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MOLECULAR CYTOGENETICS IN THE DIAGNOSTICS OF BALANCED CHROMOSOME MUTATIONS IN THE PIG (*SUS SCROFA*) – A REVIEW

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Abstract

Continually improved cytogenetic techniques (differential staining and high resolution banding techniques), complemented with the molecular genetics methods (FISH and PRINS), enable chromosomal mutations to be accurately identified in the karyotype of the pig (*Sus scrofa*). The major breeding problem are balanced mutations because of their hidden nature, as they affect the animals with normal body conformation (and normal semen parameters in boars), which transfer these aberrations to the next generations and disseminate in the population. This refers to the structural rearrangements (translocations and inversions), causing developmental abnormalities and considerably reducing fertility and productivity parameters in breeding herds, which results in substantial financial losses. Routine karyotype screening using modern cytomolecular diagnostic methods is necessary due to the potential emergence of new mutations and the rapid spread of these genetic defects in the population, especially under artificial insemination conditions.

Key words: *Sus scrofa*, banding and FISH techniques, balanced chromosome mutations, meiosis, fertility of pigs

The genome of *Sus scrofa*, including subspecies with different diploid numbers, namely the domestic pig (*Sus scrofa domestica*) and the European wild boar (*Sus scrofa scrofa*) exhibits particularly high structural instability and variation (Raudsepp and Chowdhary, 2011). In the cytomolecular analysis of chromosome structure and function in somatic, germ and embryonic cells of the pig, increasing use is made of molecular methods such as fluorescence *in situ* hybridization (FISH) and primed *in situ* labeling (PRINS) (Iannuzzi and Di Berardino, 2008; Rubeš et al., 2009). These techniques are now used to complement classical cytogenetic diagnostics based on banding techniques, in which it is necessary to identify, characterize and evaluate the biological consequences of chromosomal rearrangements (Villagómez and Pinton, 2008; Raudsepp and Chowdhary, 2011). Cytomolecular studies take on special significance because karyotype defects generally lead to infertility or reduced

fertility, making the affected boars and sows unsuitable for reproduction (Ducos et al., 2008; Basrur and Stranzinger, 2008). For this reason, many research projects using new improved analytical techniques have been undertaken, which resulted in the great amount of scientific articles. They provided the detailed and comprehensive documentation of balanced mutations and their impact on the course and products of meiosis or on the structure and dynamics of chromosome territories in germ cells in the pig, now also considered as a model species (Ducos et al., 2008; Rubeš et al., 2009). However, in recent years, the clear decline of the clinical pig cytogenetic activity and quantity of current publications may be noticed, due to the very limited number of the specialized laboratories which cumulatively report the effects of a few pig screening programs (listing the mutations identified and stating their frequency in populations under control) (Pinton et al., 2012).

Chromosomal mutations are the effect of structural alterations leading to changes in the amount of genetic material (unbalanced mutations) or the rearrangement of chromosome fragments (balanced mutations). In general, unbalanced mutations (deletions and duplications or isochromosomes) are associated with considerable deficiency or excess of genetic information, generating unbalanced gametes in meiosis, followed by defective zygotes and embryos. This leads to early embryo mortality or severe malformations of non-viable fetuses, and finally, harmfully influences fertility of the affected animal. On the other hand, balanced mutations (reciprocal, Robertsonian and tandem translocations and para- and pericentric inversions) give rise to both genetically balanced and unbalanced gametes in meiosis-I, due to specific segregation patterns of atypical pairing configurations (quadrivalent, trivalent, inversion loop) created by chromosomes involved in aberration, which usually results in the reduction of reproductive efficiency (Gustavsson, 1990; Świtoński and Stranzinger, 1998).

In the case of reciprocal translocations, the rearranged chromosomes form quadrivalent, segregated by five modes (2:2 – alternate, adjacent I, adjacent II and 3:1 or 4:0) and form gametes (18 types) with a high frequency of unbalanced ones. These segregation modes have various prevalence (depending on chromosome morphology, the size of exchanged fragments, breakpoint location, chiasma formed), but predominantly alternative and adjacent-1 occur. The alternative type produces both 50% of chromosomally balanced and normal gametes, giving viable mutation-carrier or normal fetuses, which means that the mutation is inherited in approximately 50% of offspring. The adjacent-1 and adjacent-2 segregations generate unbalanced gametes (inducing partial trisomies and monosomies in viable or non-viable embryos), whereas the 3:1 or 4:0 lead to unbalanced gametes, usually incompatible with embryonic survival. The specific, meiotic segregation pattern for particular translocation, determines the proportion of unbalanced gametes, and in consequence the extent of reduction of litter size in carriers or their mates (an average of about 40%) (Gustavsson, 1990; Świtoński and Stranzinger, 1998; Villagómez and Pinton, 2008).

In turn, in meiosis-I of Robertsonian and tandem translocation carriers trivalent is formed, which is segregated by the alternate, adjacent and 3:0 modes (creating 6 types of gametes). As a consequence of high prevalence of the alternate segregation (up to 90%) chromosomally balanced and normal gametes are produced in the

vast majority, giving viable mutation-carrier or normal embryos and fetuses, that as adult animals transmit the aberration to their progeny. Unbalanced gametes (nullisomic or disomic for one of the chromosomes involved) are formed as a result of other segregation which, after fecundation, leads to trisomic or monosomic zygotes. Such chromosomal constructions are generally not compatible with life, bringing about early embryonic losses or stillbirths, and determine the scale of reduction in reproductive performance (an average of approximately 10%) of their carriers (Gustavsson, 1990; Świtoński and Stranzinger, 1998; Villagómez and Pinton, 2008).

