Influence of sex on the meiotic segregation of a t(13;17) Robertsonian translocation: a case study in the pig

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BACKGROUND: Comparison of male versus female meiotic segregation patterns for Robertsonian translocation (RT) carriers with similar genetic background has rarely been reported in mammalian species.

METHODS: The aim of this study was to compare the segregation patterns determined for related males and females carrying a 13;17 RT in an animal model (Sus scrofa domestica L.), using dual colour fluorescence in situ hybridization on decondensed sperm nuclei and metaphases II of in vitro-matured oocytes.

RESULTS: In males, no association between the trivalent and the XY body was observed in any of the 90 pachytene nuclei studied, and the rate of unbalanced spermatozoa ranged between 2.96% and 3.83%. Female meiotic segregation analyses were carried out on 83 metaphase II oocytes. The rate of unbalanced gametes was higher in females than in males (28.91% versus 3.21%, P = 0.001). This difference was due to higher rates of diploid gametes (12.04% versus 0.05%) and unbalanced gametes produced by the adjacent segregation (16.86% versus 3.16%).

CONCLUSIONS: This study is a new scientific contribution to the comparison of segregation patterns in related males and females carrying an identical chromosomal rearrangement. It allows a better understanding of the meiotic behaviour of RTs. It also clearly illustrates the relevance of swine as an animal model for such meiotic studies.

Key words: chromosome pairing / meiosis / pig / Robertsonian translocation / segregation

Introduction

Robertsonian translocations (RTs) are among the most common constitutional structural chromosomal rearrangements in humans (Nielsen and Wohlert, 1991). They result from the centromeric fusion of two acrocentric chromosomes. Like other balanced chromosomal rearrangements, they generally have no effect on the carrier’s development and phenotype. However, these translocations can cause severe reproduction disorders.

In males, RTs can lead to spermatogenesis impairments and are frequently diagnosed in infertile patients (Martin, 2008). For instance, Mau-Holzmann (2005) reported that RTs were 15 times more frequent in an oligozoospermic group of patients than in the general population. The association of the trivalent (chromosomal configuration established during the prophase of the first meiotic division) with the XY body, transcriptionally inactive during prophase I, is generally responsible for these spermatogenesis defects (Gabriel-Robez and Rumpler, 1994; Solari, 1999). Several hypotheses have been put forward to explain the failure of meiosis as a result of this association. According to different authors, this phenomenon could be due to partial reactivation of the XY body leading to the expression of some genes located on the X chromosome (Lifschytz and Lindsley, 1972), or to spreading of the sex body (SB) inactivation towards the autosomal segments attached to the SB, without reactivation of the latter (Jaafar et al., 1993). More recent reports have provided evidence that unpaired regions, regardless of whether or not they pair with the sex chromosomes, are transcriptionally silenced during meiosis and could lead to meiotic arrest if genes critical for meiosis are present in these regions (Turner et al., 2005; Sciurano et al., 2007; Ferguson et al., 2008; Burgoyne et al., 2009).
In both sexes, RTs can also lead to the production of genetically unbalanced gametes (Roux et al., 2005). Such gametes (nullisomic or disomic for one of the chromosomes involved in the translocation) are the result of meiosis-I adjacent segregation mechanisms, and after fecundation, they give rise to monosomic or trisomic zygotes. Such chromosomal constitutions are generally not compatible with life (early embryonic losses) but some trisomic zygotes can survive to full-term pregnancies (stillbirths). During the last decade, meiotic segregations have been thoroughly studied in males using the sperm fluorescence in situ hybridization (SpermFISH) technique, allowing the analysis of large numbers of spermatozoa (Anton et al., 2007). These studies revealed a strong prevalence of the alternate segregation mode, leading to a majority of normal and balanced gametes (from 60% to 93%—Anton et al., 2007). On the other hand, for obvious ethical reasons, very few studies report female meiotic segregation results. The only available data come from the development of PGD procedures (analysis of first polar bodies or biopsied blastomeres) and generally concern limited numbers of oocytes originating from unrelated individuals (Munne et al., 2000; Durban et al., 2001; Pujol et al., 2003). From these studies, it has been observed that the estimated rates of unbalanced gametes were generally higher in females than in males. These data clearly show the necessity of cytogenetic screening of infertile couples involved in assisted reproduction programmes, ICSI.

