Similar rates of aberrant -diploid and aneuploid- secondary oocytes in two 'indigenous' cattle (*Bos taurus*) breeds as determined by dual-color fluorescent *in situ* hybridization (FISH)

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Abbreviations: MII, Metaphase II; PB, Polar Body; BAC, Bacterial Artificial Chromosome; FISH, Fluorescence In Situ Hybridization; PRINS, Primed In Situ; Xcen, X centromeric; DOP-PCR, Degenerated Oligonucleotide Primer-Polymerase Chain Reaction; COC, Cumulus Oocyte Complex; PSSC, Premature Separation of Sister Chromatids.

Abstract

In vitro-matured MII oocytes with corresponding first polar bodies (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 and Maremmana breeds, respectively, were analyzed by Fluorescence *in situ* hybridization using Xcen and 5 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 Podolian, out of 100 MII oocytes + PB analyzed, two oocytes were ''nullisomic'' for chromosome 5 (2.0%) and one disomic for chromosome 5 (1.0%). In the Maremmana, out of 100 MII oocytes + PB, one oocyte was found nullisomic for chromosome 5 (1.0%) and one was disomic for the X chromosome (1.0%). Totally, out of 200 MII oocytes + PB, the mean rate of aneuploidy (nullisomy + disomy) for the two chromosomes scored was 2.5%, of which 1.5% due to nullisomy and 1.0% due to disomy. By averaging these data with those previously reported on dairy cattle breeds the overall incidence of aneuploidy in cattle -as a species- was 2.25%, of which 1.25% 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. Comparison between cattle (Xcen-5 probes) and pig (*Sus scrofa domestica*) (1-10 probes) also reveal similar rates. Further studies are needed by using more probes in order to investigate about the inter-chromosomal effect. Establishing a 'baseline' level of aneuploidy for each species/breed could also reveal 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

The present study investigates upon the incidence of aneuploidy in *in vitro* matured bovine MII oocytes with the corresponding first polar body from two indigenous cattle (*Bos taurus*) breeds, namely, the Podolian and Maremmana, by using 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]. As known, this technique can induce technical artefacts such 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 first polar body cannot be analyzed because the chromosomes are too condensed and overlapped. The possibility to detect aneuploidy also in interphase cells (i.e., without the need to display metaphase chromosomes) came along with the fluorescence in situ hybridization (FISH) technique [2] by using chromosome- specific ‘‘painting’’ probes or Bacterial Artificial Chromosomes (BACs). This technique, in fact, if applied to MII oocytes with the corresponding first polar body, 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 polar body, which should therefore result disomic, and viceversa. The limiting factor of this technique, however, is the scarce availability of the chromosome-specific probes. The PRINS technique [3] is another interesting way to analyze aneuploidy, but because there are no specific reports on domestic animals, we preferred to use the FISH approach.

Since all the basic information we have -to date- on the aneuploidy rates in bovine oocytes has been collected by using the conventional air-drying method [4, 5, 6, 7, 8, 9], we decided to review the available data on aneuploidy in cattle oocytes by using the more resolutive FISH technique applied on in *in vitro*-matured MII oocytes plus first polar bodies and painting probes from chromosome Xcen and chromosome 5 prepared by chromosome microdissection and DOP-PCR. These probes were chosen

because they both provide strong and specific signals, and have been already 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.

In order to provide more exhaustive 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 Podolian and the Maremmana, for which no aneuploidy data are -at the present- available.

While the Podolian breed is reared in South Italy, the Maremmana 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 about 18-24 months of age, while the calving interval is over 14 months. A recent study by Ducos et al. [11] reported an incidence of chromosomal abnormalities close to 16 and 20%, respectively, in the two breeds.

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 months. Due to sanitary restrictions in Italy for BSE, it is not permitted to use females whose age is over 24 months.

