KARYOTYPE OF MAZANI WATER BUFFALO FROM IRAN

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ABSTRACT

Zoologists have introduced buffalo as "future livestock" and claim that their potentials and benefits will be more than any other livestock. River buffaloes play an impressive and crucial role in the economy of rural families. The ability of these animals to produce milk, meat and also their draft power has caused to be kept in rural areas. Mazani buffalo is classified as a river buffalo and no further karyotyping specification was made up to now. Blood samples were taken from ten (5 males and 5 females) Mazani buffaloes from Mazendaran province located in north of Iran. Blood lymphocytes cultured at 37°C for 72 h in presence of colsemid and the metaphase spreads were performed on microscopic slide. Giemsa was applied to stain chromosomes. The current study shows that 2n=50 and fundamental numbers (NF) is 60 in male and female. The types of autosome were 10 submetacentric/metacentric and 38 telocentric. The X chromosome is the largest telocentric and the Y chromosome is one of the smallest telocentric chromosomes. Also, the relative length of chromosomes ranged between 7.2 and 2.17 in Mazani buffalo. All chromosomes were found normal. The karyotype formula of Mazani buffalo is as follow: 2n (50) = 4M+6SM + 38 T+Sex chromosome.

Keywords: karyotype, chromosome, water buffalo, idiogram

INTRODUCTION

Buffalo (family *Bovidae* and tribe Bovini) can be divided into two main groups: *Bubalia* and *Syncerina*. *Bubalia* is also classified into Arni buffalo, Tamarao buffalo and Anona buffalo. Moreover, *Syncerina* consist of two subgroups called red buffalo and black buffalo. The arni buffalo is classified further two groups, the river buffalo and the swamp buffalo according to its habitat (Miyake *et al*., 1980). The diploid number of the swamp buffalo is 48 (Harisah *et al*., 1989), and the diploid number of the river buffalo is 50 (Murali *et al*., 2009; Ali *et al*., 2012).

According to climate conditions, Iranian buffaloes consist of three main categories: 1) Azeri Buffalo (Ardabil, Western and Eastern Azerbaijan provinces); 2) Mazani Buffalo (Gilan and Mazendaran provinces); 3) Khoozestani Buffalo (Khoozestan province) (Naserian and saremi, 2007). Most of these animals are kept in the states of western and eastern Azerbaijan located in northwest and state of Khoozestan located in south (Hasanzadeh and Monazzah, 2011). All of the Iranian buffaloes are riverine (Naserian and

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saremi, 2007). River buffaloes have an impressive and crucial role in the economy of rural families (Hasanzadeh and Orojee, 2003). The ability of these animals to produce milk and meat and also their draft power has caused to be kept in rural areas (Mirhoseini *et al*., 2005). The dollar value of river buffaloes in Iran is nearly equal with a pure Holstein dairy cow. Iranian water buffaloes have close appearance to Iraqi buffaloes. Hence both of them may have been originated from the same ancestor. In addition Iranian river buffaloes in northwest of the country (West Azerbaijan), have same similarity to Mediterranean river buffaloes. So, it's considered that they have descended from the same ancestor (Naserian and saremi, 2007).

Cytogenetic study is a powerful instrument to determine the normal karyotype of farm animal and to discover more about fundamental basis for abnormalities. Also, the chromosomal analysis is useful in the selection of high productive animals in farm (Ahmad *et al*., 2004). The chromosomal abnormalities in animals can be recognized and culled from breeding stock (Ahmad *et al*., 2004).

The present study was undertaken to determine the karyotype of Mazani water buffalo and compare with other river buffaloes in other countries.

MATERIALS AND METHODS

Blood samples of buffalo

Ten Mazani buffaloes (5 males and 5 females) and were used for this chromosomal analysis. The Mazani buffaloes samples were collected from Mazendaran province located in north of Iran. Figure 1, shows the Mazani water buffalo. Peripheral blood samples were aseptically taken from the jugular vein and transferred venojects containing sodium heparin.