The livestock cytogenetic screening programme carried out in France (Ducos et al., 2008), resulted in the identification of a new case of t(13;17) RT in the pig species. We propose to use the pig as an animal model to bring new insights into the meiotic behaviour of RTs. In this paper, we report the analysis of the meiotic segregation of a t(13;17) RT in related individuals with the same genetic background: 4 males (1 father, 1 brother and 2 sons) and 10 females (daughters). Chromosome pairing during the pachytene stage of male meiosis was analysed by immunolocalization and FISH. Male and female meiotic segregation patterns were estimated and compared by analysing the chromosomal content of sperm nuclei (SpermFISH) and metaphases II of in vitro-matured oocytes.

Materials and Methods

Animals

Two boars (brothers) were recruited from the national systematic control programme of young pedigree boars destined for artificial insemination centres (Ducos, et al., 2008). Classical cytogenetic analysis (GTG banding) allowed the identification of an RT t(13;17) for these two related individuals. The boars’ semen parameters (concentration, motility and morphology) were normal. Experimental inseminations were carried out with one of these boars (‘index’ boar), to obtain male and female heterozygous offspring for the translocation (2 males and 10 females; Fig. 1). The females were slaughtered after they went into first heat, i.e. at 6–8 months of age. The first two boars (brothers) and their sons were slaughtered at 14–18 months and 6–8 months, respectively.
Preparation of the probes

Male and female meiotic segregation analyses were carried out using painting probes for chromosomes 13 (SSC13) and 17 (SSC17) generated from flow sorted chromosomes (Pinton et al., 1998). The probes were labelled with biotin (SSC13), and digoxigenin (SSC17). Biotin was revealed by Alexa 594-conjugated streptavidin (Molecular Probes, Eugene, OR, USA). Digoxigenin was revealed by a mouse anti-digoxigenin antibody (Roche Diagnostic, Meylan, France) and an Alexa 488-conjugated goat anti-mouse antibody (Molecular Probes).

Fluorescence immunostaining and FISH

These experiments were carried out according to Pinton et al., (2008). Briefly, immunolocalization of the synaptonemal complex proteins (SCP) 1 and 3, the centromeres and the modified histone γH2AX was performed on spermatocytes of the ‘index’ boar using primary antibodies at 1:100 dilution in phosphate-buffered saline (PBS) and a rabbit anti-SCP1 (Abcam, Cambridge, UK), a rabbit anti-SCP3 (Abcam), a mouse anti-γH2AX (Abcam) and a human anti-Centromere (Antibodies Incorporated, Davis, CA, USA). The secondary antibodies consisted of Alexa 594-conjugated donkey anti-rabbit immunoglobulin (Ig)G (Molecular Probes), Alexa 488-conjugated goat anti-mouse IgG (Molecular Probes) and AMCA-conjugated donkey anti-human IgG (Jackson ImmunoResearch Laboratories, Grove, PA, USA) used at 1:100. Slides were mounted with antifade solution (Vector Laboratories Inc., Burlingame, CA, USA). The capture and analysis of the surface spread spermatocytes were performed using the Cytovision FISH imaging system (Applied Imaging, Sunderland, UK).

After synaptonemal complex (SC) analysis, the same cells were subjected to FISH using the SSC17 painting probes in order to localize the trivalent accurately. FISH signals were captured and analysed on the same cells for which the SCs had been analysed previously.

