

# Effects of chromosomal abnormalities on sperm



## nuclear architecture



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### Context

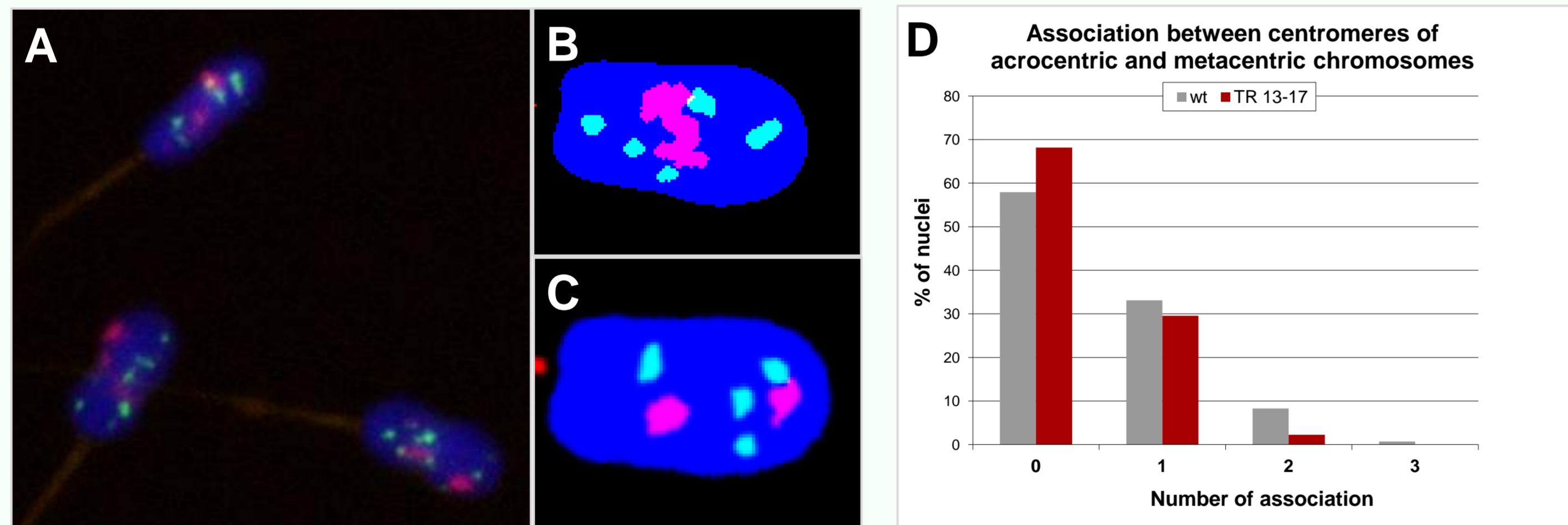
Nuclear organization in sperm has been proposed to be linked with gene expression and function after fertilization. Chromosomal territories are not randomly positioned in mammalian sperm nuclei (Foster et al. 2005) and chromatin packaging differs, with histone retention and specific histone marks at loci of developmental importance (Hammoud et al. 2009). Moreover sperm nuclear organization is altered in infertile patients suggesting a real pressure for the maintenance of such architecture for normal spermatogenesis and further embryonic development.

### Objectives

Chromosomal abnormalities have been described in patients with fertility problems. Production of genetically imbalanced gametes, meiotic silencing of unsynapsed chromatin and Y microdeletions have been proposed to be the main causes of these etiologies. However global nuclear organization has been poorly studied in such cases. In order to check whether chromosomal abnormalities may affect nuclear organization in sperm, we compared centromeres, telomeres, sexual chromosomes and 13/17 autosomes localization in sperm of normal pig and t(13;17)Robertsonian translocation carriers.