Lymphocyte culture

4.5 ml of RMPI 1640 medium was prepared with 2% phytohemagglutinin (PHA) as a mitogen

Figure 1. Mazani water buffalo.

and transferred in flask. Beside 0.5 ml of blood sample were dropped into a flask, incubated at 37°C under 5% of CO_2 environment and regularly shaken in the day and night. At the 72nd h of incubation, Closemid was added as a mitotic inhibitor and well mixed followed by further incubation for 20 minutes.

Cell harvest and banding

The mixture of blood samples was centrifuged and supernatants were discarded. Potassium chloride (hypotonic solution) was applied to the pellet for 35 minutes. KCl was discarded, cells were fixed by cool fixative (3 methanol: 1 glacial acetic acid). Fixative was discarded too, and mixtures were dropped on a clean and foggy slides by micropipette and well dried. Then, the slides stained with 20% Giemsa's solution for 25 minutes.

Chromosomal counting, karyotyping and idiograming

Chromosome counting was performed on metaphase cells under the light microscope. Fifteen clearly observable spread of each sample selected and then photographed (×1000). The length of the short arm (Ls), length of the long arm (Ll), Length of each chromosome (LT) and centromeric index (CI) were measured by Micro Measure 3.3. Other parameters like relative length (RL) were calculated by Microsoft Excel 2010. The centromeric index and arm ratio were computed to classify the types of chromosomes according to Guerra (1986). The Karyotypes were drawn by Adobe Photoshop CS6 and the idiograms were drawn by Microsoft excel 2010.

RESULTS AND DISSCUSSION

After lymphocyte culturing, cell harvesting, staining, Chromosomal counting, karyotyping and idiograming, the result show that diploid chromosomes (2n) of Mazani buffalo are 2n=50. So they are riverine (*Bubalus bubalis bubalis*). (Figure 2, and 3).

The present study showed results similar to those of previous reports on river buffalo in Iran (Khavary, 1978) and also on other river buffalo in Brazil (Pires *et al*., 1997; Rommelt, 1976), India (Murali *et al*., 2009; Bidhar *et al*., 1986; Balakrishnan and Yadav, 1984; Ramesha and Hedge 1992; Yadav *et al*., 1984; Kumar and Yadav, 1991; Gupta and chaudhri, 1978; Joshi and Govindaiah, 1999), Italy (Salerno *et al*., 1980), Pakistan (Ali *et al*., 2012), Thailand (Kenthao *et al*., 2012), Turkey (Ulbrich and Fisher, 1967), Sri lanka (Scheurmann *et al*., 1974) and Egypt (Ahmed *et al*., 2004; Cribiu and Obeidah, 1978; Hondt and Ghanam, 1971). Also it showed a similar result to the reported by Halnan (1976) that reported the diploid number is 50. It is not similar to previous reports that reported the diploid number is 2n = 48 in swamp buffalo in Japan (Miyake *et al*., 1980; Harisah *et al*., 1989; Dutt and Bhattacharya, 1952), Australia (Toll and Halnan, 1976a), Malaysia (Bongso and Jainudeen, 1979), China (Huang *et al*., 1987), Vietnam (Balakrishnan *et al*., 1988) and Thailand (Rommelt, 1977). Fischer and Ulbrich (1978) found that the diploid number of African buffalo is 52.

The fundamental number (NF) of Mazani buffalo is 60 in male and female and it is the same NF for De Hondt and Ghanam (1971); Rommelt (1976); Bongoso *et al*. (1977); Iannuzzi (1994); Kenthao *et al*. (2012).