SpermFISH

SpermFISH experiments were carried out on the first two boars as well as on two sons, according to Pinton et al., (2004). Spermatozoa decondensation was carried out according to the protocol developed by Hassanane et al., (1999), i.e. treatment with a dithiothreitol (DTT)/papain solution (1.25 g papain, Merck, plus 0.155 g DTT, Sigma, dissolved in 100 ml of 0.2 M Tris-buffer, pH 8.6) at room temperature. Optimal decondensation times were obtained between 3 and 5 min. Dual colour FISH experiments on sperm nuclei were carried out using whole chromosomes 13 and 17 painting probes (revealed by Alexa 594 and Alexa 488, respectively). The slides were observed under a Zeiss Axioskop microscope fitted with a triple bandpass filter and only sperm heads exhibiting high-intensity signals were scored.

In vitro maturation of oocytes

In vitro maturation of oocytes was carried out according to Pinton et al., (2005). The metaphase II oocytes were obtained after in vitro maturation of oocytes (Marchal et al., 2001). Briefly, the oocytes were removed from ovaries obtained from the slaughterhouse. The cumulus—oocyte complexes from follicles of 3–6 mm were initially washed four times in PBS + 0.5 mg/ml of bovine serum albumin (Sigma, St Louis, MO, USA) + 50 mg/ml of gentamycin (Sigma), then cultured in 500 ml of maturation medium composed of culture medium 199 (Sigma) supplemented with epidermal growth factor (10 ng/ml final; Sigma) and cysteamine (570 mmol/l final; Sigma). The duration of maturation was fixed at 44 h at 39°C in a 5% CO2 oven.

FISH experiments were carried out using the same set of probes as for the SpermFISH according to Pinton et al., (2005). Only the metaphase II oocytes showing clear and homogeneous hybridization signals were further considered in this study.

Statistical analyses

A conventional 2 × 2 χ2 test with the Yates correction (Dagnelie, 1975) was used to compare the proportions of the different segregation products between the individuals (between the four males, and between males and females) and to compare the disomy and nullisomy rates for chromosomes 13 and 17, respectively. Differences were considered to be significant when P < 0.05.

Results

Male meiotic pairing analysis by immunolocalization and FISH

SC analysis was carried out for 90 pachytene nuclei from the ‘index’ boar (Fig. 2). XY pair configurations (indicated by arrows) allow identification of the different pachytene substages (Solari, 1980; Villagomez, 1993; Codina-Pascual et al., 2005). Spermatocytes presented in Fig. 2a and a’ corresponded to early and late pachytene substages, respectively. No association between the trivalent and the XY body was observed, whatever the pachytene stage. The trivalents formed by the translocated and the two normal chromosomes 13 and 17 were observed as large SCs similar to those formed by large submetacentric chromosomes (Fig. 2b and b’). FISH experiments using SSC17 painting probes allowed them to be identified without ambiguity. Immunolocalization of the γH2AX protein revealed the presence of this modified histone only on the XY body. No signal was observed either on the trivalents analysed or on the autosomal bivalents (Fig. 2c and c’).

Meiotic segregation analysis in males

The hybridization rate was close to 100% in all cases, and a total of 2506, 2425, 3224 and 2633 sperm nuclei were scored for boar 1 (‘index’), boar 2 (son No.1), boar 3 (son No.2) and boar 4 (brother), respectively. Examples of the different meiotic products observed are presented in Fig. 3 and their relative proportions in Table I. The meiotic segregation patterns of the four boars were not significantly different (P= 0.44). Indeed, similar proportions of meiotic products originating from alternate segregation (normal or balanced—Fig. 3a and b) were observed between the four males analysed (96.97%, 96.16%, 96.90% and 97.04%). The rate of unbalanced meiotic products, resulting from the adjacent segregation modes (Fig. 3c–f) ranged from 2.96% to 3.83% (mean value 3.16%). The observed frequencies of nullisomy were significantly higher than those of disomies in the four carriers (P < 0.001 for chromosome 13 and P < 0.001 for chromosome 17). As diploid gametes could not be accurately differentiated from those originating from the 3:0 segregation, both meiotic products were pooled in a single group; ‘3:0 or diploid’. Their proportions were similar for each of the four boars (from 0% to 0.15%, mean 0.05%).

