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Similar rates of chromosomal aberrant secondary oocytes in two indigenous cattle (*Bos taurus*) breeds as determined by dual-color FISH

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Abstract

In vitro-matured metaphase II (MII) oocytes with corresponding first polar bodies (I pb) from two indigenous cattle (*Bos taurus*) breeds have been investigated to provide specific data upon the incidence of aneuploidy. A total of 165 and 140 *in vitro*-matured MII oocytes of the Podolian (PO) and Maremmana (MA) breeds, respectively, were analyzed by fluorescence *in situ* hybridization using Xcen and five chromosome-specific painting probes. Oocytes with unreduced chromosome number were 13.3% and 6.4% in the two breeds, respectively, averaging 10.2%. In the PO, out of 100 MII oocytes + I pb analyzed, two oocytes were nullisomic for chromosome 5 (2.0%) and one disomic for the same chromosome (1.0%). In the MA, out of 100 MII oocytes + I pb, one oocyte was found nullisomic for chromosome 5 (1.0%) and one was disomic for the X chromosome (1.0%). Out of 200 MII oocytes + I pb, the mean rate of aneuploidy (nullisomy + disomy) for the two chromosomes scored was 2.5%, of which 1.5% was due to nullisomy and 1.0% due to disomy. By averaging these data with those previously reported on dairy cattle, the overall incidence of aneuploidy in cattle, as a species, was 2.25%, of which 1.25% was due to nullisomy and 1.0% due to disomy. The results so far achieved indicate similar rates of aneuploidy among the four cattle breeds investigated. Interspecific comparison between cattle (Xcen-5 probes) and pig (*Sus scrofa domestica*) (1–10 probes) also reveal similar rates. Further studies are needed that use more probes to investigate the interchromosomal effect. Establishing a baseline level of aneuploidy for each species/breed could also be useful for improving the *in vitro* production of embryos destined to the embryo transfer industry as well as for monitoring future trends of the reproductive health of domestic animals in relation to management errors and/or environmental hazards.

Keywords: Aneuploidy; Bovine oocytes; Polar bodies; Cattle breeds; FISH analysis

1. Introduction

Estimation of the baseline level of aneuploidy in germ cells of domestic animals is, in our opinion, an important step for monitoring future trends of the reproductive health of the various species/breeds engaged in animal production, in relation to managemental errors (hormonal, nutritional and diet imbalances) and/or environmental hazards (mutagens, mitotic poisons) which are known to damage the mitotic/meiotic machinery of the cell.

The present study investigates the incidence of aneuploidy in *in vitro* matured bovine metaphase II (MII) oocytes with the corresponding first polar body (I pb)

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from two indigenous cattle (*Bos taurus*) breeds, namely, the Podolian (PO) and Maremmana (MA), by using the fluorescent *in situ* hybridization (FISH) technique. The rationale for this work is the need to review previous aneuploidy data available in cattle which were mainly based on the analysis of the metaphase II alone, according to the Tarkowski method [1]. This technique can induce technical artifacts, such as overlapping chromosomes, presence of cytoplasmic residual, compacted metaphases, chromosomal loss due to spreading, etc. which may result in ambiguous results. In addition, the chromosomal material of the I pb cannot be analyzed because the chromosomes are too condensed and overlapped. The possibility of detecting aneuploidy in interphase cells (i.e., without the need to display metaphase chromosomes) also came along with the FISH technique [2] by using chromosome-specific 'painting' probes or Bacterial Artificial chromosomes (BACs). This technique, if applied to MII oocytes with the corresponding I pb, provides a more precise estimation of aneuploidy, because the lack of any chromosome in the MII metaphase (nullisomy) should have its counterpart in the corresponding I pb, which should therefore become disomic, and vice versa. The limiting factor of this technique, however, is the scarce availability of the chromosome-specific probes. The primed *in situ* (PRINS) technique [3] is another interesting way to analyze aneuploidy, but because of a lack of data on domestic animals, we preferred to use the FISH approach.