On the other hand, both para- and pericentric inversions form a loop in the inverted segment in order to maximize the pairing of homologous *loci* between the inverted and normal homologue. The reduced frequency of interstitial cross-overs in the loop (or asynapsis and nonhomologous synapsis of inverted segment) in effect gives balanced and normal gametes (50% of each type). After fertilization, viable mutation-carriers such as zygotes and embryos and next adult animals are capable of passing this mutation on to descendants. In turn, an odd number of crossovers within the loop results in production of unbalanced gametes with a duplicated and deficient chromosome segment, which gives rise to the viable (but malformed) or non-viable fetuses affected with partial trisomy and monosomy and account for carrier decreased fertility (an average merely of several percent) (Gustavsson, 1990; Świtoński and Stranzinger, 1998; Villagómez and Pinton, 2008).

On the whole, unbalanced mutations do not represent a big economical problem in livestock farming, because they produce such great adverse phenotypical effects for their carriers that they are not inherited and hence are naturally eliminated from the population. On the contrary, balanced mutations are more important due to their hidden nature, meaning that carriers having a completely normal external appearance can transfer these aberrations in both balanced and unbalanced form to subsequent generations. Without cytogenetic controls, the mutations can be easily distributed in the population particularly when artificial insemination is used. The chromosomally unbalanced gametes produced by a balanced carrier participate in the fertilization but the embryos most often die early resulting in decreased fertility and ultimately major financial repercussions for the breeder.

Banding techniques in the evaluation of structural chromosome abnormalities

Conventional cytogenetic diagnostics is based on the microscopic analysis of metaphase chromosomes obtained from *in vitro* lymphocyte culture (Arakaki and Sparkes, 1963), which makes it possible (after routine Giemsa staining) to determine chromosome number and morphology, based on centromere location (*S. s. domestica 2n* = 38; *S. s. scrofa* 2n = 36, 37, 38) (Gustavsson, 1990). The next diagnostic stage is GTG (Wang and Fedoroff, 1972) and RBA (Dutrillaux et al., 1973) differential banding and its modifications (resolution 5–10 million base pairs) (Bickmore, 2001; Vorsanova et al., 2010), which enable homologous chromosome pairs to be identified based on G or R (reversed to G) banding patterns (Figures 1 and 2) (Gustavsson, 1980; Iannuzzi and Di Berardino, 2008). The GTG technique (inducing G bands by Giemsa-staining of protease-treated chromosomes) corresponds to the late replicat-

ing, AT-rich, condensed and rather transcriptionally inactive (of the low CpG islands and gene density) structural chromatin domains (Wang and Fedoroff, 1972; Bickmore, 2001; Iannuzzi and Di Berardino; 2008). RBA technique (generating R bands by acridine orange fluorochrome with a specificity for DNA base composition) co-localized with the early replicating, GC-rich, less condensed and very transcriptionally active (of the high CpG islands and gene density) chromosome regions (Dutrillaux et al., 1973; Bickmore, 2001; Iannuzzi and Di Berardino; 2008). The described diagnostic procedure makes it possible to determine the karyotype of a particular animal (Figures 2, 3, 4 and 5) (Rejduch et al., 2003 b, c; Danielak-Czech and Słota, 2008 a, b) by comparing an individual banding pattern with the species standard pattern of 300 G and R bands in the haploid chromosome set, developed as the standard karyotype of *Sus scrofa domestica* (Gustavsson, 1988). Standard cytogenetic analysis is expanded to include high resolution banding techniques (HRBT) (resolution 2–5 million base pairs) (Yunis, 1981; Bickmore, 2001), which yield 600 G or R bands and sub-bands (Figures 1 and 2) in the haploid set of elongated pro-metaphase chromosomes (obtained from synchronized lymphocyte culture) (Figure 4) (Danielak-Czech and Słota, 2008 a), which are compared to the standard karyotype for pro-metaphase chromosomes of the domestic pig (Rønne, 1990; Yerle et al., 1991). The above banding methods are complemented by staining which reveals the location of specific chromosome regions (Gustavsson, 1980; Iannuzzi and Di Berardino, 2008): constitutive heterochromatin blocks – C bands (CBG technique) (Sumner, 1972), nucleolar organizer regions – Ag-NOR bands (Ag-I technique) (Bloom and Goodpasture, 1976), telomere areas – T bands (THA technique) (Dutrillaux, 1973). Banding techniques are a basic tool in cytogenetic screening and diagnostics of chromosomal abnormalities in pigs. This particularly applies to structural rearrangements, which are most often generated *de novo* as a result of chromosome fragile site breaking (Riggs et al., 1993; Yang and Long, 1993; Riggs and Rønne, 2009) in response to the harmful effect of environmental factors (Rubeš et al., 1992; Słota et al., 2000; Danielak-Czech and Słota, 2004; Inglot et al., 2012; Ciotola et al., 2014).

The cytogenetic monitoring using banding techniques, which over the last forty years covered the *Sus scrofa* populations in many countries (mainly in France, Poland, the Netherlands and Hungary, and to a lesser extent in Finland, Portugal, United States of America and Canada) revealed around 200 karyotype defects. There were mainly structural chromosome aberrations (over 150 reciprocal translocations, 4 Robertsonian translocations, 1 tandem-fusion translocation and over a dozen para- and pericentric inversions) (Danielak-Czech and Słota, 2008 a; Ducos et al., 2008; Quach et al., 2009; Raudsepp and Chowdhary, 2011; Pinton et al., 2012; Kociucka et al., 2014), several cases of cell chimerism or mosaicism (Gustavsson, 1990; Padula, 2005; Pinton et al., 2011; Raudsepp and Chowdhary, 2011; Barasc et al., 2014) and a few aneuploidies (39,XYY; 39,XXY; 37,X; 40,XXXY) in the domestic pig (Gustavsson, 1990; Raudsepp and Chowdhary, 2011). Moreover, Robertsonian translocation (15;17) was identified in different animals of the wild boar population showing karyotype polymorphism (2n=36-38) for this fusion (Gustavsson, 1990; Ducos et al., 2008; Raudsepp and Chowdhary, 2011).