Meiotic segregation analysis in females

A total of 83 metaphase II oocytes taken from 10 related females were analysed by FISH after in vitro maturation and spreading. Some representative pictures of the main meiosis I products are presented in...
Fig. 4. The relative proportions of the different female segregation products are indicated in Table II. Fifty-nine (71.08%) of these oocytes were balanced, including 31 (37.34%) normal (Fig. 4a) and 28 (33.73%) ‘translocated’ (Fig. 4b), whereas 14 (16.86%) were unbalanced (Fig. 4c–f) and 10 (12.04%) diploids (Fig. 4g). Unlike the males, diploid gametes were identified unequivocally in the females.

Figure 2 Boar spermatocytes after immunolocalization and FISH experiments. (a and a’) Immunolocalization of SCP3, SCP1 (red) and centromeres (blue). (a) and (a’) correspond to an early and a late pachytene stage, respectively. XY chromosomes are indicated by arrows. (b and b’) Large pictures of the trivalent. The arrowhead indicates the centromeric region of the trivalent. (c and c’) Immunolocalization of SCP3, SCP1 and centromeres, modified histone γH2AX (yellow) and FISH with the whole chromosome 17 probe (green).

Figure 3 Examples of boar sperm nuclei of Rob t(13;17) heterozygous carriers after hybridization of the whole chromosome 13 (red) and 17 (green) painting probes. (a and b) Balanced sperm nuclei 13q/17q. (c) Nullisomic sperm nucleus for chromosome 13:17q. (d) Disomic sperm nucleus for chromosome 17:17q/der(13q;17q). (e) Nullisomic sperm nucleus for chromosome 17:13q. (f) Disomic sperm nucleus for chromosome 13:13q/der(13q;17q).
by the diploid number of chromosomes observed on the oocytes’ metaphases.

Among balanced gametes, the rates of normal and translocated oocytes were not statistically different ($P = 0.71$).

The observed frequencies of disomies were not significantly different from those of nullisomies for chromosomes 13 ($P = 0.27$) and 17 ($P = 1$).

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**Table I** Relative proportions (number, %) of the different meiotic products observed in the four related boars carriers of the Rob t(13;17)

<table>
<thead>
<tr>
<th>Segregation mode</th>
<th>Chromosomal constitution</th>
<th>In situ fluorescent phenotypes</th>
<th>Boar no. 1, number (%)</th>
<th>Boar no. 2, number (%)</th>
<th>Boar no. 3, number (%)</th>
<th>Boar no. 4, number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternate</td>
<td>13q/17q or der(13q;17q)</td>
<td>Normal</td>
<td>2430 (96.97)</td>
<td>2332 (96.16)</td>
<td>3124 (96.90)</td>
<td>2555 (97.04)</td>
</tr>
<tr>
<td>Adjacent</td>
<td>17q</td>
<td>Nullisomy 13</td>
<td>27 (1.08)</td>
<td>32 (1.32)</td>
<td>34 (1.05)</td>
<td>29 (1.10)</td>
</tr>
<tr>
<td>Adjacent</td>
<td>13q/der(13q;17q)</td>
<td>Disomy 13</td>
<td>11 (0.34)</td>
<td>18 (0.74)</td>
<td>26 (0.81)</td>
<td>10 (0.31)</td>
</tr>
<tr>
<td>Adjacent</td>
<td>13q</td>
<td>Nullisomy 17</td>
<td>24 (0.74)</td>
<td>25 (1.03)</td>
<td>22 (0.68)</td>
<td>25 (0.77)</td>
</tr>
<tr>
<td>Adjacent</td>
<td>17q/der(13q;17q)</td>
<td>Disomy 17</td>
<td>12 (0.37)</td>
<td>17 (0.70)</td>
<td>18 (0.56)</td>
<td>11 (0.34)</td>
</tr>
<tr>
<td>Total of adjacent products</td>
<td>76 (3.03)</td>
<td>93 (3.83)</td>
<td>100 (3.10)</td>
<td>78 (2.96)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3:0 or diploid</td>
<td>13q/17q/der(13q;17q) or 13q/13q/17q/17q</td>
<td>Diploidy</td>
<td>2 (0.10)</td>
<td>1 (0.05)</td>
<td>0 (0.00)</td>
<td>3 (0.15)</td>
</tr>
<tr>
<td>Total of unbalanced products</td>
<td>78 (3.11)</td>
<td>94 (3.87)</td>
<td>100 (3.10)</td>
<td>81 (3.07)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of spermatozoa scored</td>
<td>2506</td>
<td>2425</td>
<td>3224</td>
<td>2663</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Figure 4** Examples of metaphases II of sow oocytes after hybridization of the chromosome 13 (red) and 17 (green) painting probes. (a) Normal metaphase II and its corresponding first polar body with the der(13;17). (b) ‘Translocated’ metaphase II and its corresponding first polar body with the two normal chromosomes 13 and 17. (c) Unbalanced metaphase II with a chromosome 13 nullisomy. (d) Unbalanced metaphase II with a chromosome 13 disomy. (e) Unbalanced metaphase II with a chromosome 17 disomy. (f) Unbalanced metaphase II with a chromosome 17 nullisomy. (g) Diploid metaphase II.

