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FISH-BASED CHARACTERIZATION OF CHROMOSOMAL REARRANGEMENT IN THE DOMESTIC PIG

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The present study is an example of molecular approach in characterization of karyotype abnormalities in the domestic pig. Chromosome microdissection and chromosome painting techniques were applied for detailed diagnosis of reciprocal translocation in hypoprolific boar. Dual–colored FISH analysis of t(7;13)(13;q46) confirmed definitely previous identification based on high-resolution-RBA/GTG banding techniques. The experiment proved that such diagnostic refinements followed by early selection of animals carrying chromosomal rearrangements would efficiently prevent genetic defects from spreading in pig populations and limit economic effects.

Key words: CHROMOSOME MICRODISSECTION, CHROMOSOME PAINTING, FISH, RECIPROCAL TRANSLOCATIONS, KARYOTYPE ABNORMALITIES, PIG REPRODUCTION

Structural karyotype defects, in particular heritable reciprocal translocations that are highly frequent in pigs (about 150 cases reported), are a major breeding problem due to their sharp decreasing effect on pig reproduction (Danielak-Czech and Słota, 2008; Ducos et al., 2007; 2008). The reviews of numerous reciprocal translocations show that these rearrangements reduce carriers fertility by 5 to 100 % (without any visible phenotypic changes), but their individual effects depend on the morphology of the chromosomes involved, the size of exchanged chromatid fragments, and the breakpoints location (Gustavsson, 1990; Świtoński and Stranzinger, 1998; Villagomez and Pinton, 2008). In order to predict breeding consequences and prevent by early identification, translocations need to be characterized precisely by classical cytogenetic techniques supplemented with molecular methods). For better diagnosis, chromosome painting with probes derived from flow-sorted or (sporadically) microdissected chromosomes are more frequently applied recently (Ducos et al., 2002; Kubickova et al., 2002).

The aim of this paper was to apply chromosome microdissection and chromosome painting techniques for karyotype abnormalities diagnosis in pigs. The experiment was carried out on the basis of reciprocal translocation t (7;13)(q13;q46), identified previously by high-resolution-RBA/GTG banding techniques and meiotic chromosome studies with synaptonemal complex analysis (Danielak-Czech et al., 1997).

Materials and methods

Metaphase chromosomes of t(7;13)(q13;q46) boar-carrier were obtained following classical cytogenetic protocols of lymphocyte culture and GTG banding technique. The hypoprolific boar affected had normal exterior and semen parameters (including vitality) but drastically decreased mean litter size (48 %) which could cause significant financial losses in breeding population (Danielak-Czech et al., 1996).

The pig chromosome 7 and 13 — specific painting probes were prepared by way of whole autosomes microdissection (ten copies each) with glass microneedles controlled by manipulator attached to an inverted microscope. The dissected DNA material was amplified and labelled by DOP-PCR (using degenerate oligonucleotide primers: 5'- CCGACTCGAGN6ATGTGG- 3') with biotin-16-dUTP and digoxygenin-11-dUTP (Telenius et al., 1992).

The labeled PCR products were purified in Nick Columns (Amersham Biosciences AB, Uppsala, Sweden) according to the manufacturer's protocol, co-precipitated with salmon sperm DNA, and used in FISH experiments (Pinkel et al., 1986). Briefly, the chromosomes on slides were

denaturated in 70 % formamide in 2xSSC for 2,5 min at 70 °C**.** The probe was denaturated at 70 °C for 10 min. The hybridization was carried out in 37 °C, overnight. Post-hybridization washes were as follows: three times at 50 % formamide in 2xSSC and three times in 2xSSC at 42 °C.

The biotynylated probe was detected using avidin conjugated to FITC and amplified by goat biotynylated anti-avidin antibody. The digoxygenin-labelled probe was detected with goat anti-digoxygenin antibody conjugated to rhodamine (all detection products were from Roche). The chromosomes were counterstained with DAPI, and the slides were mounted in an antifade solution. Image analysis was performed under a fluorescent microscope Opton — Axiophot equipped with CCD camera, using triple attenuation filters DAPI/FITC/Rhodamine and computer image analysis system Lucia-FISH (Laboratory Imaging Ltd, Prague, Czech Republic).

Results and discussion

Dual-colored FISH with microdissected-derived chromosome painting probes confirmed previous identification of reciprocal translocation t(7;13)(q13;q46) based on high-resolution-GTG/RBA banding techniques and meiotic chromosomes studies with synaptonemal complex analysis (Danielak-Czech et al., 1997) (Fig. 1). These diagnostic refinements proved definitely such important details like size of exchanged chromosome fragments and breakpoints location — the factors determining individual effect of translocation on carrier fertility.

As known from published data, some microrearrangements, subtle pericentric exchanges as well as complex rearrangements need advanced cytogenetic molecular studies to be identified precisely, especially FISH/PRINS techniques with flow-sorted or microdissected chromosome-specific/pancentromeric probes, respectively. A number of dual-colored FISH analyses with probes prepared from thirteen flow-sorted pig chromosomes (completed with centromeric probes) have been recently carried out in France and made it possible to determine the refined formula of fifteen translocations, ascertained initially following standard G/R-banding. Owing to this chromosome painting, characterization of eight translocations has been improved and proposed hypothesis for the other ones has been maintained (Pinton et al., 1998, 2000; Ducos et al., 2002; 2007). It is worth to note, that our experiment was the second one (besides studies performed by Kubickova et al., 2002) up to now, which involved chromosome painting with microdissected chromosome-specific probes for reciprocal translocation diagnosis in hypoprolific pigs. Such molecular approach could effectively supplement classical cytogenetic techniques to be used for pig karyotype evaluation in metaphase cells, but are still sporadically applied because the commercial painting probes for this species are not available.