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 hours. Cumulus-oocyte complexes (COCs) were collected through aspiration with 21-gauge needles, washed in TC-199 medium (No. M2154; Sigma, St. Louis, MO, USA), and examined on Petri dishes under a stereomicroscope. Only oocytes with compact-intact 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 TC-199 medium + 10% fetal bovine serum (No. 10106-151; Gibco, Invitrogen, Carlsbad, CA, USA), supplemented with 0.5 mg/mL 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 pre-cleaned 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 x 60 mm coverslip, which was then air dried and treated for GTG-banding. The Xcen probe was produced by isolating the pericentromeric region, corresponding with the centromere and with the Xp11-14 region of the standardized GTG-banded karyotype (ISCNDB,2000); the probe for chromosome 5 was produced by scraping the entire chromosome. Microdissected chromosomes were amplified following the protocol of Engelen et al. [13]. Probes were labeled with digoxigenig -11-dUTP (chromosome Xcen) and biotind-16-dUTP (chromosome 5) (Roche, Mannheim, Germany. Cat. No. 11558706910 and No. 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 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 2X 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 x 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 (No. 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 (No. 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 x 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, reduced secondary oocytes without the corresponding first polar bodies were excluded from the analysis. An oocyte was defined as ‘‘nullisomic’’ when one of the two signals (either X or 5) was lacking from the MII plate but present twice in the corresponding polar body; vice versa, an oocyte was defined as ‘‘disomic’’ when one extra signal (either X or 5) was present in the MII plate but absent from the polar body. Chi-square analysis was used for statistical analysis of data.

3. Results

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

The total number of donor females used for this study was 24 and 15 for the Podolian and Maremmana breeds, respectively.

The average number of COCs collected from each cow was 12.6 in the Podolian (303/24) and 14.9 in the Maremmana (223/15), respectively. The number of cytogenetic slides prepared and successfully analyzed was 186 and 160, respectively, in the two breeds.

Totally, 526 COCs were collected through aspiration (303 and 223 in the Podolian and Maremmana, respectively). The percentage of COCs selected for *in vitro* maturation was around 75% in both breeds. Out of 221 and 175 COCs selected for maturation, respectively, in the Podolian and Maremmana, 165 and 140 reached the MII stage; the efficiency of the *in vitro* maturation process was around 80% in the two breeds. Significant ($P<0.05$) inter-individual differences were found in the yield of *in vitro*-matured MII oocytes in the two breeds analyzed.

Among the 165 Podolian oocytes at MII stage, 143 displayed haploid chromosome set. In 43 of them chromatin of the first PB was not found, therefore the final FISH analysis was done on 100 MII oocytes with corresponding first PB. Two oocytes (2%, 2/100) were nullisomic and one (1%, 1/100) was disomic for chromosome 5. 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 Maremmana oocytes at MII stage, 131 displayed haploid chromosome set. In 31 of them chromatin of the first PB was not found, so they were excluded from the final analysis.

FISH was done on 100 MII oocytes with corresponding first PB. Unreduced chromosome number was identified 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 (premature separation of sister chromatids) on chromosome 5 (Fig.1).

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, 3 oocytes were nullisomic (1.5%), 2 oocytes were disomic (1%), with an overall aneuploidy rate, for these two chromosomes, 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 first polar body were analyzed by FISH method with painting probes corresponding to bovine chromosomes X 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. [14]. No significant differences have been detected between the two species in the mean rate of diploidy, aneuploidy, disomy, nullisomy and PSSC.

4. Discussion

The present study showed that in bovine MII oocytes matured *in vitro*, from two 'indigenous' breeds, namely, the Maremmana and Podolian, the mean rates of aneuploidy for chromosomes X- and 5 were 2.0% and 3% in the two breeds, respectively. The mean rate of diploidy was 10.2% with a variation from 6.4% to 13.3% in the Maremmana and Podolian breeds, respectively. This value is 'within' the interval already reported in the literature (from 8% to 12%) by using conventional methods

[15, 4, 6]. Nullisomy was detected only in 1% of the oocytes in the former and in 2% in the latter, and concerned only chromosome 5 in the two breeds. Disomy was found in 1% of the investigated oocytes in both breeds and involved chromosome X in the Maremmana and chromosome 5 in the Podolian. Frequency of PSSC was 1% in the two breeds and concerned only chromosome 5.

