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by using specie-specific painting probes for sex chromosomes. One hundred metaphases were scored and all showed normal XY chromosomal arrangement. No metaphases with two X chromosomes were detected. The observed phenotype and the lack of cytogenetic defects led to state that this clinical case might represent a suspected condition of male pseudo-hermaphroditism. In humans, this condition is related to the androgen insensitivity syndrome (AIS). Further investigation is therefore necessary to identify at molecular level the causes of this abnormal phenotype.

P9

Cytogenetic survey in autochthonous endangered animal breeds reared in Campania region (Southern Italy): an up-date

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In the Rural Development Plan RDP 2007–2013, Misura 214, e2, project RARECa of Campania (Southern-Italy), three different Institutions (CNR, University of Naples and IZSM) are involved to study, characterize and valorise some autochthonous endangered animal breeds raised in Campania Region. In this report an up-data on the cytogenetic analyses we performed in horse (Napoletano, Salernitano and Persano breeds), cattle (Agerolese breed), pig (Casertana breed), sheep (Laticauda and Bagnolese breeds) and goat (Cilentana breed) are reported. Up to now, upon 63 Agerolese cattle four females (6.3 %) were found to be carriers of: (a) rob(1;29) (2 animals), (b) rcp(11;25) and (c) a case of partial monosomy and trisomy (2n= 60,XX,t(11;25)(q11;q14-21). All examined horses (34 animals) from Napoletana (14) and Salernitana (20) breeds showed normal karyotypes. Concerning the Laticauda sheep (46 animals), two females were found to be carriers of two new reciprocal translocations while Bagnolese sheep breed (32 animals) and Cilentana goat breed (12 animals) showed normal karyotypes. Furthermore, in Casertana pig breed 52 animals were examined and resulted with normal karyotype.

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Chromosomal abnormalities in secondary bovine oocytes matured in vitro up to 48 h

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Preliminary experiments carried out in our laboratory indicated that—by fertilizing oocytes matured in vitro for 24–32 and 48 h—the resulting blastocyst rates decreased from 22.4 % (24 h), to 14.0 % (32 h) to '0' % (48 h). The aim of this study was investigate upon the variation in the incidence of chromosomal abnormalities occurring in bovine oocytes matured in vitro for prolonged periods of time, i.e. from 24 to 32 to 48 h. Abattoir-derived oocytes were matured in vitro using standard procedures, for 24–32 and 48 h. After incubation, the COCs were treated with Ialuronidase (3 mg/ml) to eliminate the cumulus cells, swelled in hypotonic (KCl, 0.075 M) for 5–10 min, fixed individually on microscope slides with Carnoy fixative, air dried and stained with 5 % Giemsa. Conventional karyotypes were prepared from 50 matured oocytes for each time of maturation, providing the following results: chromosomal abnormalities, including unreduced diploid metaphases, hypo-haploidy and hypo-diploidy, increased from 12 % at 24 h, to 20 % at 32 h, to 36 % at 48 h. The Chi-square test (with Yates corrections) showed significant differences ($P>0.01$) in the rate of

chromosome abnormalities from 24 to 48 h of maturation as well as from 32 to 48 h, whereas the differences between 24 and 32 h were not significant. These results confirm previous data and provide further evidence that bovine oocytes to be used in IVF programs should not be matured in vitro longer than 24–32 h.

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Four cases of testicular disorder of sexual development (DSD) in cats (38,XY; *SRY*-positive)

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Disorders of sexual development (DSD) are poorly recognized in cats. In the present study we show four male cats with abnormal external genitalia. In case 1 an abnormal orifice of the urethra located close to the anus and rudimentary penis with numerous horny spines were observed. In case 2 an underdeveloped penis with rudimentary spines without scrotum was present. Case 3 had a blind ended vulva (1.5 cm long), a normal penis, but the urethra orifice was located next to the anus. In case 4 a rudimentary penis with spines and glans penis (but not covered with a prepuce) was observed. For all cases cytogenetic, molecular and histological analyses of the gonads were performed. The cytogenetic analysis, with the use of Giemsa staining, G-banding and fluorescence in situ hybridization (FISH) with a BAC probe specific for the *SRY* gene, revealed a normal male karyotype (38,XY) in all cases. The PCR detection of the *SRY* and the *ZFY* genes confirmed the presence of the Y chromosome derived sequences in all studied cats. Moreover, sequencing of three candidate genes (sex determining region Y—*SRY*, androgen receptor—*AR* and steroid-5-alpha-reductase—*SRD5A2*) was carried out. In the *SRY* of case 4 a known missense substitution g.389G>C, p.Arg130Thr was identified. In the *AR* a common tandem repeat polymorphism (CAG) in exon 1 was found, while in the *SRD5A2* gene 5 SNPs (a silent one in exon 1, 2 in introns and 2 in 3′UTR) and 2 intronic indels were observed. Histological examination of the gonads facilitated their classification as testes. In details, only Sertoli cells in seminiferous tubules were found in case 1, while in cases 2 and 3 testes with normal spermatogenetic activity were present. In case four no sperms were found in normally developed seminiferous tubules. Taking into consideration the obtained results we conclude that karyotypes of the studied cats were not altered. Thus, the cases were classified as testicular DSD (38,XY and *SRY*-positive). Further analyses of other candidate genes are postulated.

P12

Exclusion of SOX9 duplication and over expression in a horse with XX, SRY-Negative Ovotesticular DSD

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Disorders of sex development (DSD), include a broad range of atypical sexual phenotypes and have also been described in domestic animals. We investigated an equine case, which after molecular, cytogenetic, anatomic and histopathological analysis was classified as an XX, SRY-Negative Ovotesticular DSD. Since there is some empirical evidence in humans and lab animals that duplication and/or over-expression of the SOX9 gene may explain the presence of testicular tissue in absence of the testis determining factor (i.e. SRY), we explored this possibility in such an individual. DNA was extracted from blood samples, while cDNA was obtained by reverse transcription of total RNA from vulvar skin biopsies of the animals in the study. SOX9 copy number and gene expression were determined by real time PCR,