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Incidence of X-Y aneuploidy in sperm of two indigenous cattle breeds by using dual color fluorescent *in situ* hybridization (FISH)

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Abstract

The present study reports on the incidence of X-Y aneuploidy in the sperm population of two indigenous cattle breeds reared in Italy for beef purposes, the Podolian and Maremmana. Totally, more than 50.000 sperm nuclei from 10 subjects (5 from each breed) have been FISH analyzed by using Xcen and Y-chromosome specific painting probes. In both breeds, the fraction of Y-bearing sperm was significantly higher ($P<0.01$) compared to the X-counterpart. The rates of X-Y aneuploidy were 0.180% and 0.200%, respectively, in the Podolian and Maremmana. No significant inter-individual differences were found. Average frequencies of disomic and diploid sperm were 0.149% and 0.031% in the former and 0.098% and 0.102% in the latter. Significant differences

($P<0.05$) were found among the XX-XY and YY-disomy classes in both breeds, while diploidy classes were uniformly represented. In the Podolian breed, disomies were more frequent than diploidies ($P<0.05$), whereas in the Maremmana they showed similar frequencies. In both breeds disomies arising from errors in meiosis I (X-Y disomies) were more represented than those arising in meiosis II (XX and YY), while this difference was not detected for diploidies. The present study provides specific information on the incidence of X-Y sperm aneuploidy in two indigenous breeds of cattle, in order to establish a breed specific ‘aneuploidy data-base’ that could be used as reference for genetic improvement and future monitoring of the reproductive health of the breed.

Keywords: X-Y aneuploidy; sperm FISH; cattle; indigenous breeds.

1. Introduction

Chromosomal abnormalities in germ cells are known to be responsible for nearly 70% of embryonic mortality in mammals. In particular, aneuploidies have been associated with infertility, spontaneous abortions, perinatal mortality and mental retardation in humans [1, 2, 3, 4] and with embryonic and fetal mortality in farm animals [5]. Embryonic mortality has, therefore, a substantial negative impact upon fertility and reproductive health of domestic animals. In cattle, sperm chromosomal constitutions have been investigated by fluorescent *in situ* hybridization (FISH) since 1999 by using molecular probes for chromosome Y [6], cosmid PL44 for the X- and a painting probe for the Y- [7], BACs for chromosome X and the repetitive sequence BRY4a for the Y-chromosome [8]. Chromosome specific painting probes produced by chromosome microdissection and chromosome sorting followed by DOP-PCR have been also used by other authors [9, 10, 11] while Bonnet-Garnier [12] used bovine painting probes for chromosomes 1 and 29 to study the meiotic segregation of the translocated chromosome in the sperm population.

All these studies mainly demonstrated the usefulness of the fluorescent in situ hybridization technique (FISH) to validate procedures for separating the X-Y bearing sperm for embryo sexing purposes. At the present, only few studies have been carried out to investigate upon the incidence of aneuploidy in the sperm population of the various cattle breeds (or genetic types).

Previous investigations on the X-Y aneuploidy rates in sperm of cattle mainly concerned dairy breeds, such as the Holstein Friesian [7], and the Italian Friesian and Brown [13]. In order to expand our knowledge on the X-Y aneuploidy rates in domestic animals, we provide cytogenetic information on two ‘indigenous’ cattle breeds reared in Italy for beef purposes, the Podolian and Maremmana.

2. Material and methods

2.1. Xcen-Y- painting probe preparation

The Xcen and Y- painting probes were obtained by chromosome microdissection of GTG-banded prometaphase chromosome preparations, DOP-PCR and labeling, as reported in Nicodemo et al. [13].

2.2. Sperm-FISH

2.2.1. Semen samples

Indigenous breeds are known to graze freely in the pasture, therefore no frozen material is normally available from the insemination centers. For this reason, epidydimal sperm was collected from testis of slaughtered young bulls belonging to the Podolian and Maremmana breeds. Before slaughtering, sterile heparinized venous blood was drawn from the bulls for karyotyping. All bulls examined in this study resulted karyologically normal.