Because all the basic information we have on the aneuploidy rates in bovine oocytes has been collected by using the conventional air-drying method [4–9], we decided to review the available data on aneuploidy in cattle oocytes by using the more resolutive FISH technique. For this purpose, we hybridized painting probes from bovine chromosomes Xcen and 5, obtained by chromosome microdissection and degenerated oligonucleotide primer-polymerase chain reaction (DOP-PCR), on metaphase spreads from *in vitro*-matured MII oocytes and corresponding I pb. These probes were chosen because they both provide strong and specific signals, and have already been used for studying aneuploidy in two Italian dairy cattle breeds, namely the Italian Friesian and Italian Brown [10]. This investigation revealed that in these two breeds the mean rates of aneuploidy for chromosomes X and 5 were 1% for disomy and 1% for nullisomy.

To provide more precise data on the incidence of aneuploidy in *in vitro* matured bovine oocytes at the breed level, we decided to expand the investigation on two indigenous cattle (*Bos taurus*) breeds reared in Italy, namely the PO and the MA, for which no data are available at the present time.

While the PO breed is reared in South Italy, the MA is diffused in the Maremma area (Tuscany and Lazio). Both breeds are reared under extensive conditions; they graze and breed freely in the pasture. Population size includes 24,000 and 8,000 heads, respectively, inscribed in the Genealogical Books kept by the ANABIC Association (Perugia). In both breeds, sexual maturity is reached at the age of about 18 to 24 mo, while the calving interval is over 14 mo. A recent study by Ducos et al [11] reported in the PO breed an incidence of chromosomal abnormalities of 16.4% (11.7% due to the robertsonian translocation 1/29 and 4.7% due to inversions in the Y chromosome), whereas in the MA breed they found an incidence of 20.3% (18.8% due to the robertsonian translocation 1/29 and 1.5% due to XX/XY chimerism).

2. Materials and methods

2.1. Age of donor cows

The age of the donor females used in this study varied from 13 to 24 mo. Due to sanitary restrictions in Italy for Bovine spongiform Encephalopathy (BSE), the use females older than 24 mo is not permitted.

2.2. Karyotyping of donor cows

Females ready for slaughtering were previously karyotyped according to standard methods [12]. All the donors used in this study were karyologically normal.

2.3. In vitro *maturation of COCs*

Ovaries were collected from slaughtered females and transported to the laboratory within 2 h. Cumulus-oocyte complexes (COCs) were collected through aspiration with 21-gauge needles, washed in TCM-199 (No. M2154; Sigma, St. Louis, MO, USA), and examined on Petri dishes under a stereomicroscope. Only oocytes with several compact cumulus cell layers and good morphology were selected for the study. Groups of oocytes selected from each donor were transferred into 50-ml droplets of maturation medium consisting of TCM-199 + 10% fetal bovine serum (Number 10106-151; Gibco, Invitrogen, Carlsbad, CA, USA), supplemented with 0.5 mg ml^{-1} follicle-stimulating hormone (FSH; No. F8174; Sigma), 5 mg/ml luteinizing hormone (LH; No. L5269; Sigma), covered with sterile mineral oil (No. M5310; Sigma) and allocated in a

humidified atmosphere containing 5% CO_2 in air at 39 °C for 24 h.

2.4. Oocyte fixation

After 24 h maturation, the COCs were incubated for a few minutes in a hyaluronidase solution (1 mg/ml; No. H4272; Sigma) to remove the cumulus cells, washed in Phosphate Buffered Saline (PBS), and exposed to a hypotonic sodium citrate solution (0.8% wt/vol) for 3 min, followed by KCl (75 mM) treatment for 3 min. The fixation was carried out using cold methanol/glacial acetic acid (1:1) solution. Oocytes were individually fixed at the center of a precleaned slide, air-dried, and kept at −20 °C until analysis.