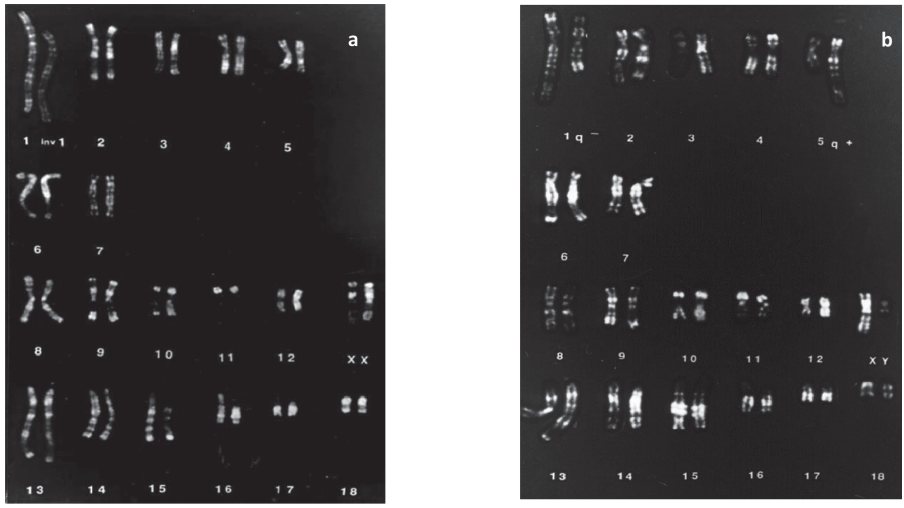

Figure 1. The RBA-HRBT banded karyotype of a boar carrying pericentric inversion inv(1)(p22;q11) (a); the RBA banded karyotype of a boar carrying reciprocal translocation rcp(1;5)(q21;q21) (b) (B. Danielak-Czech)

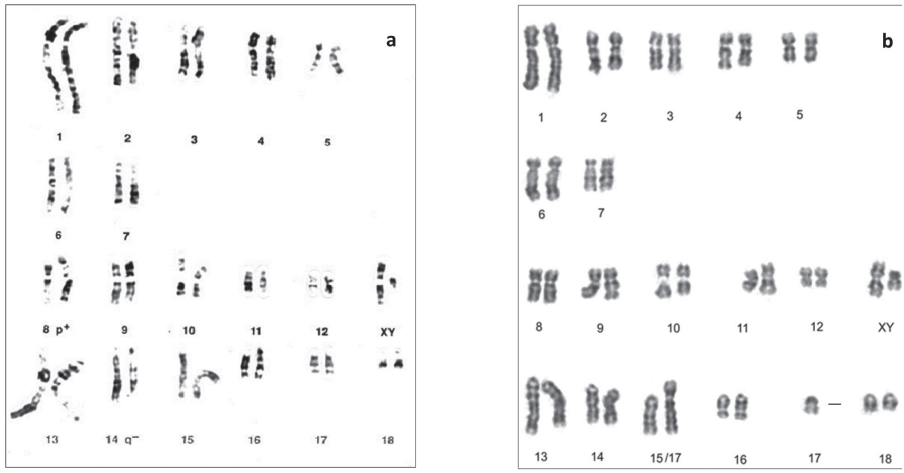

Figure 2. The GTG-HRBT banded karyotype of a boar carrying reciprocal translocation rcp(8;14) (p21;q25) (a) (B. Danielak-Czech); the GTG-banded karyotype of a wild boar heterozygous for – rob(15;17) Robertsonian translocation (b) (Rejduch et al., 2003 b)

Out of the approximately 200 chromosome mutations described to date, ten were diagnosed in Poland (Table 1): two inversions (Figure 1) (Świtoński, 1991; Danielak-Czech et al., 1996 a, b; Świtoński et al., 1998), six reciprocal translocations (Figures 1, 2, 3, 4, 6, 7) (Danielak-Czech et al., 1994, 1996 c, 1997; 2006; 2013; Danielak-Czech and Słota, 2007, 2008 a; Rejduch et al., 2003 c, 2006) and one tandem-fusion translocation (Figure 5) (Danielak-Czech and Słota, 2008 b; Danielak-Czech et al.,

2010 a, b) in the domestic pig (*S. s. domestica*) as well as a hetero- and homozygous Robertsonian translocation (2n=36–38) in the European wild boar (*S. s. scrofa*) (Figures 2, 6) and in the wild boar and the domestic pig hybrids (Rejduch et al., 2003 a, b; 2010 b; Wnuk et al., 2005).

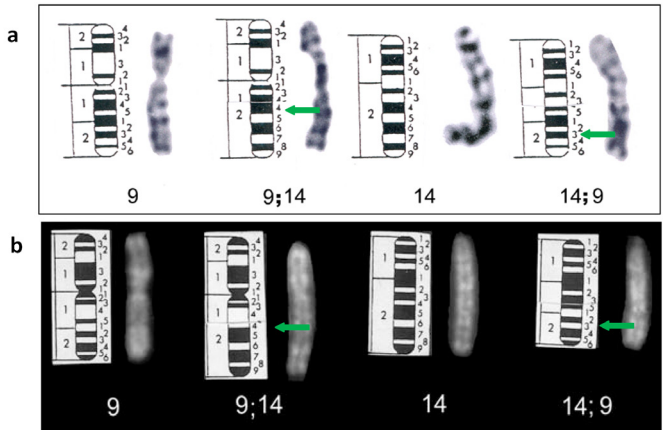

Figure 3. The breakpoints on chromosomes 9 and 14 of a boar carrying rcp(9;14)(q14;q23): the GTG technique (a); the RBA technique (b) (Rejduch et al., 2003 c)