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**Comparison between males and females**

As no inter-individual variation of the meiotic patterns was observed between the four boars analysed, the male data were pooled and compared with the female data (Table II).

The rate of balanced (normal and ‘translocated’) gametes was lower in females (71.08%) than in males (96.78%, on average). This difference was partly due to the higher rate of diploid gametes in
females (12.04%) compared with diploid or double disomic gametes in males (0.05% on average). Even if the diploid or 3:0 gametes were not considered, the rate of unbalanced gametes was significantly higher in females (19.18%) than in males (3.16%) ($P < 0.001$).

**Discussion**

**Immunostaining analysis of male meiotic pairings**

In the few studies carried out in fertile (normozoospermic) human males (Oliver-Bonet et al., 2005), the rate of association between the multivalent and the XY body was generally low. Conversely, variable degrees of association between the XY body and multivalents have been observed among subfertile patients (Gabriel-Robez and Rumpler, 1994). In the case of RTs, a correlation between the degree of XY body association and spermatogenesis problems has been observed (Johannisson et al., 1987; Johannisson et al., 1993; Gabriel-Robez and Rumpler, 1994). This association between the trivalent and the XY body seems to increase as pachytene progresses (Sciurano et al., 2007) and is inhibited by the heterosynapsis between the free arms of the trivalent (Gabriel-Robez and Rumpler, 1994; Solari, 1999). More recently, it has been shown that heterosynapsed chromosome regions lose the variant histone $\gamma$H2AX (Turner et al., 2005; Turner et al., 2006; Sciurano et al., 2007).

In our case, no association of the trivalent with the XY body was observed in any of the 90 spermatocytes analysed, whatever the pachytene substage. This concurs with the normal semen parameters observed in the boars studied. This result is also consistent with that of Shan et al. (1994) who studied the same rearrangement and reported heterosynapsis of the two short arms of the trivalent.

This could be explained by the particular nature of the swine acrocentric chromosomes, presenting very tiny short arms when compared with human acrocentrics (Villagomez et al., 1993). However, this phenomenon of early heterosynapsis, preventing the association between the multivalent and the XY body, has been observed in other cases of reciprocal translocations in pigs (Gabriel-Robez et al., 1988; Jaafar et al., 1989; Jaafar et al., 1992; Villagomez et al., 1993) and seems to be a particular feature of swine male meiosis (Villagomez and Pinton, 2008).

Overall, the currently available meiotic pairing data (showing that the association between the multivalent and the XY body is frequent in humans, whereas it has rarely been rarely reported in pigs—Villagomez et al., 1995) suggest that there are major differences in the meiotic behaviour of chromosomal rearrangements between the two species. However, this conclusion should be considered with caution, for the following reasons. First, when compared with humans, the number of studies carried out in pigs still remains very limited, and conclusions should thus be drawn carefully. Second, the majority of pig studies carried out so far involved fertile (normozoospermic) individuals. Further studies in boars exhibiting abnormal semen parameters (azoospermia, oligozoospermia etc.) should be carried out to clearly validate this ‘between species difference’ hypothesis.