2.2.2. Fluorescent in situ hybridization

Decondensation of spermatozoa, sperm-FISH analysis, scoring, validation of the data and statistical analysis were all performed as reported in Nicodemo et al. [13]. Briefly, sperm with one signal (green or red) were scored as normal haploid; sperm with 2 signals were classified as disomic (XX, YY and XY depending on the 2 signal colors). Diploid sperm were distinguished from disomic sperm on the basis of their size. Since the decondensation process might not be uniform along the slide, size comparison was made strictly within the same microscopic field where the diploid sperm were found. In addition, as reported in Nicodemo et al.[13], to verify if this could lead to errors in the estimation of aneuploidy, an additional hybridization experiment was carried out on two samples (one for each breed, previously analyzed with Xcen and Y probes) by using a probe for chromosome 6.

3. Results

Table 1 shows the number and frequency (%) of X- and Y- bearing sperm and the rates of XY aneuploidy in sperm of bulls of the Podolian and Maremmana breeds of cattle. The efficiency of the FISH procedure, in terms of Xcen- and Y-signal visualization, was close to 99% in both breeds.

3.1. X/Y ratio

Out of more than 25.000 sperm analyzed for each breed, the fraction of the Y- bearing sperm was significantly ($P<0.01$) higher compared to the X- counterpart in both breeds: 51.05 *vs* 48.76% in the Podolian and 50.87 *vs* 48.93% in the Maremmana, respectively. ‘Within’ each breed, inter-individual variations in the X/Y ratio resulted statistically significant ($P<0.05$).

3.2. X-Y aneuploidy rates

In the Podolian breed, the mean rate of X-Y aneuploidy varied from 0.079 to 0.271, with an average of 0.180. Interindividual differences were not statistically significant. In the Maremmana breed, the mean rate of X-Y aneuploidy varied from 0.115 to 0.340, with an average of 0.200. Similarly to the Podolian breed, no significant differences were detected among the five bulls investigated.

3.3. Disomy vs diploidy

In the Podolian breed, the incidence of total disomy varied from 0.079 to 0.220, with an average of 0.149, while diploidy varied from 0 to 0.080, with an average of 0.031. In the Maremmana breed, the corresponding values for disomy varied from 0.058 to 0.141, with an average of 0.098, while diploidy varied from 0.035 to 0.226, with an average of 0.102. In both breeds, no significant differences were found among the five bulls investigated in the mean rate of disomy and diploidy.

3.4. XY-XX-YY class comparison

Table 2 shows the statistical significance of the comparisons 'within' each breed in the frequency of the different aneuploidy classes. In both breeds, the incidence of the YY disomic sperm was significantly higher ($P<0.05$) compared to the XY and XX counterparts: (0.101 *vs* 0.024 each in the Podolian, 0.64 *vs* 0.17 each in the Maremmana). On the contrary, the frequency of the YY, XY and XX diploid sperm was quite similar in the two breeds.

To analyze possible differences in the occurrence of errors during meiosis I (XY disomic/diploid sperm) or meiosis II (XX and YY disomic/diploid sperm) we applied the Mann-Whitney test. Meiotic errors giving rise to disomies were significantly more frequent ($P<0.05$) in M-II than in M-I (0.125 *vs* 0.024 in the Podolian, and 0.81 *vs* 0.17 in the Maremmana). Concerning the diploidy, the differences between M-I and M-II were not statistically significant.

3.5. Interbreed comparison

To investigate about possible interbreed differences, we compared the data achieved in the Podolian and Maremmana with those previously reported on the Italian Friesian and Brown [13] and on the Swedish Friesian [7] (Table 3). No significant differences were found among the breeds analyzed in the overall incidence of X-Y aneuploidy, which varied from 0.142 in the Brown to 0.200 in the Maremmana, as well as in the overall incidence of disomy which varied from 0.079 in the Brown to 0.149 in the Podolian, and in the overall incidence of diploidy which varied from 0.031 in the Podolian to 0.102 in the Maremmana.