2.5. Chromosome microdissection and probes preparations

Metaphase cells for the production of probes via microdissection were prepared according to the standard cytogenetic techniques [12]. For microdissection, the fixed lymphocyte suspension was spread onto a precleaned 24 × 60 mm coverslip, which was then air dried and treated for GTG-banding. The Xcen probe was produced by scraping the pericentromeric region, corresponding to the centromere and the Xp11-14 region of the standardized GTG-banded karyotype; [13] the probe for chromosome 5 was produced by scraping the entire chromosome. The chromosomal DNA was amplified following the protocol of Engelen et al [14]. Probes were labeled with digoxigenin-11-dUTP (chromosome Xcen) and biotin-16-dUTP (chromosome 5) (Roche, Mannheim, Germany. Cat. Number 11558706910 and Number 11093070910, respectively) in a second DOP-PCR reaction using 2 μl of products from the first reaction as template.

2.6. In situ hybridization

The Xcen and 5 probes were hybridized simultaneously on lymphocyte metaphase plates for validation and subsequently used for oocytes analysis. Probes were precipitated in the presence of 10 mg salmon sperm DNA (No. D7656; Sigma) and 10 mg of calf thymus DNA (No. D8661; Sigma) dissolved in 15-μl hybridization solution (50%formamide in 2X SSC + 10% dextran sulfate; No. F7503 and No. D8906, respectively; Sigma) (SSC = standard saline citrate), denatured at 72 °C for 10 min, and incubated at 37 °C for 90 min. Fixed oocytes were denatured for 2 min in a solution of 70% formamide in 2× SSC (pH 7.0) at 72 °C for 3 min. The hybridization mixture containing the Xcen and 5 probes was applied on the slides and covered with 24 × 24 mm coverslips. The slides were hybridized in a moist chamber at 37 °C overnight. After hybridization and slide washing, the biotin-labeled probe was revealed using a green Alexa 488 fluorochrome conjugated to streptavidin (Number S-11223; Invitrogen, Carlsbad, CA, USA), and the digoxigenin-labeled probe was revealed using a red rhodamine fluorochrome conjugated to an antidigoxigenin antibody from sheep (Number 11207750910; Roche). Slides were counterstained with DAPI (40,60-diamidino-2-phenylindole, 0.24 mg/ml) (No. D9542; Sigma) in Antifade (No. H1000; Vector Laboratories, Burlingame, CA, USA).

2.7. Fluorescence analysis and scoring

The slides were observed at 100× magnification with a Leica (Wetzlar, Germany) DMRA fluorescence microscope equipped with DAPI, Fluorescein isothiocyanate (FITC), and Texas Red (TXRD) specific filters, the DAPI/FITC/TXRD triple filter, and phase-contrast optics. Digital images were captured using the Leica Q4000 software. To avoid possible bias, secondary oocytes with haploid chromosome set but lacking the I pb chromatin were excluded from the analysis. An oocyte was considered nullisomic when one of the two signals (either X or 5) was absent on the MII plate but present in duplicate on the corresponding I pb. In case of disomy, an additional signal (either X or 5) was present on the MII plate but absent from the I pb. χ^2 analysis with Yates' correction was used for statistical analysis of data.

3. Results

The results of the present study are shown in Table 1 and in Figure 1. The total number of donors used for this investigation was 39 (24 PO and 15-MA females). The average number of COCs collected from individual donors was 12.6 in the PO (303/24) and 14.9 in the MA (223/15), respectively. The number of cytogenetic slides prepared and successfully analyzed was 186 and 160, respectively, in the two breeds.

In total, 526 COCs were collected through aspiration (303 and 223 in the PO and MA, respectively). The percentage of COCs selected for *in vitro* maturation was around 75% in both breeds. Out of 396 COCs selected for IVM (221 PO and 175 MA), 305 reached the MII stage (165 PO and 140 MA). The efficiency of the *in vitro* maturation process was around 80% in the two breeds. Significant ($P < 0.05$) interindividual dif-

Table 1
Incidence of aneuploidy in bovine secondary oocytes matured in vitro of the Podolian and Maremmana breeds (only oocytes with the corresponding first polar body were analyzed by FISH method with painting probes corresponding to bovine chromosomes X and 5).

