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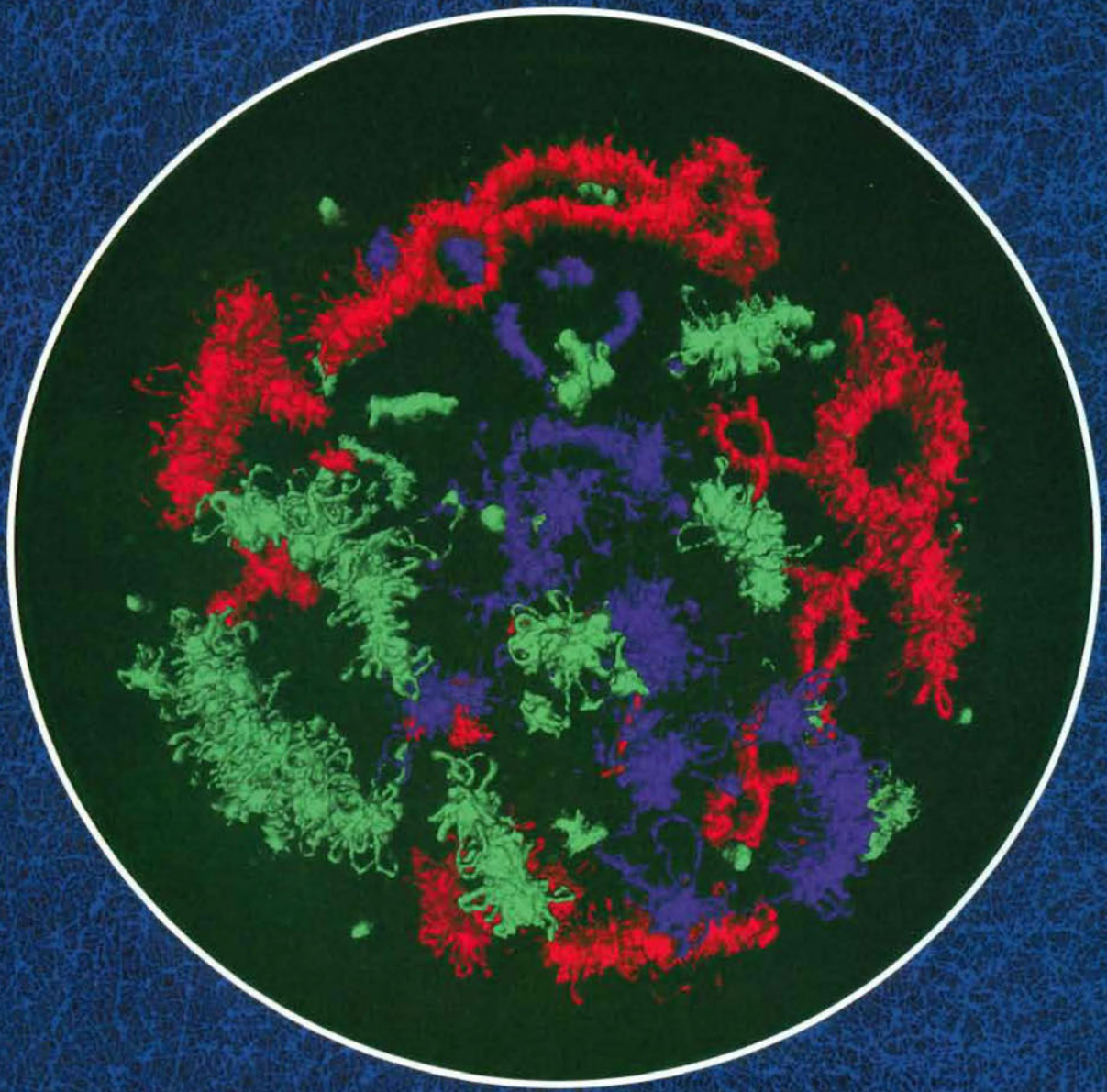
CHROMOSOME RESEARCH

The Biology of Chromatin and Chromosomes



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ABSTRACTS

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unbalanced gametes. Such unbalanced gametes may have the potential to influence other semen parameters. An analysis of semen including morphology, morphometry, sperm viability and sperm motility using CASA are therefore included in this study.

Fertility data from Danish boars are used to identify approximately 25 Yorkshire boars and 10 Landrace boars with reduced fertility. The control group consists of 50 Landrace and 50 Yorkshire boars with normal fertility. Karyotyping is done by RBA-banding of lymphocyte chromosomes. Testis biopsies from boars with reduced fertility are obtained at slaughter and used for meiotic studies by immunohistochemistry using antibodies to meiosis-specific proteins and for testicular cell suspensions if indicated by karyotyping or semen analysis.

The experiment was initiated in October 2011 and at present we have identified 12 Yorkshire boars and 5 Landrace boars with low fertility. Data from the ongoing study will be presented and discussed at our poster.

PIII-16

Analysis of meiotic recombination by immunostaining and FISH in pigs.

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Early stages of male meiosis and especially the pachytene stage of the first meiotic division have been analyzed by direct immunostaining approaches in many species (mouse, human, cat, shrew…). These methods allow visualizing the synaptonemal complexes and crossing-over foci by immunolabeling of meiotic specific proteins using, for instance, antibody against SCP3 or MLH1. In addition, these approaches can be combined with FISH and allow studying recombination and interference for each autosomal pair. To date, only humans and mice have been subjected to this kind of analysis.