4. Discussion

The present study provides specific information on the incidence of X-Y sperm aneuploidy in two indigenous breeds of cattle, the Podolian and Maremmana, which -in so far- were never investigated under this point of view. The two breeds showed X/Y ratios which were significantly in favour of the Y-bearing fraction compared to the X-counterpart ($P<0.01$). This is basically similar to previous results reported by Nicodemo et al. [13] on the Italian Friesian and Brown breeds of cattle and by Hassanane et al. [7] in the Holstein Friesian breed. The preponderance of the Y-signal compared to the X- is somewhat intriguing. In the paper by Hassanane, the X- signal was due to a PL44 cosmid (approximately 50Kbp long) whereas that of the Y- was due to a painting probe; so, in that case the difference might have been due to the signal intensity. In the present case, as well as in that previously reported by Nicodemo et al. [13], both X- and Y- signals were from painting probes which are known to provide strong fluorescent signals. More work should be done to clarify this aspect.

The mean rate of X-Y aneuploidy was found to be quite similar in the two breeds analyzed: 0.180 and 0.200, respectively, in the Podolian and Maremmana. The difference between these two breeds was not statistically significant. By averaging them together (Table 3), we can assume the value of 0.190 as 'mean'rate of X-Y aneuploidy in the 'indigenous' breeds reared in Italy, so far.

In the previous paper by Nicodemo et al. [13] the X-Y aneuploidy rates in the Italian Friesian and Brown breeds were 0.162 and 0.142, respectively, whereas in the Holstein Friesian analyzed by Hassanane et al. [7] the corresponding rate was 0.170. Again the differences among the breeds were not significant. By averaging them together (Table 3), we can assume the value of 0.159 as 'mean'rate of X-Y aneuploidy in the 'dairy' cattle breeds analyzed, in so far.

Even though the difference between the mean rate of X-Y aneuploidy in the indigenous breeds (0.190) and that of the dairy breeds (0.159) is not statistically significant, the present data do indicate that in the dairy bulls the incidence of X-Y aneuploidy is less compared to the indigenous ones, probably as the result of selection. This finding is also confirmed by Rybar et al. [14] who report that aneuploidy frequencies in young dairy bulls candidates for artificial insemination are relatively low. Such an hypothesis can be explained by the fact that dairy bulls are selected for various functional traits, among which the 'calving interval' is known to be directly related to sperm aneuploidy. Finally, by taking into consideration all the available data from table 3, the mean rate of X-Y aneuploidy in the sperm population of cattle as a species (*Bos taurus*) is 0.167, of which 0.110 due to disomy and 0.057 due to diploidy.

Studies on aneuploidy in sperm of domestic animals should be implemented also by using autosomal probes in order to test possible inter-chromosomal effects [15], age related effects [16] and extended also to other domestic species, in order to detect possible 'inter-specific' differences.

Preliminary investigations, in fact, on the X-Y aneuploidy rates in some domestic species [17, 9, 18, 19] indicate interesting differences in the X-Y aneuploidy rates among pig (0.188), cattle (0.150), river buffalo (0.468), sheep (0.033), goat (0.394) and horse (0.316) which are worth to be further investigated in order to better understand the genetic causes of aneuploidy and their impact on the reproductive and productive efficiency of domestic animals. Furthermore, the information acquired in the present study will allow us to establish a breed specific 'aneuploidy data-base' that could be used as reference for genetic improvement and future monitoring of the reproductive health of the breed.

Acknowledgements

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Table 1 - Number and frequency (%) of X- and Y- bearing sperm and rates of XY aneuploidy in sperm of bulls of the Podolian and Maremmana breeds of cattle.