Donor	Age	Number of analyzed oocytes											
		Collected	Selected for IVM	Slides analyzed	Tot MII (a)	Reduced				Aneuploid			PSSC % on (c)
						Unreduced % on (a)	Total (b) % on (a)	PB % on (b)	+PB (c) % on (b)	Nullisomic % on (c)	Disomic % on (c)	Tot % on (c)	
Podolian breed													
1	24	17	12	11	9	3	6	1	5	—	—	—	—
2	21	21	14	13	10	2	8	3	5	—	—	—	1[5]
3	20	12	8	7	6	1	5	1	4	—	—	—	—
4	19	14	12	10	6	—	6	2	4	—	—	—	—
5	15	15	9	9	9	—	9	3	6	—	—	—	—
6	17	17	12	10	7	—	7	1	6	1[5]	—	1	—
7	17	15	12	11	10	1	9	2	7	—	—	—	—
8	14	22	15	13	10	2	8	4	4	—	—	—	—
9	19	11	8	7	5	—	5	—	5	—	1[5]	1	—
10	13	8	6	6	5	—	5	—	5	—	—	—	—
11	19	9	7	7	7	—	7	2	5	—	—	—	—
12	23	33	25	23	20	4	16	5	11	—	—	—	—
13	14	15	10	10	8	1	7	3	4	—	—	—	—
14	20	27	22	18	16	3	13	3	10	—	—	—	—
Group[a]	18	67	49	40	37	5	32	13	19	1[5]	—	1	—
Total		303	221	186	165	22 (13.3)	143 (86.7)	43 (30.1)	100 (69.9)	2 (2.0)	1 (1.0)	3 (3.0)	1 (1.0)
Maremmana breed													
1	13	15	13	13	12	—	12	4	8	1[5]	—	1	—
2	20	9	8	8	7	1	6	—	6	—	—	—	1[5]
3	15	22	16	14	12	—	12	3	9	—	—	—	—
4	15	17	12	12	11	—	11	3	8	—	—		—
5	18	29	20	18	17	2	15	4	11	—	—	—	—
6	17	14	12	10	8	—	8	1	7	—	1[X]	1	—
7	22	24	16	14	11	2	9	1	8	—	—	—	—
8	22	40	36	32	27	1	26	7	19	—	—	—	—
9	20	10	8	8	7	1	6	2	4	—	—		—
10	18	16	14	13	12	—	12	—	12	—	—	—	—
Group[b]	18	27	20	18	16	2	14	6	8	—	—	—	—
Total		223	175	160	140	9 (6.4)	131 (93.6)	31 (23.7)	100 (76.3)	1 (1.0)	1 [10] (1.0)	2 (2.0)	1 (1.0)
Total for the two breeds		526	396	346	305	31 (10.2)	274 (89.8)	74 (27.0)	200 (73.0)	3 (1.5)	2 (1.0)	5 (2.5)	2 (1.0)

[a] Group of 10 animals with < 4 analyzed oocytes; [b] Group of five animals with < 4 analyzed oocytes