The autosomes consist of 10 submetacentric/

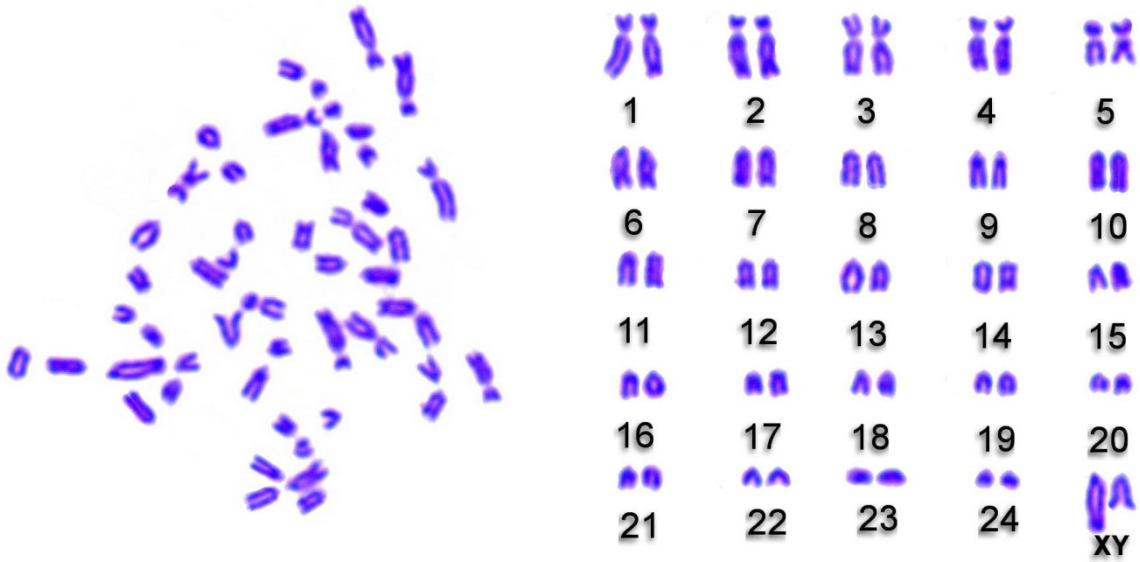

Figure 2. Chromosome spread (left) and karyotype (right) of male Mazani buffalo (*Bubalus bubalis*) 2n (diploid) = 50.

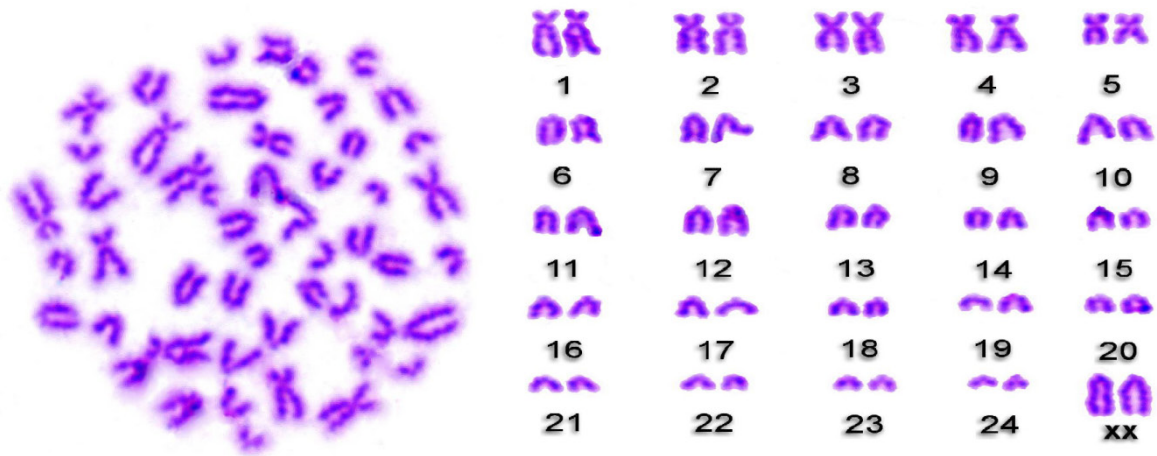

Figure 3. Chromosome spread (left) and karyotype (right) of female Mazani buffalo (*Bubalus bubalis*) 2n (diploid) = 50.