Bulls	Sperm													
	Analyzed	Without Signal (1)	With Signal (1)	Normal (2)		X-Y Aneuploid (2)								
						disomic				diploid				total
	(a)		(b)	X	Y	XY	XX	YY	total	XY	XX	YY	total	
Podolian breed														
1	5,109	105	5,004	2,414	2,579	0	2	9	**11**	0	0	0	**0**	**11**
		(2,055)	(97.945)	(48.241)	(51.539)	(0)	(0.040)	(0.180)	**(0.220)**	(0)	(0)	(0)	**(0)**	**(0.220)**
2	5,199	99	5,100	2,516	2,580	1	3	0	**4**	0	0	0	**0**	**4**
		(1.904)	(98.096)	(49.333)	(50.589)	(0.020)	(0.059)	(0)	**(0.079)**	(0)	(0)	(0)	**(0)**	**(0.079)**
3	5,225	74	5,151	2,443	2,701	0	0	6	**6**	0	0	1	**1**	**7**
		(1.416)	(98.584)	(47.428)	(52.436)	(0)	(0)	(0.116)	**(0.116)**	(0)	(0)	(0.019)	**(0.020)**	**(0.136)**
4	5,225	83	5,142	2,554	2,574	4	1	6	**11**	1	2	0	**3**	**14**
		(1.589)	(98.411)	(49.670)	(50.059)	(0.078)	(0.019)	(0.117)	**(0.213)**	(0.019)	(0.039)	(0)	**(0.058)**	**(0.271)**
5	5,120	5	5,115	2,514	2,591	1	0	5	**6**	3	1	0	**4**	**10**
		(0.098)	(99.902)	(49.150)	(50.653)	(0.020)	(0)	(0.098)	**(0.117)**	(0.059)	(0.020)	(0)	**(0.080)**	**(0.197)**
All	***25,878***	***366***	***25,512***	***12,441***	***13,025***	***6***	***6***	***26***	***38***	***4***	***3***	***1***	***8***	***46***
		(1.414)	***(98.586)***	***(48.765)***	***(51.055)***	***(0.024)***	***(0.024)***	***(0.101)***	***(0.149)***	***(0.015)***	***(0.012)***	***(0.004)***	***(0.031)***	***(0.180)***
Maremmana breed														
1	5.334	41	5,293	2,592	2,683	2	0	4	**6**	2	7	3	**12**	**18**
		(0.769)	(99.231)	(48.970)	(50.690)	(0.038)	(0)	(0.076)	**(0.114)**	(0.038)	(0.132)	(0.056)	**(0.226)**	**(0.340)**
2	6.381	4	6,377	3,187	3,174	1	1	7	**9**	5	1	1	**7**	**16**
		(0.063)	(99.937)	(49.976)	(49.773)	(0.015)	(0.015)	(0.112)	**(0.141)**	(0.079)	(0.015)	(0.015)	**(0.110)**	**(0.251)**
3	5.163	62	5,101	2,418	2,674	0	1	2	**3**	5	0	1	**6**	**9**
		(1.200)	(98.800)	(47.402)	(52.421)	(0)	(0.019)	(0.040)	**(0.058)**	(0.098)	(0)	(0.019)	**(0.118)**	**(0.176)**
4	6.126	16	6,110	2,990	3,113	1	3	1	**5**	1	1	0	**2**	**7**
		(0.261)	(99.739)	(48.936)	(50.949)	(0.016)	(0.051)	(0.016)	**(0.082)**	(0.016)	(0.016)	(0)	**(0.033)**	**(0.115)**
5	5.708	75	5,633	2,765	2,861	1	0	4	**5**	1	0	1	**2**	**7**
		(1.314)	(98.686)	(49.086)	(50.790)	(0.018)	(0)	(0.070)	**(0.089)**	(0.018)	(0)	(0.018)	**(0.035)**	**(0.124)**
All	***28.712***	***198***	***28,514***	***13,952***	***14,505***	***5***	***5***	***18***	***28***	***14***	***9***	***6***	***29***	***57***
		(0.690)	***(99.310)***	***(48.930)***	***(50.870)***	***(0.017)***	***(0.017)***	***(0.064)***	***(0.098)***	***(0.049)***	***(0.032)***	***(0.021)***	***(0.102)***	***(0.200)***

(1) percentage values refer to column (a); (2) percentage values refer to column (b);

Table 2 - Statistical significance of the comparisons 'within' each breed in the frequency of the different aneuploidy classes.

