Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Journal of Equine Veterinary Science 89 (2020) 103028
https://doi.org/10.1016/j.jevs.2020.103028

In vitro matured horse oocytes from aged mares show weakened centromeric cohesion and a higher incidence of aneuploidy

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Embryonic aneuploidy of meiotic origin is a major contributor to implantation failure, miscarriage and congenital birth defects in women of advanced age. Similarly, old mares show reduced fertility and an increased incidence of early pregnancy loss (EPL). Both in vivo and in vitro matured oocytes from old mares have been shown to display chromosome misalignment, however it is not clear whether maternal age increases the likelihood of oocyte or embryo aneuploidy. Age-related loss of chromosomal centromeric cohesion and a consequent premature separation of the sister chromatids is thought to be the main mechanism that contributes to aneuploidy of meiotic origin. The cohesin complex works as molecular “glue”, forming a ring-like structure that surrounds and holds the sister chromatids together, thereby preventing premature separation and mis-segregation. We hypothesized that increased mare age may predispose to loss of chromosomal cohesion and thereby to aneuploidy. Cumulus oocyte complexes recovered from slaughtered mares were divided into mare age groups (young, 2-14 y.o., mean ± SD: 9.4 ± 3.0 years; old, 16-27 y.o., mean ± SD: 20.5 ± 3.6 years), matured in vitro for 26h and denuded. Only oocytes that reached Metaphase II (MII) were used for further studies. RNA was extracted and cDNA synthesized from pools of 10 oocytes (n=4 per age group). mRNA expression for cohesion complex components (REC8, STAG3, WAPL, SGO1 and SGO2) was evaluated by qRT-PCR, using PGK1 and SRP14 as housekeeping genes. Thirty two MII oocytes from young and 36 from old mares were analyzed after immunofluorescent staining (IF) of chromosome spreads with probes for DNA (Hoechst 33342) and kinetochores (CREST). The chromatid-kinetochore count and inter-kinetochore distance (iKD) were evaluated by confocal microscopy and 3D image analysis (Imaris 8.3). Gene expression for the centromeric cohesion protector SGO1 was reduced in oocytes from aged mares. Moreover, in vitro matured oocytes from aged mares showed a higher incidence of aneuploidy than oocytes from young mares (20/36 [54.6 %] versus 5/32 [15.2%]; $p<0.05$) and an increased iKD (mean ± SD, 1.96 ± 0.74 versus 1.33 ± 0.40 μm; $p<0.0001$). Furthermore, 70% (14/20) of the old mare aneuploid oocytes had an uneven number of chromatids, consistent with unbalanced premature separation of sister chromatids (PSSC) during meiosis I. Although reduced SGO1 function would predispose to deterioration of centromeric cohesion and could explain a higher incidence of PSSC, the molecular origin of the cohesion defect remains to be proven. In conclusion, advanced mare age predisposes to reduced centromeric cohesion, as evidenced by an increased iKD, and increases the risk of oocyte aneuploidy, most likely via premature separation and random segregation of the sister chromatids during the first meiotic division. The age-related increase in aneuploidy observed in horse oocytes presumably contributes to reduced fertility and an increased incidence of EPL in old mares.