**Meiotic segregation in males**

In humans, RTs mainly involve chromosomes 13 and 14, on the one hand, and chromosomes 14 and 21, on the other hand. Rob t(13;14) is the most frequent translocation in the general population (0.7/100—Roux et al., 2005). The development of heterspecific fertilization and SpermFISH procedures allowed analysis of the meiotic segregation in males carrying RTs. The results obtained indicated that balanced sperm nuclei were significantly more frequent than

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**Table II** Distribution of the different meiotic segregation products for male and female swine heterozygous carriers of the Rob t(13;17)

<table>
<thead>
<tr>
<th>Segregation mode</th>
<th>Chromosomal constitution</th>
<th>In situ fluorescent phenotypes</th>
<th>Sows, number (%)</th>
<th>Sows, corrected rate (without diploids) in %</th>
<th>Boars (pooled data), number (%)</th>
<th>Boars, corrected rate (without diploids) in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternate</td>
<td>13q/17q</td>
<td>Normal</td>
<td>31 (37.34)</td>
<td>42.46</td>
<td>10 441 (96.78)</td>
<td>96.83</td>
</tr>
<tr>
<td>Alternate</td>
<td>der(13q;17q)</td>
<td>Translocated</td>
<td>28 (33.73)</td>
<td>38.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total of alternate products</td>
<td></td>
<td></td>
<td>59 (71.08)</td>
<td>80.82</td>
<td>10 441 (96.78)</td>
<td>96.83</td>
</tr>
<tr>
<td>Adjacent</td>
<td>17q</td>
<td>Nullisomy 13</td>
<td>2 (2.40)</td>
<td>2.74</td>
<td>122 (1.13)</td>
<td>1.13</td>
</tr>
<tr>
<td>Adjacent</td>
<td>13q/der(13q;17q)</td>
<td>Disomy 13</td>
<td>6 (7.22)</td>
<td>8.22</td>
<td>65 (0.60)</td>
<td>0.60</td>
</tr>
<tr>
<td>Adjacent</td>
<td>13q</td>
<td>Nullisomy 17</td>
<td>2 (2.40)</td>
<td>2.74</td>
<td>96 (0.88)</td>
<td>0.89</td>
</tr>
<tr>
<td>Adjacent</td>
<td>17q/der(13q;17q)</td>
<td>Disomy 17</td>
<td>3 (3.61)</td>
<td>4.11</td>
<td>58 (0.53)</td>
<td>0.53</td>
</tr>
<tr>
<td>Total of adjacent products</td>
<td></td>
<td></td>
<td>14 (16.86)</td>
<td>19.18</td>
<td>341 (3.16)</td>
<td>3.16</td>
</tr>
<tr>
<td>3:0 or diploid</td>
<td>13q/17q/der(13q;17q) or 13q/13q;17q/17q</td>
<td>Diploidy</td>
<td>10 (12.04)</td>
<td>6 (0.05)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total of unbalanced products</td>
<td></td>
<td></td>
<td>24 (28.91)</td>
<td>19.18</td>
<td>347 (3.21)</td>
<td>3.16</td>
</tr>
<tr>
<td>Total number of gametes scored</td>
<td></td>
<td></td>
<td>83</td>
<td>73</td>
<td>10 788</td>
<td>10 782</td>
</tr>
</tbody>
</table>
unbalanced ones for the two translocations previously mentioned, but also for other uncommon translocations (Pellestor, 1990; Rives et al., 2005; Moradkhani et al., 2006a, b). Our results, showing a preponderance of alternate segregation products, are in accordance with human data. Previous studies have shown that this preponderance does not seem to be influenced by the nature of the acrocentrics involved in the translocation, but more by the configuration adopted by the chromosomes during the prophase of the first meiotic division (Vidal et al., 1982; Luciani et al., 1984; Navarro et al., 1991).