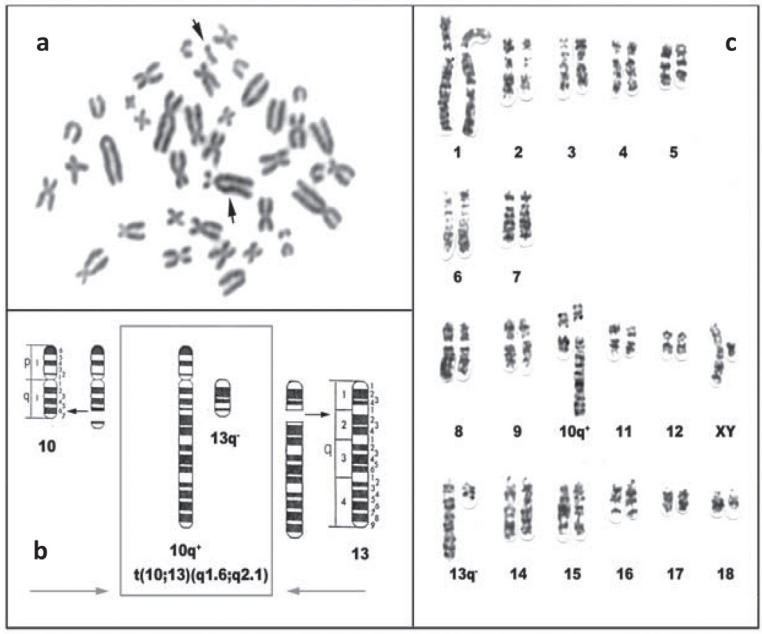

Figure 4. Metaphase spreads of a boar with rcp(10;13)(q16;q21) – conventional Giemsa staining (a); G-banded ideograms of rearranged chromosomes (arrows indicate the chromosomes involved and translocation breakpoints, respectively) (b); high resolution GTG-banded (GTG-HRBT) karyotype (c) (Danielak-Czech and Słota, 2008 a)

Table 1. Structural chromosome aberrations in *Sus scrofa* diagnosed in Poland

Type of chromosome aberration	The case of diagnosed aberration	Diagnostic techniques	Decrease of the mean litter size (%)	Source
Inversion	inv(8)(p11;p12)	GTG; Ag-NOR; synaptonemal complexes analysis (LM)	not estimated	Świtoński, 1991
	inv(1)(p22;q11)	GTG and GTG-HRBT; RBA and RBA-HRBT (Figure 1); CBG; synaptonemal complexes analysis (LM and EM)	0	Danielak-Czech et al., 1996 a, b; Świtoński et al., 1998
Reciprocal translocation	t(7;13)(q13;q26)	GTG and GTG-HRBT; RBA and RBA-HRBT; QFQ; Ag-NOR; CBG; conventional meiotic chromosome analysis; synaptonemal complexes analysis (LM); FISH (Figure 7); PRINS	48	Danielak-Czech et al., 1994, 1996 c, 1997, 2006, 2013
	t(8;14)(p21;q25)	GTG and GTG-HRBT (Figure 2); RBA and RBA-HRBT; QFQ Ag-NOR; CBG; conventional meiotic chromosome analysis; synaptonemal complexes analysis (LM)	25	Danielak-Czech et al., 1994, 1997
	t(1;5)(q21;q21)	GTG and GTG-HRBT; RBA (Figure 1) and RBA-HRBT; QFQ Ag-NOR; CBG	not estimated	Danielak-Czech et al., 1994
	t(9;14)(q14;q23)	GTG (Figure 3); RBA (Figure 3); synaptonemal complexes analysis (EM) (Figure 6)	100	Rejduch et al., 2003 c, 2006
	t(10;13)(q16;q21)	GTG and GTG-HRBT (Figure 4); Ag-NOR; CBG	not estimated	Danielak-Czech and Słota, 2007, 2008 a
	t(1;6)(p13;q23)	GTG; FISH; sperm-FISH	20	Kociucka et al., 2014
Tandem fusion-translocation	der(14;17)(q29;q10)	GTG (Figure 5); CBG (Figure 5); Zoo-FISH	not estimated	Danielak-Czech and Słota, 2008 b; Danielak-Czech et al., 2010 a, b
Robertsonian translocation	rob(15;17)	GTG (Figure 2); synaptonemal complexes analysis (EM) (Figure 6); Zoo-FISH	not estimated	Rejduch et al., 2003 b, 2010 b; Wnuk et al., 2005

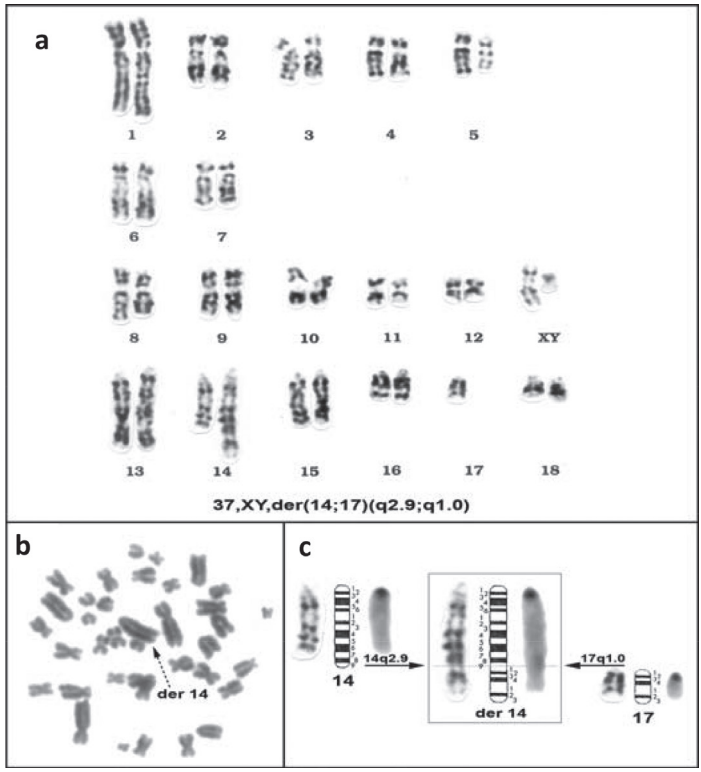

Figure 5. Metaphase chromosomes of a boar with tandem fusion der(14;17)(q29;q10): GTG-banded karyotype (a); conventional Giemsa staining (arrow indicates derivative chromosome 14) (b); G bands, C bands and ideograms of chromosomes involved in the tandem fusion (arrows indicate break-points) (c) (Danielak-Czech and Słota, 2008 b)