Comparison	Podolian			Maremmana		
	%	comparison	P	%	comparison	P
Disomy						
XY (1)	0.024	(1-2)	NS	0.17	(1-2)	NS
XX (2)	0.024	(2-3)	<0.05	0.17	(2-3)	<0.05
YY (3)	0.101	(1-3)	<0.05	0.64	(1-3)	<0.05
Total (4)	**0.149**	(4-10)	<0.05	**0.98**	(4-10)	NS
M-I (5)	0.024	(5-6)	<0.05	0.17	(5-6)	<0.01
M-II (6)	0.125	-	-	0.81	-	-
Diploidy						
XY (7)	0.015	(7-8)	NS	0.049	(7-8)	NS
XX (8)	0.012	(8-9)	NS	0.032	(8-9)	NS
YY (9)	0.004	(7-9)	NS	0.021	(7-9)	NS
Total (10)	**0.031**	-	-	**0.102**	-	-
M-I (11)	0.015	(11-12)	NS	0.049	(11-12)	NS
M-II (12)	0.016	-	-	0.053	-	-

Table 3 - Number and frequency (%) of X- and Y- bearing sperm and rates of XY- aneuploidy in sperm of bulls of 'indigenous' and 'dairy' cattle breeds.

Breed	Bulls	Sperm With signals	Normal		X-Y aneuploid						
			X	Y	disomic			diploid			Total
					M-I	M-II	total	M-I	M-II	total	
A. Indigenous breeds											
Podolian[1]	5	25,512	12,441 (48.765)	13,025 (51.055)	6 (0.024)	32 (0.125)	38 (0.149)	4 (0.016)	4 (0.016)	8 (0.031)	46 (0.180)
Maremmana[1]	5	28,514	13,952 (48.930)	14,505 (50.870)	5 (0.017)	23 (0.081)	28 (0.098)	14 (0.049)	15 (0.053)	29 (0.102)	57 (0.200)
Total A	***10***	***54,026***	***26,393 (48.852)***	***27,530 (50.958)***	***11 (0.020***	***55 (0.102)***	***66 (0.122)***	***18 (0.033)***	***19 (0.035)***	***37 (0.068)***	***103 (0.190)***
B. Dairy breeds											
Ital.Friesian[2]	10	51,885	24,793 (47.784)	27,008 (52.053)	16 (0.031)	42 (0.081)	58 (0.112)	15 (0.025)	11 (0.021)	26 (0.050)	84 (0.162)
Ital.Brown[2]	10	50,835	24,313 (47.827)	26,450 (52.031)	6 (0.012)	34 (0.067)	40 (0.079)	21 (0.041)	11 (0.022)	32 (0.063)	72 (0.142)
Swed.Friesian[3]	5	53,224	26,316 (49.044)	26,816 (49.976)	16 (0.029)	52 (0.096)	68 (0.125)	n.d. n.d.	24 (0.045)	24 (0.045)	92 (0.170)
Total B	***25***	***155,944***	***75,422 (48.365)***	***80,274 (51.476)***	***38 (0.024)***	***128 (0.082)***	***166 (0.106)***	***36 (0.023)***	***46 (0.030)***	***82 (0.053)***	***248 (0.159)***
A + B											
ALL	***35***	***209,970***	***101,815 (48,490)***	***107,804 (51,342)***	***49 (0.023)***	***183 (0.087)***	***232 (0.110)***	***54 (0.026)***	***65 (0.031)***	***119 (0.057)***	***351 (0.167)***

(1) Present study; (2) Nicodemo et al.,2009; (3) Hassanane et al.(1999); n.d.= not detected.