For the 13/14 translocation, which has been thoroughly documented in humans, we can observe a variation in the segregation profiles between the different patients studied. Indeed, the rates of normal/balanced spermatozoa reported by the authors ranged from 73.6% to 91%, whereas the rates of unbalanced gametes originating from adjacent segregation ranged between 9% and 23.3% (Roux et al., 2005; Ogur et al., 2006; Chen et al., 2007). Some authors suggested that the different technical approaches carried out (type of probes, scoring criteria etc.) could be responsible for the discrepancies observed between one study and another (Anton et al., 2004). However, these analyses were mainly carried out in individuals that were not related and we cannot exclude the influence of the genetic background on the meiotic segregation process of RT carriers. Our experiments, carried out on four closely related boars, allowed analysis of the meiotic segregation patterns of individuals having quite similar genetic backgrounds. Our results revealed a lack of interindividual variation in the meiotic segregation of this RT, as similar meiotic profiles were observed in the four boars studied.

Among the unbalanced meiotic products, a deviation from the theoretical 1:1 ratio between disomic and nullisomic spermatozoa has frequently been observed in humans. Indeed, excess of nullisomic spermatozoa compared with disomic has been reported on many occasions. Similar results were obtained for the four boars analysed in this study. Different hypotheses have been proposed to explain such results. The first concerns the technical approach and suggests that hybridization failures could be responsible for an overestimation of the proportion of nullisomic meiotic products (Rousseaux et al., 1995; Morel et al., 2001; Anton et al., 2004). The possible intervention of a meiotic checkpoint has been suggested as a second explanation (Honda et al., 2000; Anton et al., 2004). This checkpoint could explain a preferential cell maturation arrest of the disomic cells. However, the normal sperm concentrations observed in the boars analysed in this study would seem to discredit this second hypothesis.

**Meiotic segregation in females**

In humans, female meiotic segregation of RTs has been studied thanks to the first polar body analysis of ~10 cases of the Rob t(13;14) and 5 cases of Rob t(14;21) (Munne et al., 2000; Durban et al., 2001; Pujo et al., 2003). These studies revealed a high rate of unbalanced gametes produced [32% and 42% for the Rob t(13;14) and Rob t(14;21), respectively], with a wide variation from 7.6% to 52.3% (Munne et al., 2000). Moreover, an excess of normal versus translocated oocytes has been observed among the alternate segregation products, suggesting a transmission ratio distortion in favour of the normal chromosomes. Other studies, carried out on offspring of RT carriers, found the opposite result, i.e. preferential transmission of the translocated chromosomes (Pardo-Manuel de Villena and Sapienza, 2001).

Studies in mice also showed differences in the transmission rate of the translocated chromosomes. They revealed a preferential segregation of the translocated chromosomes to the polar body, leading to a prevalence of progeny with normal chromosomes (Aranha and Martin-DeLeon, 1994; Underkoffler et al., 2005; Schulz et al., 2006).

In our case, we found no transmission ratio distortion in the alternate products of the 13/17 translocation, results that are similar to those obtained in cows carrying Rob t(1;29) (Bonnet-Garnier et al., 2008).

Moreover, we did not observe any premature separation of sister chromatids (PSSCs) (Angell, 1991). It has been suggested that the age of the female can influence the occurrence of PSSCs (Pujo et al., 2003). In our case, we can hypothesize that the animals analysed were too young to observe PSSCs.

Finally, the rate of diploid oocytes identified (12.04%) is consistent with earlier results obtained in sows with normal karyotypes (from 12.8% to 27.7%) (Vozdova et al., 2001; Sosnowski et al., 2003; Lechniak et al., 2007).

**Comparison between male and female meiotic segregation patterns**

If we still consider the Rob t(13;14) in humans, meiotic segregation patterns have been studied for ~30 males and 10 females to date. The rate of unbalanced gametes originating from adjacent segregation ranged from 9% to 23.3% in males, and from 8% to 60% in females (Munne et al., 2000; Roux et al., 2005; Ogur et al., 2006; Chen et al., 2007). Similar results were obtained for the Rob t(14;21). These results clearly showed a higher rate of unbalanced gametes (diploids, as well as unbalanced gametes produced by the adjacent segregation) in females than in males. In the case of the Rob t(13;17) analysed in this study in swine, we also observed a higher rate of unbalanced gametes in females than in males. This difference is mainly due to the higher rate of diploid gametes produced in females when compared with males (12.04% versus 0.05%), but also to unbalanced gametes produced by the adjacent segregation (16.86% versus 3.16% on average).

