder promptly selects these animals without cytogenetic control), numerical sex chromosome aberrations are more tolerated by the species due to the genetic inactivation of one X-chromosome for the gene dosage compensation. Most often, these abnormalities are cause of sterility, especially in the females, and the carriers almost always have a normal morphological constitution.

In river buffalo, in collaboration with the Veterinary Medicine Faculty of Naples, we are studying females with reproductive problems (females that have reached the fertility age but do not remain pregnant also in presence of the bull). So far, 20% of these females showed sex chromosome abnormalities such as X-monosomy, X-trisomy, sex reversal syndrome, mosaicism XX/XY (freematinism, the most common) (Iannuzzi et al., 2000b, 2001b, 2004, 2005; Di Meo et al., 2008). All these females carrying sex chromosome abnormalities were phenotipically normal with some exception in some female showing some male trait (head, horns, prominent wither, tight pelvis). While external sex genitalia were normal, internal sex adducts were abnormal varying between the atrophy of Muller ducts to complete absence of internal sex adducts (with closed vagina). All these females were sterile (reviewed in Di Meo et al., 2008). This means that the breeders kept for long time (also 5-6 years) sterile females with high economic damage. Thus, the importance to cytogenetically study all females with reproductive problems in early fertile age, including those showing longer inter-births. Indeed, the female may carrier balanced autosome abnormalities which reduced the fertility in the carrier (Di Meo et al., 2010). In Italy, in collaboration with the National Buffalo Breeder Association (ANASB), the most important bulls, especially those used in both natural and artificial insemination, a cytogenetic screening with advanced banded karyoypes is performed and certified to select the bulls. This adds economic value to this important species, allowing also to seal certified semen coming from caryological normal bulls. Cytogenetic test as those of CA (chromosome breaks, chromatid breaks, fragments, aneuploidy) and SCE (sister chromatid exchange) are also very useful to test the buffalo population exposed to environmental pollution or to check the animal welfare. Indeed, lower levels of CA and SCE in the animals, compared to animal control (animals raised in on polluted areas), mean a stable genome and appropriate environmental conditions for breeding, and viceversa.

Molecular cytogenetics

Assignments of both type I (known genes) and type II loci (generally microsatellies) have been performed in river buffalo using a somatic cell hybrid panel (El Nahas et al. 1996) and FISH-mapping techniques (Iannuzzi 1998; Iannuzzi et al. 2003). The first genetic map for river buffalo with only 54 loci, mostly assigned by FISH, was reported by Iannuzzi (1998). In this first genetic map, at least one bovine molecular marker (and associated syntenic group) was assigned to each river buffalo chromosome or chromosome arm). Improved genetic maps with 99 (El Nahas et al. 2001) and 293 (Iannuzzi et al. 2003) loci were later established, until we reached the most recent one with 388 loci by Di Meo et al. (2008). Figure 3 shows a comparison between the cytogenetic and radiation hybrid (RH) maps of river buffalo chromosome. These maps still remain poor needing to be noticeably expanded. These map are very useful for comparative mapping studies among bovids and between bovids and humans to transfer useful genetic information from richer genomes (humans) to those of domestic animals, as well as to better anchor the radiation hybrid maps to specific river buffalo chromosomes (Figure 3; Perucatti et al. 2009).

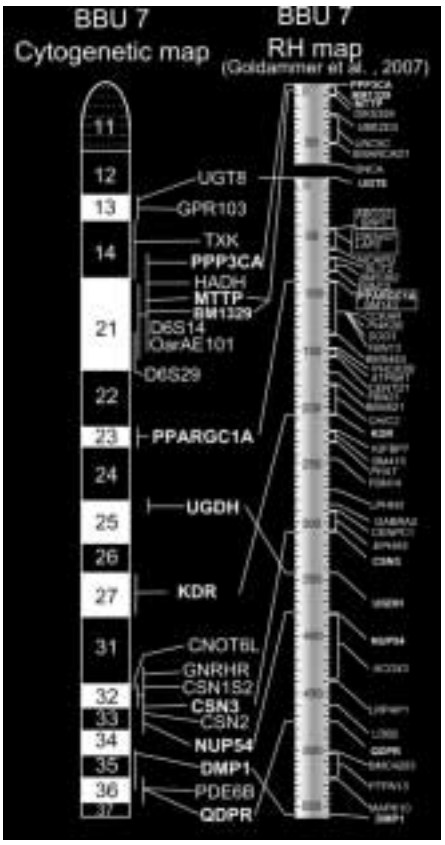

Figure 3: Cytogenetic map of river buffalo chromosome 7 (Perucatti et al., 2009) compared with the previous published RH-map (Goldammer et al., 2007). Both maps agree but four loci (UGDH and KDR - bands 7q25 and 7q27, as well as DMP1 and QDPR - bands 7q35 and 7q36) have been erroneously positioned in the RH-map (from Perucatti et al., 2009).

Furthermore, the availability of molecular markers and of the FISH-technique allow to follow the chromosome abnormalities also in the germinal lines, in particular in the sperms. These markers can be found useful application also in the production of in vitro embryos. Indeed, since about 50% of these embryos are polyploid (and abnormal), the cytogenetic control can be very useful to check abnormal embryos so to improve the technique producing embryos reducing the percentages of abnormal embryos.

Conclusions

Although several genetic studies have been performed in river buffaloes, more work is necessary to genetically improve this species. Cytogenetic approaches are very useful to explain the reproductive problems often found by the breeders during the animal breeding. Cytogenetic test are also very useful to test the genome stability of animals and, indirectly, the animal welfare and food chain. Indeed, when animals are exposed to mutagenic compounds, cytogenetic test reveal a higher chromosome fragility, compared to that of animal not exposed (control). The use of molecular markers and the FISH-technique not only can improve our knowledge on buffalo genome, but they can be used to better study the chromosome abnormalities and to check both sperms and embryos before their use. A better collaboration among, breeders, veterinary practioners, cytogeneticists and breeder associations is necessary if we want to bring up the genetic improvement of buffaloes. Acknowledgements. This study was in part supported by DG-RSTL.083.001, DG-RSTL.083.002 and by National Buffalo Breeder Association (ANASB).

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