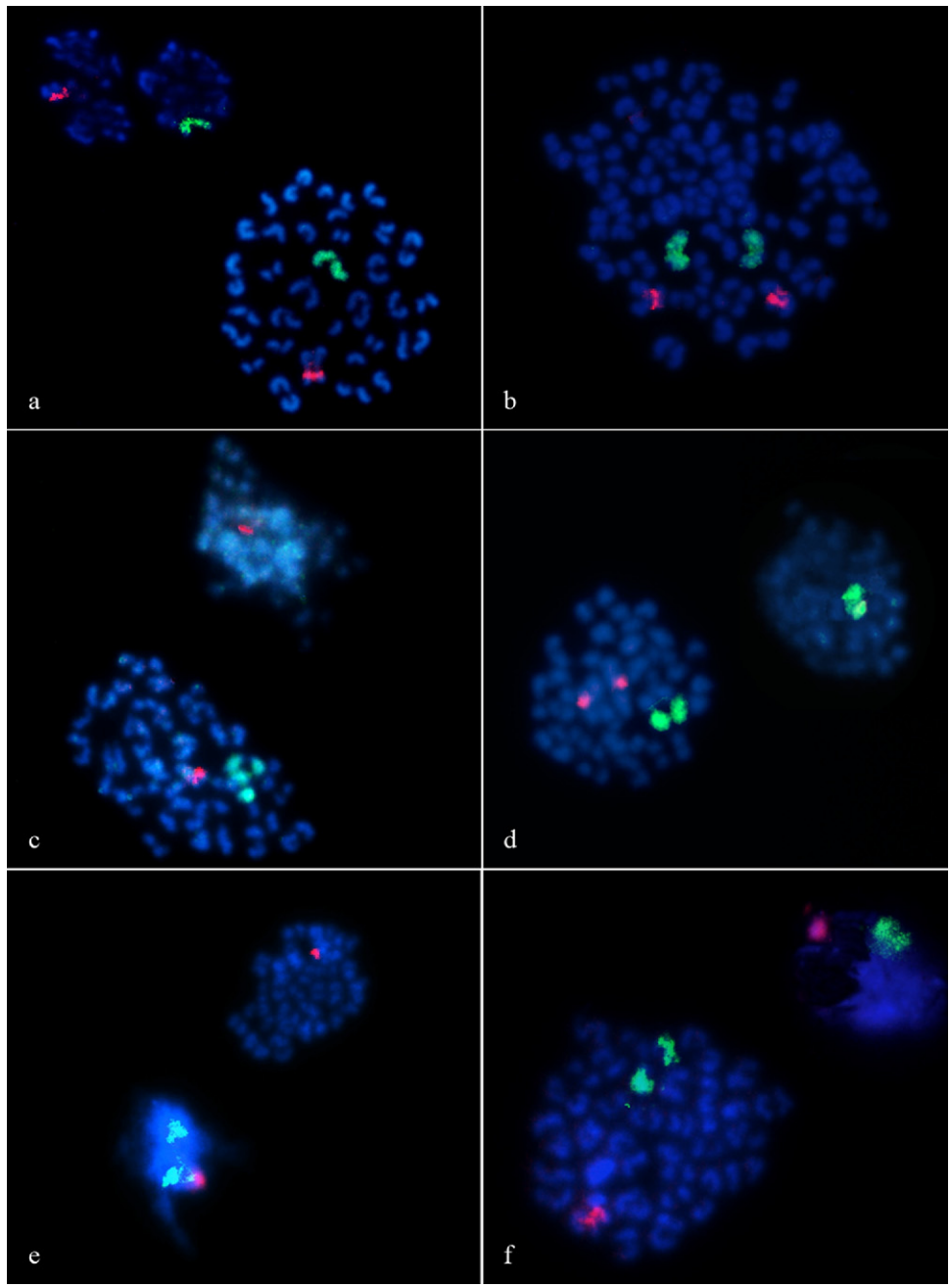

Fig. 1. Metaphases and corresponding first polar bodies of *in vitro*-matured secondary oocytes after FISH showing signals for chromosome X (red) and chromosome 5 (green): (a) normal, (b) unreduced, (c) disomic for chromosome 5, (d) disomic for chromosome X, (e) nullisomic for chromosome 5, (f) PSSC for chromosome 5.

ferences were found in the yield of *in vitro*-matured MII oocytes in the two breeds analyzed.