Several hypotheses have been proposed to explain such differences between the two sexes.

The first is that a strong selective disadvantage affects the unbalanced products in the male, but not in the female meiosis (Munne et al., 2000). Indeed, it has been postulated that specific trivalent configurations that are more prone to produce an unbalanced segregation are also those that preferably induce an association of the trivalent with the XY body, which results in germ cell failure. However, this has not been confirmed in recent human studies (Sciurano et al., 2007). This hypothesis is not relevant in our study where no association between the trivalent and the XY body and normal sperm concentrations were observed in the males.

The second hypothesis, also formulated by Munne et al. (2000), is that there are more unbalanced gametes produced in females than males for the same reasons that there are more aneuploid gametes produced in female than in male non-carriers. Indeed, it now seems well established that female meiosis is more prone to meiotic errors due to less efficient pachytene and spindle assembly checkpoints (Roeder and Bailis, 2000; Eaker et al., 2001; Hunt and Hassold, 2002; Morelli and Cohen, 2005; Burgoyne et al., 2009).
Finally, earlier results in swine showed that the recombination rate of the female is greater than that of the male in most of the chromosomal regions (Archibald et al., 1995; Marklund et al., 1996; Rohrer et al., 1996; Mikawa et al., 1999; Guo et al., 2009). In the most recent study by Guo et al. (2009), the average female:male recombination ratio for autosomes was 1.34:1, i.e. slightly lower than that estimated in humans (between 1.6:1. Kauppi et al., 2004 and 2:1. Li et al., 1998). Such differences between the two sexes could explain the observed meiotic segregation differences between males and females in the case of structural chromosomal rearrangements (discussed in Pinton et al., 2005 for reciprocal translocations). Direct analyses of the frequency and localization of chiasmata (crossing-over) using immunolocalization of MLH1 foci (e.g. Sun et al., 2006) may help to better document this point and should be considered in future studies. However, carrying out such analyses in swine will require important technical/methodological adjustments, especially for females.

In conclusion, the analysis of human meiosis, in the case of constitutional chromosomal rearrangements for instance, is strongly constrained by the difficulty of collecting the necessary biological material. Although access to male samples (sperm and testicular biopsies) is generally possible, the situation is much more critical in females. Otherwise, it is generally very difficult or even impossible in humans to get data on more than one individual carrying the same rearrangement. The comparison of male versus female segregation patterns for the same rearrangement for individuals of the same genetic background, for instance, is almost impossible. Moreover, it should be pointed out that most if not all of the individuals undergoing gamete testing in human studies will have experienced reproductive difficulty and thus the data from their gametes may not necessarily be applicable to the larger number of carriers with apparently normal fertility.

The recourse to animal models allows these limitations to be overcome. For instance, we think that the choice of a fertile male in our study aiming at assessing the effect of sex on the meiotic segregation of an RT was relevant, because it avoids the bias that would have occurred with an infertile male. The laboratory mouse, intensively used as a model by geneticists, is not so relevant for cytogeneticists, due to its karyotypic structure very different from that of human. In this respect, swine seems to be a much better model than mouse. Indeed, not only does the karyotypic structure of swine resemble the human structure (except for the specific features of the acrocentrics mentioned above), but the females of this species are relatively prolific and the generation interval is relatively short (~2 years), which makes the experimental production of individuals with particular karyotypes possible at reasonable expense. The present paper, as well as the one published earlier by the same group (Pinton et al., 2005) dealing with reciprocal translocations, shows that extensive studies can be carried out in this species, allowing us to improve our knowledge concerning the meiotic behaviour of constitutional chromosomal rearrangements in mammalian species.

### References


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