**The Time Doesn't Stop: Impact of Paternal Aging on Fertility in a Mouse Model**

Mayra E. O. D. Assumpção[1]; Larissa A. Stábile[1]; Camilla M. Mendes[1]; Thais R. S. Hamilton[1]; Marcelo D. Goissis[1]; Álvaro M. Alves[1]; Marcílio Nichi[1]; Heriberto Barbosa-Moyano[1]; Mariana S. A. G. Gomes[2]; Claudia M. Mori[2]

1. Department of Animal Reproduction, School of Veterinary Medicine and Animal Science, University of Sao Paulo, Sao Paulo, Brazil
2. Department of Animal Pathology, School of Veterinary Medicine and Animal Science, University of Sao Paulo, Sao Paulo, Brazil

This study worked with the hypothesis that aged mice present alterations in testosterone levels, sexual behavior, and sperm physiology, negatively influencing fertilization processes and early *in vitro* embryo development. Three experiments were conducted using male C57BL/6J mice aged 4 (young/control), 19, and 24 (senile/experimental) months. Data were analyzed using SAS software. Were performed ANOVA One-Way and the post-hoc test LSD. A level of 5% significance was considered. In the first experiment (4, n=6; 19, n=5; 24-month, n=7), behavioral tests were performed, including open field, elevated T maze, and social interaction with synchronized young females. The experimental groups exhibited a decrease in rearing (p=0.023), and center distance moved (p=<.0001), as well as in the frequencies of entry into the central zone (p=0.002) and the peripheral zone (p=0.002). The time in the central area was longer in the aged groups (p=0.001), while the movement time (p=0.001) and average speed (p=<.0001) in that area were lower. In the social interaction test, the aged groups showed a shorter social interaction time (p=0.010) and a lower frequency of female chasing (p=0.003), and there was a trend for a decrease in mating attempt frequency (p=0.082). In the second experiment (4, n=11; 19, n=5; 24-month, n=9), male reproductive capacity was evaluated. Serum testosterone levels, testicular weight, sperm morphology, CASA motility, integrity of plasma and acrosomal membranes (FITC/IP), oxidative stress (CELLROX green), mitochondrial membrane potential (JC1), susceptibility of chromatin to acid challenge (modified SCSA), and respective condensation (CMA3), sperm

capacitation status (CTC), and impacts on *in vivo* fertilization rate were assessed. The 24 months group showed lower serum testosterone levels (p=0.018). Aged groups exhibited a higher percentage of individual (p=0.0001), multi-defects (p=0.0361), and total defects (p=<.0001) compared to the control group. The 19-month group demonstrated a higher percentage of tail defects (p=0.023), and the 24-month group had a higher percentage of cells with midpiece defects (p=0.006). Males at 19 months exhibited higher intermediate mitochondrial membrane potential (p=0.032) and damaged plasma membrane with oxidative stress (p=0.003). The 24-month group showed a higher percentage of deprotaminated sperm (p=0.045). The percentage of capacitated sperm (p=<.0001) was decreased in aged groups compared to the control, with the 19-month group showing increased acrosome reaction (p=0.016). In the third experiment (4, n=5; 19, n=5; 24-month, n=7), *in vitro* embryo development rate, blastocyst stages, and the first cell differentiation (marked with CDX2 and SOX2) from young females mated with males from control and experimental groups were analyzed. It was found that aged groups had lower cleavage rates (D1.5; p=0.0290), blastocyst (p=0.0070), and embryo development rates (D4.5; p=0.0120). In the evaluation of specific blastocyst stages, the 19-month group showed more early blastocysts (EB; p=0.0051), and aged groups had less expanded (BX; p=0.0402) and hatched (HB; p=0.0003) blastocysts. There was a reduction in the total cell number (p=0.004) and trophectoderm (CDX2-positive; p=0.016) in the aged groups. The 24-month group had fewer cells for inner cell mass (SOX2-positive; p=0.035). Aged groups exhibited few fluorescence intensities for CDX2 (p=<.0001). Specifically, the 24-month group showed lower fluorescence intensity for SOX2 (p=<.0001). This study demonstrated the negative effect of paternal age on sexual behavior, and sperm quality, potentially leading to delayed first-cell division and consequently poorer early embryonic development *in vitro*. This work was supported by the Coordination of Improvement of Higher Education Personnel (CAPES).