### Results

#### ➤ Centromeres of acrocentric and metacentric chromosomes do not cluster together



3D-SpermFISH experiments were performed with centromeric probes AC6 (acrocentric chromosomes, red) and SSCSR2A (metacentric chromosomes, green) probes

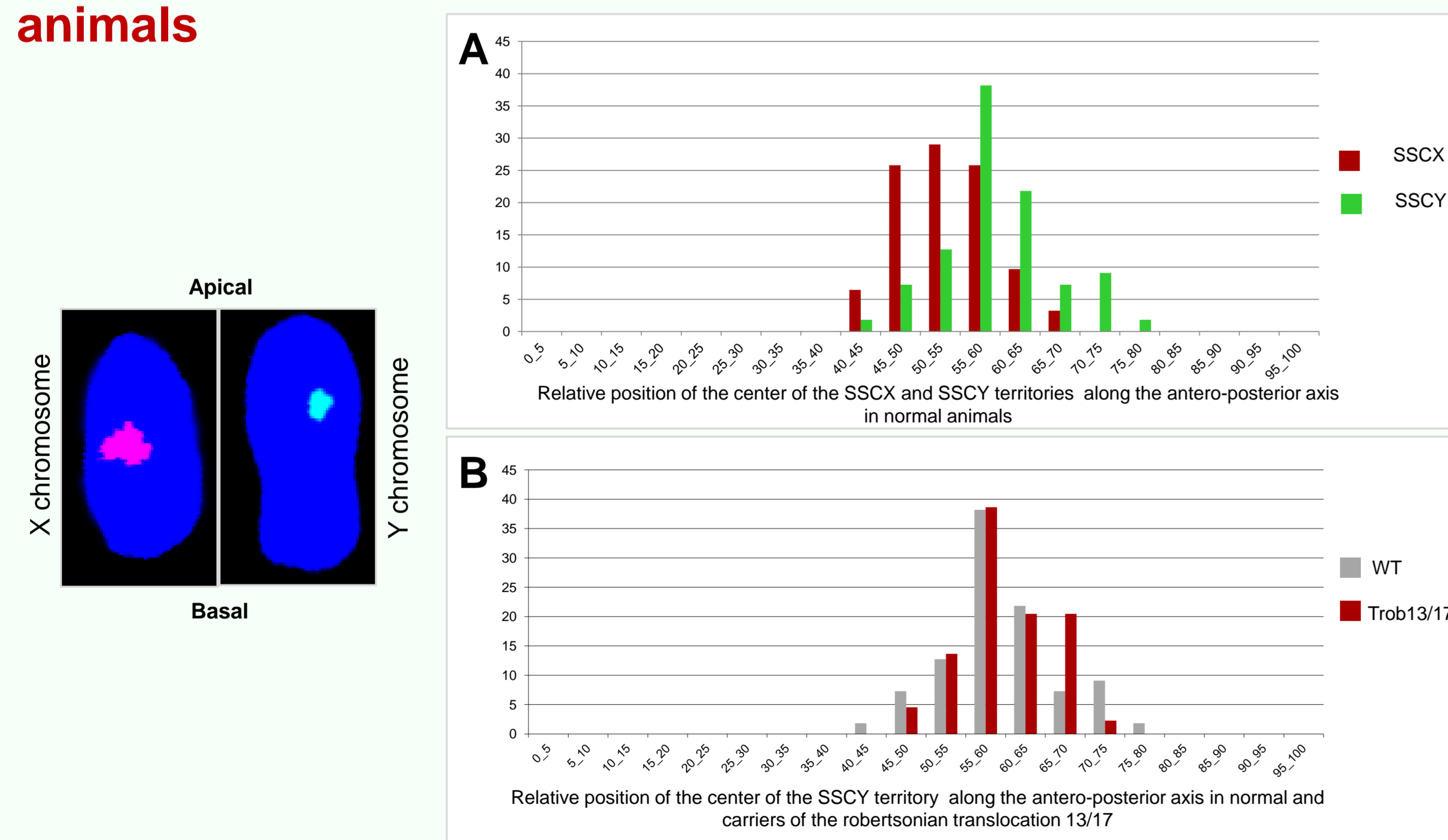
A: average projection of a confocal Z-stack acquisition

B-C: Segmented clusters of centromeres (using Nemo software)

D: Quantification of association between SSCSR2A and AC6 segmented objects.

