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Nuclear localisation dynamics of centromeres, chromocentres and nucleoli of antral oocytes of homozygous and Robertsonian heterozygous mice during aging.

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Several studies have demonstrated the existence of a functional correlation between nuclear architecture and nuclear functions both in mammalian and non-mammalian somatic cells.
Current models of nuclear architecture of mammals establish that chromosomes in cell nucleus are organized as "chromosome territories" (CTs). This organization is cell- and stage-specific during differentiation of both somatic and germinal cells [1; 2; 3].
Two types of mouse oocytes at the germinal vesicle stage can be distinguished on the basis of their chromatin morphology: surrounded nucleolus (SN), in which the nucleolus is surrounded by a rim of Hoechst positive chromatin and not-surrounded nucleolus (NSN), in which this rim is absent [4].
This morphological difference has a biological relevance; NSN oocytes are tanscriptionally active, yet incapable of development beyond the 2-cell stage. Whereas SN oocytes, which are transcriptionally inactive, are capable of development to blastocyst stage *in vitro* [5; 6].
While it has been shown that Robertsonian translocations can influence nuclear architecture of male gamete during his maturation [7], there is not evidence during oocyte maturation.
The Robertsonian (Rb) translocation is one of the most common chromosomal rearrangements in mammals [8; 9]. The interest in Rb translocations derives not only from their high frequency in mammals, but also from their influence on fertility [10]: heterozygotes for Rb chromosomes have a tendency to be infertile or to produce offspring with birth defects.
Aim of this research work was to study the nuclear organisation of antral oocytes of mice carriers of Robertsonian translocations during aging. We have analysed the changes of the nuclear organization of antral oocytes isolated from 2n=32 (2n=40 X 2n=32) heterozygous mice compared to oocytes isolated from 2n=40 and 2n=24 female three, five and seven months after birth in order to define the influence of karyotype and age on the nuclear architecture.
Investigation was focused on three nuclear subcompartments: kinetochores, chromocentres and nucleoli. Antibodies that recognise centromere proteins, HP1-β protein and the fluorochrome (DAPI) were used to localise kinetochores, heterochromatin and nucleoli. Their number and reciprocal nuclear distribution were analysed. Results obtained by confocal microscopy allowed to characterise the changes that the mouse oocyte nuclear architecture undergoes during aging. To perform such 3D study we developed a novel method in order to maintain the 3D nuclear organisation of the nucleus of single oocytes isolated from ovaries.
As expected, SN and NSN configuration was found at the end of oocytes maturation but unexpected findings regarding centromeres clustering with others sub-regions analyzed are been noted. Our work represent the first model showing non-associated centromeres to nucleoli or chromocenters. We have called these centromeres "disperse" in nucleus.
It is well known that during folliculogenesis heterochromatin and in particular centromeres are clusterized. Our results suggest that this chromatin association is variable between karyotypes and ages. To explain these characteristics it would be very interesting to know, for example using the chromosome-painting, if the CREST signals that appear not-associated to nucleoli or heterochromatin regions correspond to an acrocentric or metacentric chromosome.
The results presented provide the first step to know how karyotype structure can influence nuclear architecture of female gamete at the end of his maturation.

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