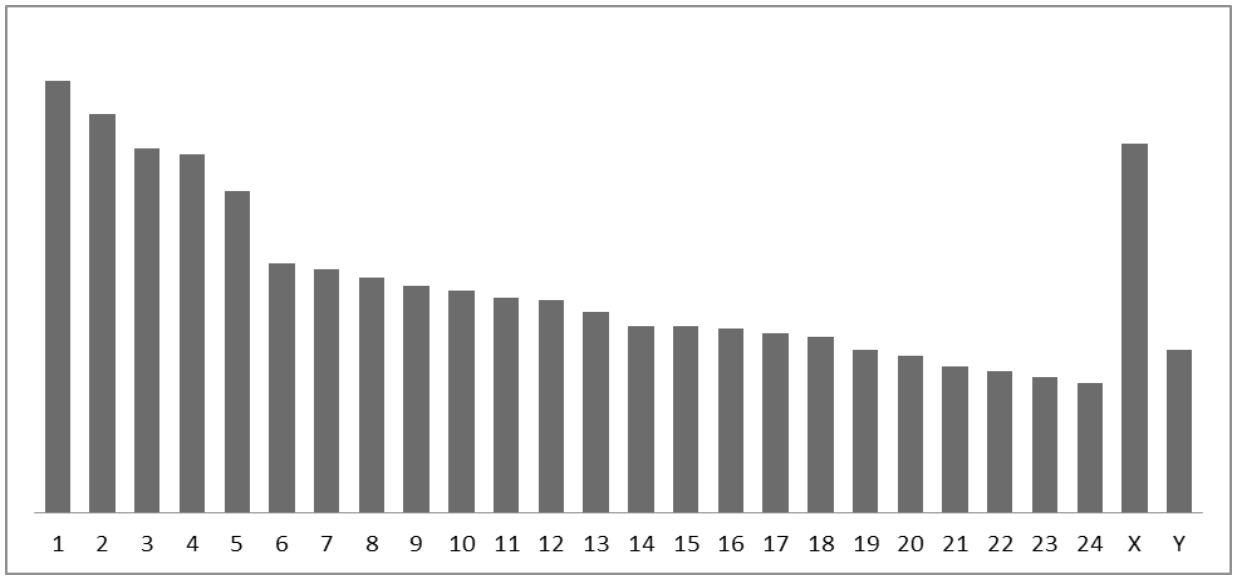

Figure 4. Idiogram of Mazani buffalo using the relative length.

metacentric (pair Nos. 1 to 5) and 38 telocentric chromosomes (pair Nos. 6 to 24). This is similar to Kenthao *et al*. (2012) that reported there are 10 submetacentric and 38 telocentric in Mehsani buffaloes. Also, this is not in agreement with Ali *et al*. (2012) that reported the autosomes of Pakistani river buffalo (*Bubalus bubalis bubalis*) contains 10 metacentric/submetacentric chromosomes whereas the rest of the autosomes were classified as acrocentric ones. Also this is not in agreement with Cribiu (1987) that reported the autosomes of Egyptian river buffalo (*Bubalus bubalis bubalis*) contains 5 pairs metacentric/submetacentric chromosomes and 19 pairs acrocentric, like Pakistani river buffalo. Maybe it is because they did not use of MicroMeasure and only predict from slides.

The pair of sex chromosomes was XX in the female and XY in the male. From karyotypes it appeared that the X was the largest telocentric while the Y was one of the smallest telocentric. It is in agreement with Kenthao *et al*. (2012) that reported the X is largest telocentric and Y is small telocentric in Mehsani buffaloes from Thailand; However, the X chromosome is the largest acrocentric and Y chromosome is the acrocentric in Pakistani river buffalo (Ali *et al*., 2012), Indian river buffalo (Murali *et al*., 2009; Nair *et al*., 1986; Iannuzzi, 1994), Brazilian river buffalo (Pires *et al*., 1997) and Egyptian river buffalo (Cribiu, 1978). Also it is not in agreement with Meo *et al*. (2005) that reported the Y chromosome is acrocentric in river buffalo.