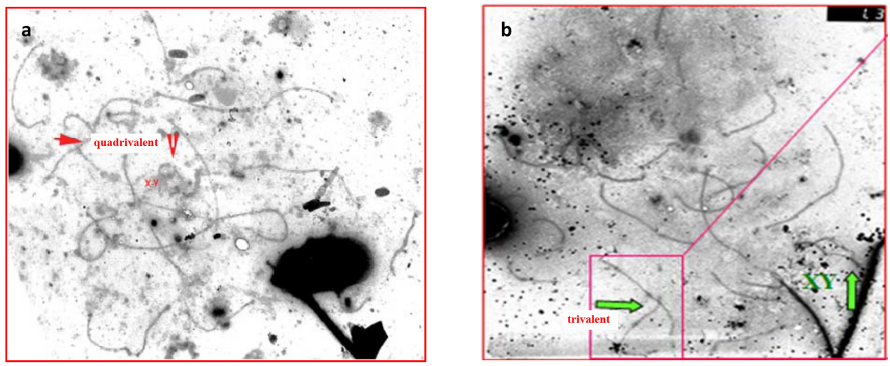

Figure 6. Synaptonemal complexes (EM) of the reciprocal translocation t(9;14)(q14;23) boar-carrier – arrows indicate quadrivalent 9;14 and X-Y bivalent (a) (Rejduch et al., 2003 c); Synaptonemal complexes (EM) of pairing chromosomes in the primary spermatocyte of the wild boar (15;17) translocation carrier (heterozygous form) – trivalent consisting of 15, 15;17 and 17 chromosome (b) (Rejduch et al., 2003 b)

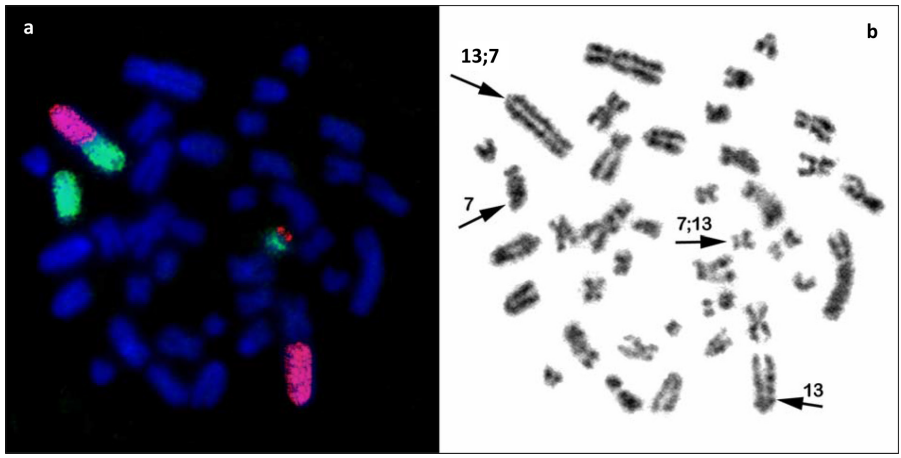

Figure 7. Hybridization signals on chromosomes of the reciprocal translocation t(7;13)(q13;q46) boar-carrier: dual-color chromosome painting by FISH technique with microdissected whole 7 and 13 chromosome probes (a); GTG-banded chromosomes (b) (Danielak-Czech et al., 2006)

Balanced reciprocal translocations constitute a serious breeding problem due to their hereditary nature and the resultant dramatic decrease (5 to 100%) in the number of piglets per litter. In turn, Robertsonian translocations or tandem fusions reduce fertility by 5–22%, whereas inversions, only in a few cases, slightly decrease reproductive efficiency (Raudsepp and Chowdhary, 2011). The direct cause of the poorer fertility parameters in the carriers of these karyotype defects is the disturbed course of gametogenesis caused by abnormal pairing and the segregation of untypical chromosome structures (tetravalent, trivalent, univalent instead of typical bivalents), which leads to the formation of gametes, and after their fertilization to the production of embryos with unbalanced karyotype, which are eliminated in the early stages of development (Gustavsson, 1990; Basrur and Stranzinger, 2008). Therefore, in the case of boars with chromosomal aberrations, classical cytogenetic diagnostics is expanded to include additional analytical procedures which enable the course of spermatogenesis in testicular tissues to be evaluated (Świtoński and Stranzinger, 1998; Villagomez and Pinton, 2008). These procedures involve analyzing the pairing process in pachytene primary spermatocytes during prophase of meiosis I, using light microscopic (LM) (Świtoński, 1991; Danielak-Czech et al. 1994, 1997; Barasc et al., 2012; Mary et al., 2014) or electron microscopic (EM) (Świtoński et al., 1998; Rejduch et al., 2006; Villagomez et al., 2008) observations of synaptonemal complexes (Figure 6) (Rejduch et al., 2003 b, c), as well as microscopic analysis of (Giemsa-stained) chromosome segregation at metaphase I and II during conventional evaluation of meiosis in spermatocytes (Danielak-Czech et al., 1994, 1997).