**Centromeres of acrocentric and metacentric chromosomes respectively cluster together.** We observed a mean of 2.2 centromeres/cluster for metacentric chromosomes (5.9 clusters for 13 metacentric chromosomes) and 3.6 centromeres/cluster for acrocentric chromosomes (1.7 clusters for 6 acrocentric chromosomes) in normal animals. **However, in more than 60% of the nuclei, centromeres from acrocentric and metacentric chromosomes do not associate together.**

#### ➤ Sexual chromosome localization is unchanged in Trob(13;17) animals



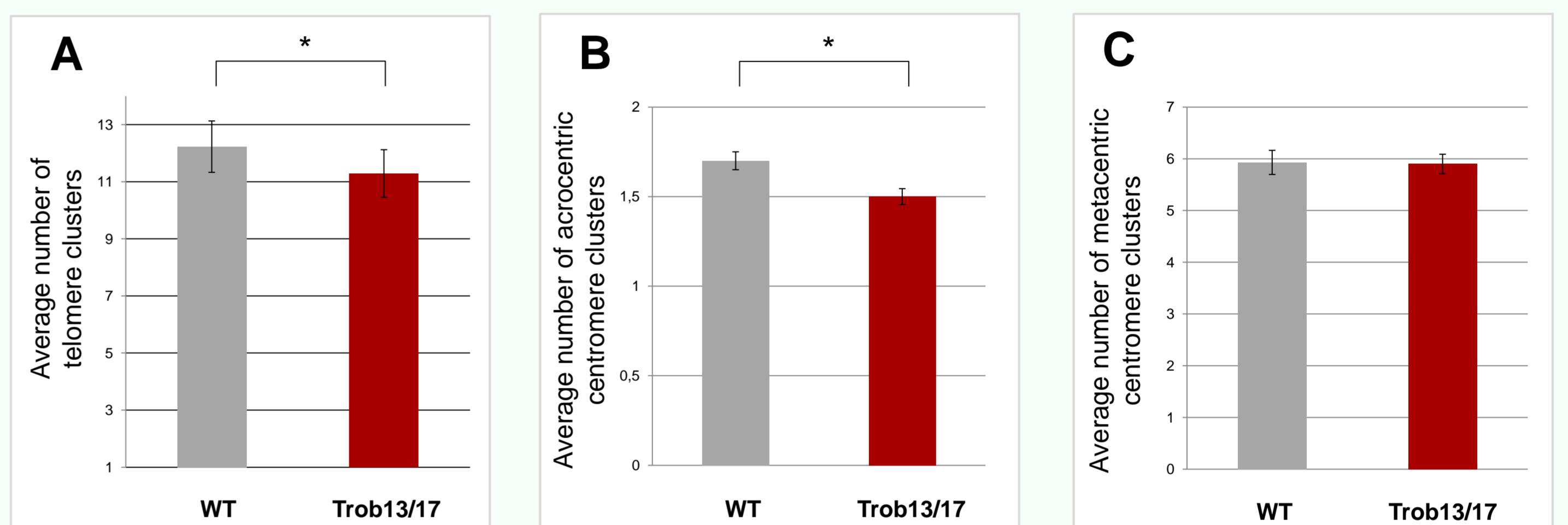
3D-SpermFISH experiments were performed either with SSCY (green) or SSCX (red) paints.

Position of chromosomal territories (CT) was calculated relatively to the apico-basal axis.

A. The X territory is located in the median part of the nuclei as the Y is more apical but never at the tip.

B. No significative differences were seen between normal and translocated animals for the Y position.

#### ➤ Less centromere (from acrocentric chromosomes) and telomere clusters were observed in sperm from t(13;17) translocated boars