Among the 165 PO oocytes at MII stage, 143 displayed haploid chromosome set. In 43 of them chromatin of the I pb was not found, therefore the final FISH analysis was done on 100 MII oocytes with corresponding I pb. Two oocytes (2%, 2/100) were nullisomic and one (1%, 1/100) was disomic for chromosome five. The overall frequency of aneuploidy (nullisomy and disomy) was 3% (3/100). Besides, one haploid oocyte (1%, 1/100) was affected by PSSC (premature separation of sister chromatids) on chromosome

5 (Fig 1f). Unreduced, diploid set of chromosomes was identified in 22 secondary oocytes (13.3%, 22/165).

Among the 140 MA oocytes at MII stage, 131 displayed haploid chromosome set. In 31 of them chromatin of the I pb was not found, so they were excluded from the final analysis. FISH was done on 100 MII oocytes with corresponding I pb. Unreduced chromosome number was found in 9 out of 140 secondary oocytes (6.4%, 9/140). One oocyte (1%, 1/100) was nullisomic for chromosome 5 and one (1%, 1/100) was disomic for chromosome X. The overall frequency of aneuploidy (nullisomy and disomy) was 2% (2/100). Besides, one haploid oocyte (1%, 1/100) was affected by PSSC.

By averaging the data from the two breeds, 31 oocytes out of 305 (10.2%) were found to be unreduced; out of 200 MII + pb analyzed, three oocytes were nullisomic (1.5%), two oocytes were disomic (1%), with an overall aneuploidy rate of 2.5%.

Table 2 shows the incidence of aneuploidy in the four cattle breeds analyzed by FISH, so far, for a total of 400 bovine secondary oocytes matured *in vitro* (only oocytes with corresponding I pb were analyzed by FISH with painting probes corresponding to bovine chromosomes Xcen and 5). The comparison shows that among the four breeds there are no significant differences in the mean rate of diploidy, aneuploidy, disomy, nullisomy and PSSC.

Table 3 shows a comparison between the aneuploidy data achieved by FISH in cattle and those reported in the pig by Vozdová et al [15] and Lechniak et al [16]. No significant differences have been detected between the two species in the mean rate of aneuploidy, disomy and nullisomy, whereas the rate of diploidy was significantly higher ($P \leq 0.01$) in the pig compared to cattle (28.32% vs 11.67%).

4. Discussion

The present study provides additional data on the rate of aneuploidy (nullisomy + disomy) in secondary oocytes of two indigenous cattle breeds reared in Italy mainly for their meat, namely the Podolian and Maremmana. Furthermore, the present data are compared with previous results achieved on two dairy cattle breeds, namely the Italian Friesian and Italian Brown, to detect possible interbreed differences. Finally, the available data in cattle (*Bos taurus*) have been compared with those already published in the pig (*Sus scrofa domestica*), to examine possible interspecific differences.

Table 2
Incidence of aneuploidy in bovine secondary oocytes matured *in vitro* of four cattle breeds (only oocytes with corresponding first polar body were analyzed by FISH method with painting probes corresponding to bovine chromosomes X and 5).

Oocytes	Breed					
	Friesian* n	Brown* n	Podolian† n	Maremmana† n	Total	
					n	%
Donors used	23	19	24	15	81	—
Age range‡	13–24	z14–24	13–24	13–22	13–24	—
COCs collected	295	254	303	223	1075	—
IVM selected	204	179	221	175	779	—
Slides prepared	180	168	186	160	694	—
MII	159	144	165	140	608	—
Unreduced	16	24	22	9	71	
MII + PB	100	100	100	100	400	100.00
Normal	98	98	97	98	391	97.75
Aneuploid	2	2	3	2	9	2.25
Disomy chrom X	0	0	0	1	1	0.25
Disomy chrom 5	1	1	1	0	3	0.75
Total disomy	1	1	1	1	4	1.00
Nullisomy chrom X	1	0	0	0	1	0.25
Nullisomy chrom 5	0	1	2	1	4	1.00
Total nullisomy	1	1	2	1	5	1.25
PSSC for chrom X	0	1	0	0	1	0.25
PSSC for chrom 5	2	0	1	1	4	1.00
Total PSSC	2	1	1	1	5	1.25

* Nicodemo, et al (2010); † Present study; ‡ Months.