FISH and PRINS techniques in studies of structural chromosome rearrangements in somatic and germ cells

Over the last dozen years or so, molecular analysis using fluorescence *in situ* hybridization (FISH) (Pinkel et al., 1986) has been an essential diagnostic tool for balanced chromosome mutations (inversions, reciprocal and Robertsonian translocations, tandem fusions) (resolution 0.5–10 million base pairs) with molecular probes with different specificity levels (probes for whole chromosomes or their fragments, sub-telomere, telomere and centromere regions, and certain chromosome *loci*) (Rubeš et al., 2009; Raudsepp and Chowdhary, 2011). The karyotype rearrangements of pigs are most often studied using chromosome painting probes as well as probes obtained by cloning genomic DNA inserts from genomic libraries (cosmid probes with DNA inserts < 20–40 kb and bacterial probes with DNA insert sizes of 100–300 kb) (Fahrenkrug et al., 2001; Shizuya and Kouros-Mehr, 2001; Iannuzzi and Di Berardino, 2008; Rubeš et al., 2009). Chromosome-specific painting probes are generated by flow sorting of chromosomes (Telenius et al., 1992; Langford et al., 1993; Yerle et al., 1993), and through needle (Pinton et al., 2003) or laser (Kubicková et al., 2002) microdissection of chromosomes or their fragments and DOP-PCR or PARM-PCR amplification. These procedures gave rise to a set of painting probes for all *S. s. domestica* chromosomes. In addition, the microdissection technique allowed generating probes specific for certain chromosome arms or bands (Chaudhary et al., 1998). Oligonucleotide probes (primers) used in PRINS technique (Pellestor et al., 1995) are also available, which enable labeling telomere (TTAGG)n repeats (Gu et al., 1996) and centromere sequences, both in the set of acrocentric chromosomes (primer AC6) and sub-metacentric/metacentric chromosomes (primer SSCR2A) (Miller et al., 1993; Rogel-Gaillard et al., 1997), as well as in certain autosomal pairs (1, 9, 11, 14) and Y heterosome (Rubeš et al., 2009). Furthermore, the possibility of applying human genome probes in interspecific *in situ* hybridizations (Zoo-FISH) considerably broadens the scope of cytomolecular studies of the *Sus scrofa* species (Chowdhary et al., 1998). However, it should be emphasized that for accurate identification of complex or subchromosomal rearrangements (involving defining of breakpoints), species-specific FISH probes are always preferable (primarily, available flow sorted paints like in pigs) (Langford et al., 1993; Yerle et al., 1993), and ought to be applied to improve cytogenetic diagnostics of livestock.

The identification of over 20 chromosomal aberrations (at first based on analysis of G/R banding patterns) has been confirmed or verified using FISH with painting probes for flow-sorted chromosomes and PRINS with probes for centromere sequences and comprehensive cytomolecular diagnosis (banding techniques/FISH) for many new rearrangements was performed (Konfortova et al., 1995; Pinton et al., 1998, 2000; Ducos et al., 2002 a, 2008; Rubeš et al., 2009; Raudsepp and Chowdhary, 2011). The first attempt to paint chromosomes using the single-color FISH method clearly determined the chromosome fragments that had been rearranged in the case of t(7;15)(q24;q12) reciprocal translocation (Konfortova et al., 1995). The next experiment, using flow-sorted probes for dual-color painting of chromosomes, involved in eight different reciprocal translocations (initially diagnosed with GTG or RBA/RBG banding techniques) in 3 sows and 5 AI boars with reduced

fertility (30–43%) (Ducos et al., 1997 a, 1998), confirmed the exchange of very small fragments of chromosome pairs 6, 5 and 17 as well as breakpoints in six rearrangements: t(6;13)(p15;q41), t(13;17)(q41;q11), t(6;14)(q27;q21), t(3;5)(p13;q23), t(2;14)(q13;q27), t(15;17)(q13;q21), and verified diagnosis of the t(11;16)(p14;q14) translocation while demonstrating the corrected breakpoints in regions p12 and q12 (Pinton et al., 1998). In the case of reciprocal translocation with centromere repositioning – t(6;16)(q11;q11), hybridization with chromosome-specific painting probes for SSC6 and SSC16 failed to provide sufficient proof of the earlier identification, therefore diagnostic procedure included PRINS technique using oligonucleotide probes AC6 and SSCR2A for centromere sequences. This made it possible to determine breakpoints in the pericentromeric region 6q11 and in the centromeric region 16q11, and revealed the presence of SSC6-centromere linked to a part of SSC16-centromere in one of the rearranged chromosomes and only a portion of the centromere of chromosome 16 in the second rearranged, minute marker chromosome (Pinton et al., 1998). In another experiment, diagnosis of three reciprocal translocations: t(1;7)(q17;q26), t(1;6)(q17;q35), t(4;12)(p13;q13) in boars intended for reproduction or used in AI (with the average litter size decreased to 36%) was substantiated using GTG and RBG banding techniques and supplemented with molecular analysis by FISH with flow-sorted painting probes, thus correcting the previous hypotheses concerning chromosome breakpoints (Pinton et al., 2000). Similarly, GTG banding and dual-color chromosome painting techniques with flow-sorted probes were applied for cytomolecular identification of seven new reciprocal translocations revealed in 2 AI boars that showed decreased fertility (over 20%) and in 5 young boars that were cytogenetically tested prior to using AI (Ducos et al., 2002 a). In the case of five rearrangements: t(4;6)(q21;p14), t(7;8)(q24;p21), t(2;6)(p17;q27), t(5;8)(p11;p23), t(3;15)(q27;q13), the FISH technique allowed the detection of small changes in chromosomal material and pinpointed breakpoints, thus slightly modifying the characteristics of the aberrations suggested by the banding pattern, whereas in the case of two translocations: t(5;8)(p12;q21) and t(5;17)(p12;q13) it fully confirmed the rearranged karyotypes defined by G-banding analysis (Ducos et al., 2002 a). The identical diagnostic procedure was also used to characterize the next autosomal translocations, t(12;14)(q13;q21) (Pinton et al., 2005; Ducos et al., 2002 b), t(9;14)(p24;q27), t(1;6)(p22;q12) and (provisionally) translocation t(10;13) (Quach et al., 2009) in AI boars (showing fertility decreased to 26%, 39% and above 40%) as well as the first case in the pig – Y-autosome reciprocal translocation t(Y;14)(q11;q11) (Ducos et al., 2007; Pinton et al., 2008) in an azoospermic boar.