The goal of our study was to apply these approaches in pigs in order to analyze the meiotic recombination in this species. Immunostaining techniques were adapted to the pig species and three sets of six probes were developed in order to identify the 18 autosomal porcine chromosomes. The first results obtained on a karyotypically normal boar indicated an average of 30.2 MLH1 foci per spermatocyte on pig autosomes. Specific distributions of MLH1 foci were observed for the different autosomal pairs with the presence of recombination hotspots. Moreover a significant difference between the recombination patterns was observed between the different types of chromosomes (acrocentric versus metacentric).

In conclusion, to our knowledge our results are the first reported in the pig species but complementary analyses are in progress on individuals of different genetic background and age, and on a reciprocal translocation carrier t(12;13).

PIII-17

Mitotic index and aneuploidy variation in growing day 2- to day 7- IVP bovine embryos of the Agerolese breed of cattle

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The present study reports on the variation of the mitotic index in growing day 2- to day 7- IVP bovine embryos of the Agerolese breed of cattle. After IVM (24 h), COCs were transferred into 25/well with 300 μl IVF-TALP and covered with mineral oil. Frozen sperm from a bull were selected by centrifugation on a Percoll discontinuous gradient (45–80 %). The pellet was diluted in IVF medium and added to the COCs at the concentration of 1×10^6 sperm/mL. Gametes were co-incubated for 20–22 h at 39 °C, in 5 % CO_2 in air. After co-incubation, presumptive zygotes were vortexed to remove cumulus cells and randomly allocated in six groups, each into 400 μl of SOF medium, with 30 μl/ml essential amino acids, 10 μl/ml non-essential amino acids, 0.34 mM tri-sodium citrate, 2.77 mM myo-inositol, and 5 % BS. Zygotes were incubated in a humidified mixture of 5 % CO_2, 7 % O_2 and 88 % N_2 in air at 39 °C for 20–22 h. Starting on Day 0 (IVF day), embryos were taken out

of the incubator at day 2(48 h),3 (72 h),4 (96 h), 5 (120 h), 6(144 h) and 7(168 h), examined under a stereomicroscope, treated in a lysing buffer (0.01 N HCl, 0.1 % Tween 20) for 30 s, transferred in a small droplet to a precleaned slide and fixed with methanol-acetic acid (3:1). Out of 178 embryos (3,100 cells) analyzed, the mitotic index was 19.6 % (28/143 cells) at day 2, 18.6 % (41/221 cells) at day 3, 10.3 % (27/263 cells) at day 4, 7.1 % (26/367 cells) at day 5, 0.7 % (5/681 cells) at day 6 and 0.6 (9/1425 cells) at day 7. FISH analysis is undergoing and will be reported elsewhere.

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PIII-18

Study of synaptonemal complex in hybrids of red fox (*Vulpes vuples*) and arctic fox (*Alopex lagopus*)

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The synaptonemal complex is a protein structure that enables pairing of homologous chromosomes in meiosis of mammalian germ cells. It is present during prophase of the first division. Observation of these cytological structures allows for the analysis of chromosome pairing—not only its regularity but also some aberrations which may occur. Formation of univalents or multivalents can be seen, what reflects some abnormal pairing of chromosomes due to a translocation or aneuploidy. The observation of synaptonemal complexes formation can be especially useful in the analysis of meiosis in interspecies hybrids such as a hybrid of red fox and arctic fox. These two species have different number and morphology of chromosomes: 2n=48–50 in arctic fox and 34+B chromosomes in red fox. It results in variable number of chromosomes in their hybrids and hence different patterns of homologous chromosome association in meiosis.

In the present study testicular material from 4 interspecies hybrids of red fox and arctic fox was obtained and used to prepare specimens for the synaptonemal complexes analysis. Then, the preparations were silver-stained and immunofluorescently labeled with antibody specific to SCP1—one of protein forming the structure of the synaptonemal complex. We were able to observe not only usual homologous bivalents but also univalents, trivalents or other anomalous connections, which can lead to abnormal segregation during meiosis and, as a result, to forming of aberrant gametes.

PIII-19

Sister chromatid exchange (SCE) in Calabrian and Large White pig breed and in their crosses: preliminary results

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Aim of this study was to compare genome stability among an italian native pig breed, Calabrian, a breed for intensive pig farming, Large White, and their crosses. Frequencies of sister chromatid exchanges (SCE) in peripheral blood lymphocytes were investigated in 10 Calabrian pigs, 10 Large White pigs and 7 Calabrian x Large White pigs. All animals were aged between 8 and 12 months, reared in the same conditions in a farm located in the province of Cosenza. Whole blood was added to RPMI 1640 medium enriched with FCS (10 %), L-Glutamine (1 %) and Lectin (1,5 %) and incubated at 37.5 °C for 72 h. BrdU (10 μg/ml) and Colcemid were added 16 h and 1 h before harvesting respectively. SCEs were counted in 35 metaphase plates per animal and average difference evaluated with *t*-test. In Calabrian pigs, on 350 cells and 13,088 chromosomes examined, the mean number of SCEs was 7.29±3.25 per cell and 0.19 per chromosome; in Large White, on 350 cells and 13,287 chromosomes examined, the mean number of SCEs was 6.45±2.74 per cell and 0.17 per chromosome; in Calabrian X Large White, on 245 cells and 9,303 chromosomes examined, the mean number of SCEs was 6.72