When the results of this study on two 'indigenous' breeds are compared to those previously reported by Nicodemo et al. [10] on two 'dairy' breeds (Italian Friesian and Italian Brown) (Table 2), it is quite evident that among the four breeds investigated there are no significant differences in the mean rate of diploidy, aneuploidy, disomy, nullisomy and PSSC. This finding, however, needs to be further investigated by increasing the number of MII oocytes as well as the number of chromosome-specific probes.

Previously, 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% [16] to 14.2% [17] in pig. In other mammalian species, the rate of aneuploidy was found to be 5.8% in the horse [18] and rabbits [19], 1.8% in the hamster [20], and 2.7% in the mouse [21].

To re-examine inter-specific 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. [14] (Table 3). Even though in this paper there is no information upon the age of the donor gilts as well as about the breeds they belong, these data are the only ones available for comparison, so far. Despite the pronounced difference in the total number of MII oocytes analyzed so far (400 in cattle *vs* 1,189 in the pig), the rate of aneuploidy is quite similar in the two species: 2.25 *vs* 2.86, respectively, while the rate of disomy was 1.00 *vs* 1.68, whereas that of nullisomy was 1.25 *vs* 1.18, respectively. However, more recent studies on pig oocytes analyzed by FISH demonstrate that the rate of aneuploidy is around 7%, varying from 6.3% (sows 1.3%; gilts 10.8%) [22] to 6.7% (prepubertal gilts) to 8.5% (cycling gilts) [23].

In humans, the aneuploidy rates detected by FISH vary among different laboratories, with the highest value reaching 47% [24]; 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 5 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 inter-chromosomal differences in the rate of non-disjunction.

Although theoretically all chromosomes may participate at similar frequency in non-disjunction events, the evidence on humans [24, 25] 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 [16] 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. [22] and Pawlak et al. [23] revealed a significant predominance of the chromosome 10 in porcine aneuploid oocytes.

Premature separation of sister chromatids (PSSC) can be an additional source of aneuploidy in the resulting embryo. In the present study, a balanced PSSC was observed in 2% of the oocytes, involving chromosome 5 in both breeds analyzed. As known, balanced PSSCs are not considered to be 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 *in vitro*-matured oocytes is strongly dependent upon the culture system [21] and the age of donor [26]. Previous studies on pig oocytes by Lechniak et al. [22]

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. [27] 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 months; so, 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 in *in vitro* matured MII oocytes with corresponding fist polar body among the various cattle breeds analyzed so far, as well as between cattle and pig. Further studies, however, are needed to expand investigations to other species/breeds by using more animals, more oocytes as well as more chromosomal probes in order to cover a major fraction of the genome.

Estimation of the baseline level of aneuploidy in germ cells of domestic animals is -to 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 management errors (hormonal unbalancements, nutritional and dietetical mistakes) and/or environmental hazards (mutagens, mitotic poisons) which are known to damage the mitotic/meiotic machinery of the cell.

5. References

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Figure caption

Figure 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.