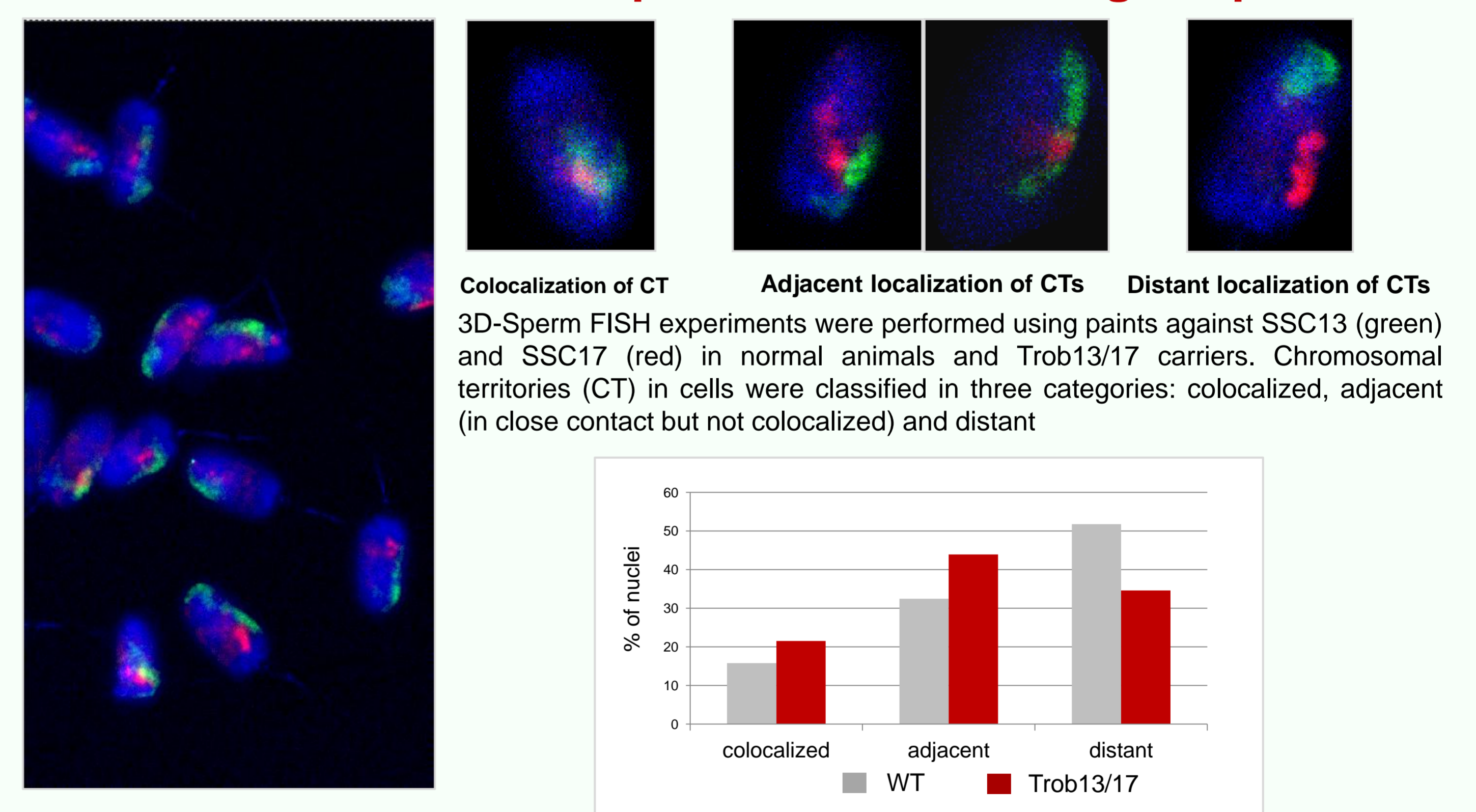


3D-SpermFISH experiments were performed using AC6 and SSCSR2A oligonucleotides (centromeres) or a biotinylated LNA modified oligonucleotide (TTAGGG)<sub>7</sub> (telomeres).

Average numbers of clusters corresponding to telomeres and centromeres in normal and translocated animals were estimated (ANOVA analysis, \* p-value<0.05)

As expected, significantly less telomere clusters (A) and centromere clusters of acrocentric chromosomes (B) were observed in translocated sperm cells due to at least to centromere fusion. The number of metacentric centromere is unchanged in both type (C).

#### ➤ Autosomes 13/17 are displaced from their original positions



Comparison between normal animals and Trob13/17 carriers clearly show differences between the localization of SSC13 and SSC17 territories. As in normal animals around 50% of the cells present distant CTs, this value decrease to 30% in Trob13/17 animals. In Trob13/17 animals CTs are closer and in more cases colocalized. More detailed analysis (in process) will confirm whether SSC17 territory moves to the SSC13 (more peripheral) territory.

### Methodology

**Animal Model:** sperm from normal boars and t(13;17) Robertsonian carriers (Pinton et al. 2009)

**Probes:** painting probes: Sus Scrofa chromosome 13, 17, X and Y (Yerle et al. 1993)  
telomere: biotinylated LNA modified oligonucleotide (TTAGGG)<sub>7</sub>  
centromere : SSCSR2A (metacentric chromosomes) and AC6 (acrocentric chromosomes) oligonucleotides respectively labeled with Alexa488 and Cy3 (Rogel-Gaillard et al. 2007)

**3D image analysis:** with a dedicated software NEMO (Iannuccelli et al. 2010). A segmented image from each object was created from the raw image and allowed us to obtain the percentage of colocalization (overlapped signals) of two markers and 3D distance between the different objects.

#### References:

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