The mean of the short arm (Ls), long arm (Ll), chromosome length (LT), relative length (RL), arm ratio (Ll/Ls) and centromeric index (CI) of Mazani buffalo are shown in Table 1.

The relative length of chromosomes ranged between 7.20 and 2.17 in Mazani buffalo (Table 1), it means difference of range relative length (DRL) is 5.03. It is very different from DRL of Toda buffalo that is 3.95 (Murrali *et al*., 2009).

All chromosomes from this population were found normal. The Mazani buffalo karyotype

Table 1. Mean of the short arm (Ls), long arm (Ll), chromosome length (LT), relative length (RL), arm ratio (Ll/Ls) and centromeric index (CI) from metaphase chromosomes of Mazani male and female buffalo.

Chromosome pairs	Ls (µm)	Ll (µm)	LT (µm)	CI	RL	Arm ratio (Ll/Ls)	Type of Chromosome
1	5.46	11.53	16.99	0.32	7.20	2.11	Submetacentric
2	4.34	11.36	15.70	0.28	6.66	2.62	Submetacentric
3	5.17	9.16	14.33	0.36	6.08	1.77	Submetacentric
4	6.02	8.07	14.09	0.43	5.97	1.34	Metacentric
5	5.16	7.49	12.65	0.41	5.36	1.45	Metacentric
6	0.00	9.80	9.80	0.00	4.16	∞	Telocentric
7	0.00	9.56	9.56	0.00	4.05	∞	Telocentric
8	0.00	9.26	9.26	0.00	3.93	∞	Telocentric
9	0.00	8.93	8.93	0.00	3.79	∞	Telocentric
10	0.00	8.74	8.74	0.00	3.71	∞	Telocentric
11	0.00	8.46	8.46	0.00	3.59	∞	Telocentric
12	0.00	8.38	8.38	0.00	3.55	∞	Telocentric
13	0.00	7.88	7.88	0.00	3.34	∞	Telocentric
14	0.00	7.34	7.34	0.00	3.11	∞	Telocentric
15	0.00	7.32	7.32	0.00	3.10	∞	Telocentric
16	0.00	7.26	7.26	0.00	3.08	∞	Telocentric
17	0.00	7.03	7.03	0.00	2.98	∞	Telocentric
18	0.00	6.90	6.90	0.00	2.93	∞	Telocentric
19	0.00	6.40	6.40	0.00	2.71	∞	Telocentric
20	0.00	6.18	6.18	0.00	2.62	∞	Telocentric
21	0.00	5.75	5.75	0.00	2.44	∞	Telocentric
22	0.00	5.54	5.54	0.00	2.35	∞	Telocentric
23	0.00	5.31	5.31	0.00	2.25	∞	Telocentric
24	0.00	5.11	5.11	0.00	2.17	∞	Telocentric
X	0.00	14.52	14.52	0.00	6.16	∞	Telocentric
Y	0.00	6.40	6.40	0.00	2.71	∞	Telocentric

can be formula as follow:

2n (50) = 4M + 6SM + 38 T + Sex chromosome

The idiogram of Mazani buffalo are presented in Figure 4 and it is similar to the idiogram of Toda buffalo from Indian (Murrali *et al*., 2009).

Many breeds of the buffalo live in South East of Asia, Australia, North Africa, South America, the Middle East and the Mediterranean coasts. Due to inadequacy of the cytogenetic study and chromosomal analysis of domestic buffaloes, it is not possible to determine the origin of buffaloes in various countries. According to available data, we know that the diploid number is 50 and NF or number of arms is 60 in the river buffalo.

In the future we expect an increase in the number of cytogenetic studies of many kind of buffaloes from many countries, and using the various chromosomal band techniques.

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CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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