There are much fewer examples of using the FISH technique with pig-specific probes isolated by microdissection to analyze structural chromosome aberrations in the *Sus scrofa* species. The application potential of laser microdissection for probe production and for painting pig metaphase chromosomes was documented through identification of reciprocal translocation t(7;18) (Musilova et al., 2014). In turn, Danielak-Czech et al. (2006, 2013), who used FISH painting probes for chromosome pairs 7 and 13 obtained by manual microdissection of (G-banded) chromosomes (Figure 7) and human PRINS oligonucleotide telomere probe labeling interstitial (TTAGG)n sequences at the 7q13 breakpoint, have confirmed the diagnosis of re-

ciprocal translocation t(7;13)(q13;q46). It should be added that this rearrangement was identified previously by classical and high-resolution GTG/RBA-HRBT banding techniques and methods of the conventional analysis of meiotic chromosomes and synaptonemal complexes (Danielak-Czech et al., 1994, 1997). Complementary molecular analysis of the t(7;13)(q13;q46) translocation has conclusively confirmed the location of previously defined chromosome breakpoints that specify the size of rearranged fragments, which (in addition to the morphological type of chromosomes involved in the translocation) determines the scale of disturbances in chromosome pairing and segregation during gametogenesis, the frequency of produced gametes and embryos with unbalanced karyotype, the reduced number of piglets per litter, and the extent of fertility decline (determined to be 48% compared to herd fertility). Indirectly, this allows a conclusion that hypothetical financial loss from the use of one boar carrying this translocation (around 8 000 US dollars for natural mating and 162 000 US dollars for artificial insemination in the active population) was estimated correctly and provided sufficient justification for eliminating the carriers of this karyotype defect from reproduction (Danielak-Czech et al., 1996 c; Danielak-Czech and Słota, 2008 a).

An interesting example of using the FISH technique with painting probes specific for chromosome arms 4p and 4q (obtained by needle microdissection) is the precise diagnosis of pericentric inversion inv(4)(p14;q23) (Pinton et al., 2003) in boars and sows with normal fertility parameters. In this case, the aberration was identified previously by GTG/RBG banding and fluorescence *in situ* hybridization with a cosmid probe (BHT12) corresponding to telomere band 4p15 (beyond the inverted fragment) (Ducos et al.,1997 b).

Few attempts have also been made to identify structural chromosome rearrangements by FISH using species-specific probes cloned in bacterial artificial chromosome (BAC)-Vectors. Such analysis was performed on paracentric inversion inv(9)(p12;p22) detected *de novo* in intersex animals based on G-banding patterns (GTG technique). In that case proximal chromosomal breakpoint 9p12 was fine mapped using BAC probes corresponding to the microsatellite markers *SW2571* and *SW539* from the critical region potentially involved in the pig intersexuality (Pinton et al., 2002). The second example is the recently reported identification of the t(6;16)(p13;q23) reciprocal translocation (Kociucka et al., 2014) in a boar with lowered fertility (20%) and considerable perinatal mortality of piglets with congenital malformations (presumably with unbalanced karyotype). The FISH experiments in lymphocyte cells and gametes (sperm-FISH) were performed using 3 differently labeled BAC probes (CHORI-242 Porcine Genomic BAC Library) (http://bacpac.chori.org/porcine242.htm) flanking chromosome breakpoints as well as a telomere probe. The investigation confirmed the preliminary diagnosis (based on G bands) and made it possible to analyze meiotic segregation and estimate the frequency (%) of genetically unbalanced spermatozoa in the boar carrying this translocation. A particularly high frequency of adjacent-I meiotic segregation (41.9%) was found, which generally results in the production of chromosomally unbalanced gametes and smaller litters (Kociucka et al., 2014).

Other examples of using FISH in germ cells are the analysis of synaptonemal complexes and meiotic segregation in spermatocytes (for the assessment of the interchromosomal effect and inter- or intra-individual variation of segregation patterns with regard to fertility) as well as the frequency (%) of genetically unbalanced spermatozoa in male-carriers of chromosomal rearrangements: reciprocal translocations t(Y;1) (Barasc et al., 2012), t(Y;14)(q11;q11) (Pinton et al., 2008), t(3;15)(q27;q13) (47.83%) (Pinton et al., 2004; 2005; Massip et al., 2008; Bonnet-Garnier et al., 2009), t(12;14)(q13;q21) (24.33%) (Pinton et al., 2004; 2005; Bonnet-Garnier et al., 2009), centric fusion t(13;17) (2.96%-3.83%) (Pinton et al., 2009), pericentric inversions inv(4)(p14;q23) (4.08%) (Massip et al., 2010), inv(2)(p11;q11) (0.62%), inv(2)(p11;q21) (1.30%), inv(1)(p21;q210) (3.05%), inv(1)(p24;q29) (1.27%) and paracentric inversions: inv(2)(q13;q25) (4.12%), inv(1)(q12;q24) (0.84%) (Massip et al., 2009). These studies, broadened by analysis of sperm nuclear organization (3D-spermFISH) in t(13;17) carrier (Acloque et al., 2013), made use of painting probes isolated by microdissection (SSC4p, SSC15, SSCX) (Pinton et al., 2005; Massip et al., 2010) or flow sorting (SSC1, SSC3, SSC10, SSC11, SSC12, SSC13, SSC14, SSC17, SSC18, SSCX, SSCY) (Pinton et al., 2005, 2009; Bonnet-Garnier et al., 2009; Acloque et al., 2013) as well as probes specific for gene, sub-telomere and microsatellite sequences cloned in BAC vectors (Pinton et al., 2004; Massip et al., 2008, 2009, 2010) or centromere and telomere oligonucleotide probes (Pinton et al., 2008; Acloque et al., 2013). The FISH technique, along with the same probes, was also used to investigate meiotic chromosomes in females with chromosome abnormalities, which aimed to determine the frequency of aneuploidies (%) in *in vitro* maturing metaphase II oocytes in sows carrying two reciprocal translocations t(3;15)(q27;q13) (28.6%), t(12;14)(q13;q21) (38.5%) (Pinton et al., 2005), centric fusion t(13;17) (28.91%) (Pinton et al., 2009) and inversion inv(4)(p14;q23) (3.69%) (Massip et al., 2010). In addition, cytomolecular comparative analysis of male and female meiotic segregation profiles, performed using the example of these karyotype defects, made it possible to evaluate the effect of sex on meiotic segregation of identical structural chromosomal rearrangements (such as reciprocal and Robertsonian translocations) as well as peri- and paracentric inversions (Pinton et al., 2005, 2009; Massip et al., 2010).