Table 3
Comparison between cattle (*Bos taurus*) and pig (*Sus scrofa domestica*) in the incidence of aneuploidy in MII oocytes matured *in vitro* with corresponding first polar body as detected by the FISH method.

Parameter	Cattle		Pig					
	N*	%*	N†	%†	N‡	%‡	N†+‡	%†+‡
Tot MII	608		1668		214		1882	
Unreduced	71	11.67	479	28.71	54	25.23	533	28.32
MII oocytes + PB	400	100.0	1189	100.0	160	100.0	1349	100.0
Normal oocytes	391	97.75	1155	97.14	150	93.75	1305	96.74
Aneuploid oocytes	9	2.25	34	2.86	10	6.25	44	3.26
Disomic for chromosome X	1	0.25	—	—	—	—	—	—
Disomic for chromosome 5	3	0.75	—	—	—	—	—	—
Disomic for chromosome 1	—	—	12	1.00	2	1.25	14	1.03
Disomic for chromosome 10	—	—	8	0.68	4	2.50	12	0.89
Total disomic	4	1.00	20	1.68	6	3.75	26	1.93
Nullisomic for chromosome X	1	0.25	—	—	—	—	—	—
Nullisomic for chromosome 5	4	1.00	—	—	—	—	—	—
Nullisomic for chromosome 1	—	—	8	0.68	—	—	8	0.59
Nullisomic for chromosome 10	—	—	6	0.50	4	2.50	10	0.74
Total nullisomic	5	1.25	14	1.18	4	2.50	18	1.33

* Nicodemo et al (2010) +present study; † Vozdová et al (2001); ‡ Lechniak et al (2007).

The mean rates of aneuploidy for chromosomes Xcen- and 5 were 2.0% in the MA and 3% in the PO, whereas the mean rate of diploidy was 10.2%, with a variation from 6.4 to 13.3% in the MA and PO breeds, respectively. Diploidy was the predominant chromosomal abnormality observed in this study; however, this value falls into the range of previously published data (from 8 to 12%) by using conventional Giemsa staining methods [17,4,6]. Nullisomy was detected only in 1% and 2% of the oocytes in the PO and MA breeds, respectively, and concerned only chromosome five in the two breeds. Disomy was found in 1% of the investigated oocytes in both breeds and involved chromosome X in the MA and chromosome 5 in the PO. Frequency of PSSC was 1% in the two breeds and concerned only chromosome 5.

When the results of the present study are compared with those previously reported by Nicodemo et al [10] on two dairy breeds (Italian Friesian and Italian Brown) (Table 2), it is quite evident that these four breeds do not differ significantly with regard to the mean rate of the analyzed chromosomal aberrations.

This lack of differences among the four breeds analyzed might be of interest, since the Podolian and Maremmana breeds are less intensively selected compared to the Italian Friesian and Italian Brown breeds. It is known, in fact, that in the last two decades, a negative trend for fertility has been observed in dairy cattle, mainly caused by a network of genetic, environmental and managerial factors. Over the last 30 years, such decline has been recorded at the same time as the introduction to genetic selection programs for increased milk production [18]. This finding, however, needs to be further investigated by increasing the number of MII oocytes as well as the number of chromosome-specific probes to cover a major fraction of the genome.

Conventional cytogenetic methods provided rates of aneuploidy in MII oocytes matured *in vitro* variable from 2.9% [4] to 7.1% [7] in cattle, and from 4.9% [19] to 14.2% [20] in pig. In other mammalian species, the rate of aneuploidy was found to be 5.8% in the horse [21] and rabbits [22], 1.8% in the hamster [23], and 2.7% in the mouse [24].