The studies reported originate construction of microdissected pig painting probes panel for currently developing «sperm-FISH» technique, which enables an accurate estimation of the rate of chromosomally unbalanced spermatozoa in boar semen. It would allow predict *in vitro* the potential effect of different reciprocal translocations and the others chromosomal rearrangements in young boars before reproduction (Ducos et al., 2002; Pinton et al., 2004). Such analyses followed by early selection of boars carrying karyotype abnormalities would efficiently prevent genetic defects from spreading in a population and limit economic effects.

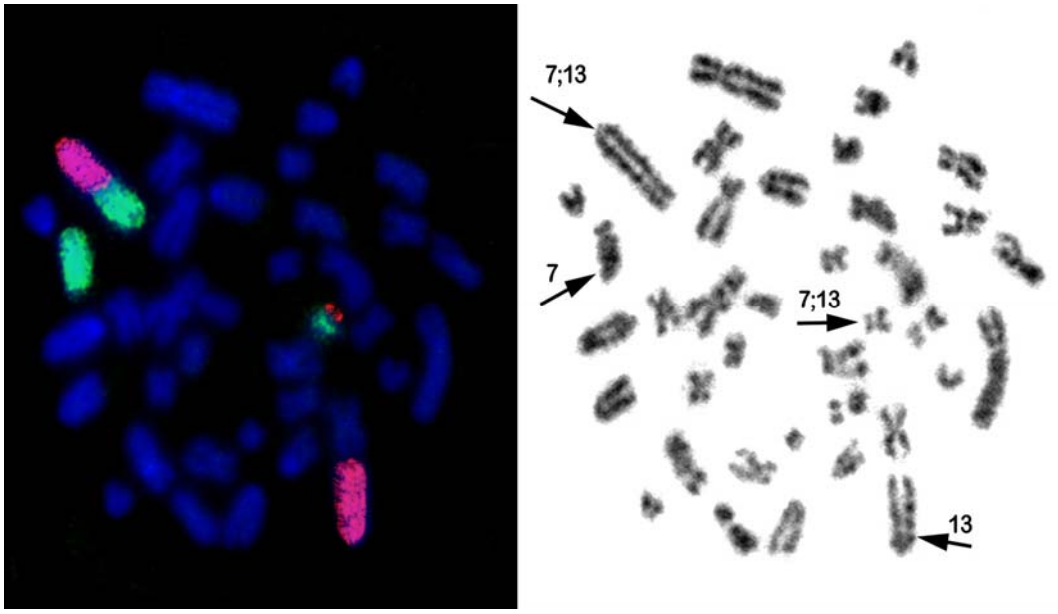

Fig. 1. Dual-color chromosome painting (7 and 13 chromosomes) and GTG-banded karyotype of t(7;13)(q13;q46) boar-carrier (arrows indicate rearranged chromosomes)

Summary

Dual-colored FISH analysis performed in boar-carrier of reciprocal translocation t(7;13)(13;q46) confirmed definitely previous identification based on high-resolution RBA/GTG banding techniques. The experiment carried out proved the usefulness of chromosome microdissection and chromosome painting methods for detailed diagnosis of karyotype abnormalities in the domestic pig.

S t r e s z c z e n i e

Dwukolorowa analiza FISH wykonana u knura-nosiciela translokacji wzajemnej t(7;13)(13;q46) definitywnie potwierdziła wcześniejszą identyfikację opartą na wysoko-rozdzielczych technikach prążkowych RBA/GTG. Przeprowadzony eksperyment wykazał przydatność metod mikrodysekcji chromosomów i malowania chromosomów do szczegółowej diagnozy nieprawidłowości kariotypu u świni domowej.
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FISH-БАЗОВАНА ХАРАКТЕРИСТИКА ПЕРЕДИСЛОКУВАННЯ ХРОМОСОМ У ДОМАШНІХ ПОРОСЯТ

Р е з ю м е

Дослідження є прикладом молекулярного наближення у характеристиці каріотипних аномалій у домашніх поросят. Мікроаналіз хромосом та техніки маркування хромосом використовувались для детального діагностування взаємних переміщень у низько плідних кнурів. Двоколірний FISH-аналіз t(7;13)(13;q46) достовірно підтвердив попередню ідентифікацію, базовану на високо-дозвільних технологіях RBA/GTG. Дослід довів, що така діагностична очистка разом з маркуванням хромосом є важливими для діагностики аномалій каріотипу у домашніх свиней.

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FISH-БАЗИРОВАННАЯ ХАРАКТЕРИСТИКА ПЕРЕДИСЛОКАЦИИ ХРОМОСОМ В ДОМАШНИХ ПОРОСЯТ

А н н о т а ц и я

Исследование является примером молекулярного приближения в характеристике кариотипних аномалий у домашних поросят. Микроанализ хромосом и техники маркировки хромосом использовались для детального диагностирования взаимных перемещений у низко

плодотворных боровов. Двухцветный fish-анализ t(7;13)(13;q46) достоверно подтвердил предыдущую идентификацию, базированную на высоко разрешительных технологиях Rba/gtg. Опыт доказал, что такая диагностическая очистка вместе с маркировкой хромосом является важной для диагностики аномалий кариотипу у домашних поросят.

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