To date, owing to the fact that pig flow karyotype is available (Langford et al., 1993; Yerle et al., 1993), interspecific *in situ* hybridization technique (Zoo-FISH) has been rarely used for the identification of chromosomal mutations in *Sus scrofa*. This type of experiment was performed using 3 differently labeled human painting probes (for autosome pairs 10, 12 and 20) to confirm the diagnosis of tandem fusion-translocation der(14;17)(14q29;17q10) in AI boar (the first case of tandem fusion in pigs), which had been identified earlier using GTG high-resolution banding technique (Figure 5) (Danielak-Czech and Słota, 2008 b). The fluorescent signals observed in homologous chromosome regions: SSC14q22→tel and 10q, SSC14q14→16 and SSC5p14→qtel, SSC17q10→tel and SSC17 evidenced autosome fusion and pinpointed the localization of the preliminarily determined breakpoints in the telomere and centromere region, characteristic for tandem fusions (Danielak-Czech et al., 2010 a, b). A similar diagnostic approach was used for Robertsonian

translocation 15;17 in European wild boar with karyotype 37,XY,rob(15;17) (Rejduch et al., 2010 b), in which rearranged chromosomes were identified using 3 differently labeled human painting probes for chromosome pairs 2, 8 and 20, according to the initial diagnosis based on G banding (Figure 2) and the analysis of synaptonemal complexes (Figure 6) (Rejduch et al., 2003 b). The described experiments, similar to the results obtained in the other species (e.g. cattle) (Di Meo et al., 2000; Iannuzzi et al., 2001), confirmed chromosome homology and genetic conservatism of the human and animal genomes (Hayes, 1995; Rettenberger et al., 1995; Chowdhary et al., 1998; Frönicke and Wienberg, 2001), which determines the suitability of human painting probes and the FISH technique for diagnosis of translocations and other chromosomal rearrangements. Nevertheless, animal chromosome- and species-specific painting probes (most of all available flow sorted ones, especially commercial) (Doležel et al., 2012) may simplify and accelerate the karyotype analysis, and should be the standard for clinical molecular cytogenetics.

A summary of numerous publications concerning veterinary cytogenetics shows that both classical banding techniques and *in situ* hybridization methods are essential components of genome studies in farm animals. In addition to karyotype control and diagnosis of chromosomal rearrangements (Rezácová et al., 2003; Słota et al., 2003, 2004 b) it also includes defining interspecific homology and syntenic conservation of chromosome fragments (Goureau et al., 1996; Chowdhary et al., 1998; Kozubska-Sobocińska et al., 2008), physical localization of genes (Danielak-Czech et al., 2014; Kozubska-Sobocińska et al., 2014), microsatellite sequences and gene constructs (Słota et al., 1996, 2004 a). Moreover, this cytomolecular approach is the basis for the generation of cytogenetic and integrated species maps and interspecific comparative maps (Fahrenkrug et al., 2001; Di Meo et al., 2007; Rubeš et al., 2009; Raudsepp and Chowdhary, 2011).

It should be emphasized that the continually improving cytogenetic techniques, complemented with elements of molecular genetics, enable very accurate and reliable analysis of karyotype analysis, whereas normal karyotype is one of the main requirements to qualify the boars for reproductive purposes. Moreover, cytogenetic screening allows the detection and early elimination from breeding animals with heritable chromosomal mutations, which cause developmental abnormalities and considerably reduce fertility and productivity parameters in breeding herds, resulting in significant financial losses. For this reason, many years ago (e.g. more than 40 years ago in France, and 8 years ago in Poland) some European and North American countries have implemented pig karyotype control systems, particularly for AI boars (Danielak-Czech and Słota, 2008 a, b; Ducos et al., 2008). However, because potential new chromosomal aberrations may emerge ("*de novo*") and rapidly spread in breeding populations, especially when artificial insemination is used, cytogenetic control of each boar-candidate for sire is needed prior to its first AI service. Hence for some time, cytogenetic monitoring covered also some young prospective breeding boars (whereby 13 structural aberrations, among them 8 reciprocal translocations, were identified in France between 1995 and 2001) (Ducos et al., 2002, 2007). In France, the analyses in pigs are not mandatory except for the animals issued from litters smaller than 6 piglets (male genetic types) or 7 piglets (female genetic types)

(Ducos et al., 2002, 2007). The intensified control program involving young boars resulted in 67 new chromosomal rearrangements (including 56 reciprocal translocations) being detected in a short period (2002–2006) in the French population of 7700 putative sires, which means that one boar out of 200 waiting to be used in AI station carries a heritable structural karyotype defect with significant impact on fertility (Ducos et al., 2007). Furthermore, based on the extensive screening studies performed in France (which revealed a further 44 balanced mutations, including 40 reciprocal translocations in the period 2007–2010), the prevalence of balanced chromosomal mutations in a group of young boars was estimated to be 0.47% (Ducos et al., 2007; Pinton et al., 2012). This frequency corresponds to the similar incidence (0.46%) calculated for the population of young boars covered by cytogenetic monitoring in Poland (Danielak-Czech and Słota, 2008).

In summary, cytogenetic diagnostics provides unique tools to identify hidden balanced mutations responsible for low fertility of phenotypically normal animals, while precise cytomolecular analysis of such rearrangements in germ cells enables to accurately predict their impact on carrier reproductive efficiency. In turn, the intensification of chromosomal control of pig populations, combined with the selection of the affected reproducers, has a substantial effect on breeding and economic success. Overall, it may be considered that cytogenetics is still a useful biotechnology to be applied in the genetic improvement of pigs.

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