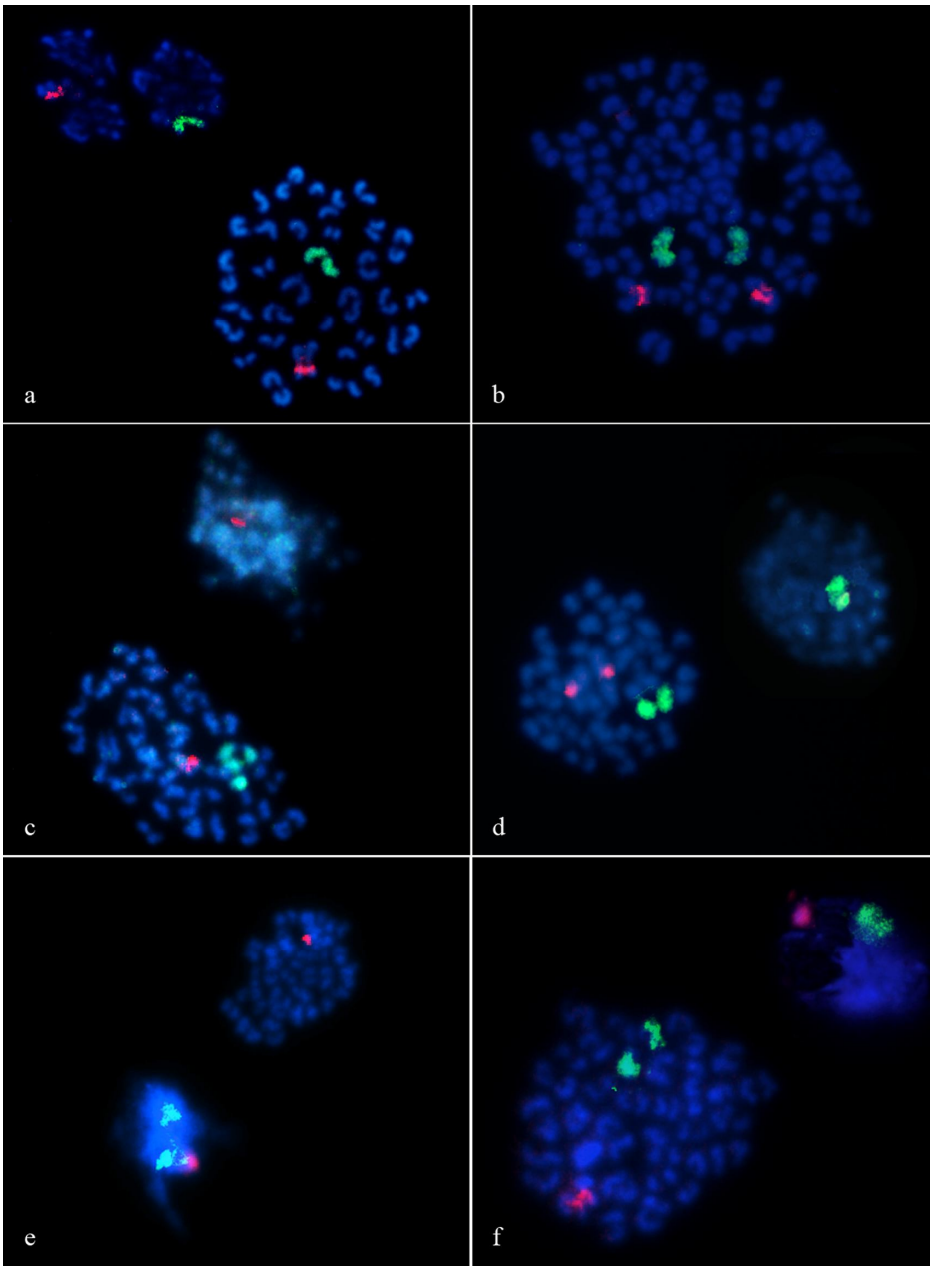

Table 1 - Incidence of aneuploidy in bovine secondary oocytes matured *in vitro* of the Podolian and Maremmana breeds (only oocytes with the corresponding first polar body were analyzed by FISH method with painting probes corresponding to bovine chromosomes X and 5).

Donor	Age	Number of analyzed oocytes											
							Reduced			Aneuploid			PSSC
		Collected	Selected for IVM	Slides analyzed	Tot MII (a)	Unreduced	total (b)	-PB	+PB (c)	Nullisomic	Disomic	Tot	
						% on (a)	*% on (a)*	*% on (b)*	*% on (b)*	*% on (c)*	*% on (c)*	*% on (c)*	*% 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**^{X} ***(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 5 animals with <4 analyzed oocytes

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([1])	Brown([1])	Podolian([2])	Maremmana([2])	Total	
	N	N	N	N	N	%
Donors used	23	19	24	15	81	-
Age range([3])	13-24	14-24	13-24	13-22	13-24	-
COCs collected	295	254	303	223	1,075	-
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 N. of PSSC	***2***	***1***	***1***	***1***	***5***	***1.25***

([1])Nicodemo et al.(2010); ([2]) Present study; ([3]) 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[a]	%[a]	N[b]	%[b]	N[c]	%[c]	N[b+c]	%[b+c]
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***

[a]Nicodemo et al. (2010) + present study; [b]Vozdová et al. (2001); [c]Lechniak et al. (2007)