To re-examine interspecific differences on the basis of FISH-data, we compared the results obtained in cattle by our works with those previously reported in the pig by Vozdová et al [15] and Lechniak et al [16] (Table 3). Despite the pronounced difference in the total number of MII oocytes + PB analyzed so far (400 in cattle vs 1349 in the pig), the rate of aneuploidy was quite similar in the two species: 2.25% vs 3.26%, respectively. No significant differences were found in the rate of disomy (1.00% vs 1.93%, respectively) and in the incidence of nullisomy (1.25% vs 1.33%, respectively). On the contrary, the frequency of diploidy was significantly ($P \leq 0.01$) higher in the pig compared to cattle (28.32% vs 11.67%, respectively). This result is not surprising since in domestic animals such aberration is the most frequent abnormality reported in oocytes matured *in vitro* [4,6,9,15,16,17,21].

Recently, studies on pig oocytes analyzed by FISH demonstrated that the rate of aneuploidy is around 7%,

varying from 6.3% (sows 1.3%; gilts 10.8%) [16] to 6.7% (prepubertal gilts) to 8.5% (cycling gilts) [25].

In humans, the aneuploidy rates detected by FISH vary among different laboratories, with the highest value reaching 47% [26]. In this case, however, it must be considered that unfertilized oocytes are normally recovered from patients with reproductive disorders, which is not the case in animals.

In the present study, chromosome five was found four times more often involved in non-disjunction process compared with the X chromosome (2.0% vs 0.5%, respectively). Even though the difference was not statistically significant, this finding might suggest that also in cattle there are interchromosomal differences in the rate of non-disjunction.

Although theoretically all chromosomes may participate at similar frequency in non-disjunction events, the evidence on humans [26,27] and, recently, on pig oocytes showed that some chromosomes (usually of smaller size) are more often involved in non-disjunction. The results of at least three studies on porcine oocytes showed an unequal participation with the smaller chromosome pairs to be more often involved in non-disjunction. Sosnowski et al [19] used the conventional Giemsa staining and pointed at smaller chromosomes to be more often present in aberrant numbers in porcine oocytes. The studies of Lechniak et al [16] and Pawlak et al [25] revealed a significant predominance of the chromosome 10 in porcine aneuploid oocytes.

PSSC can be an additional source of aneuploidy in the resulting embryos. In the present study, a balanced PSSC was observed in 2% of the oocytes, involving chromosome five in both breeds analyzed. As known, balanced PSSCs are not considered directly responsible for aneuploidies, although they may indicate a predisposition to non-disjunction. On the contrary, unbalanced PSSC can lead to embryonic aneuploidy in 50% of the cases, depending upon the behavior of the extra chromatid during the second meiotic division. However, no oocytes with unbalanced PSSC were observed in this study.

As known, aneuploidy in oocytes matured *in vitro* is strongly dependent upon the culture system [24] and the age of donor [28]. Previous studies on pig oocytes by Lechniak et al [16] demonstrated that the rate of aneuploid oocytes differed significantly between mature sows (1.3%) and young gilts (10.8%) which suggests a significant effect of the donor age. On the contrary, a recent work by Hornak et al [29] failed to observe an increase in the aneuploidy rate in almost 7 year old sows.

On the basis of these considerations, we specify that in the present study, as well as in the previous one by Nicodemo et al [10], the culture system was the same and the donor's age, due to sanitary restrictions, was never above 24 mo; therefore, the influence of these factors can be considered as minimal.

In conclusion, on the basis of the data so far accumulated, there seem to be no significant differences in the incidence of aneuploidy (disomy + nullisomy) in MII oocytes matured *in vitro* with corresponding I pb among the four cattle breeds analyzed so far. Interspecific comparison between cattle and pig also revealed similar rates of aneuploidy, with a significantly higher ($P \leq 0.01$) incidence of diploidy in the pig compared to cattle (28.32% vs 11.67%, respectively). Further studies are needed to expand investigations to other species/breeds by using more animals, more oocytes as well as more chromosomal probes to cover the entire genome.

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