SUMMARY

Introduction: Infertility is a serious health problem among young people, affecting about 10 – 15% of couples. Epidemiological data indicate that in approximately 50% of couples, the absence of pregnancies is caused by a male factor. Semen analysis allows the assessment of the number of sperm, their movement and morphology. It can be assumed that the dysfunction of Sertoli cells is responsible for abnormal spermatogenesis, which then impairs the course of spermatogenesis. So far, there are no recognized biomarkers to assess the functional state of Sertoli cells. The products of Sertoli cell metabolism and factors regulating spermatogenesis are present in sperm plasma. Studies in recent years indicate that AMH and micro–RNA present in extracellular vesicles (exosomes) contained in the sperm plasma may be particularly useful in diagnostics.

Aims of the study: 1. Identification of testicular function hormonal biomarker(s) related to spermatogenesis insperm plasma; **2.** Selection of miRNAs potentially correlating with disfunction of spermatogenesis; **3.** Demonstration of the effect of selected miRNAs and AMH on Sertoli cells in an experimental model in cell culture.

Materials and methods: The studies were carried out in sperm plasma in men (n = 310) with azoospermia, oligoasthenoteratozoospermia, oligozoospermia and with normal parameters according to 2010 WHO criteria. The evaluation of semen parameters (sperm count, morphology, motility) was performed using the computer assisted semen analysis system (CASA). In all study groups, hormone concentration profiles (testosterone, AMH, prolactin, estradiol and progesterone) were assessed by electrochemiluminescence (ECLIA) and androstenedione by chemiluminescence (CLIA). By through ultracentrifugation, the isolated sperm plasma exosomes were calculated by the ELISA method. The expression profile of selected miRNAs (miR–let–7a, miR–let–7b, miR–let–7c, miR–100, miR–181a, miR–181b, miR–518f) in sperm plasma exosomes was analyzed using the qRT–PCR method. The effect of AMH on miR–181a and miR–181b expression was investigated in the murine Sertoli MSC–1 cell line cultures. The research was approved by the Bioethics Committee at the Medical University of Bialystok –act number R–I–002/148/2018.

Results: The AMH concentration in sperm plasma was significantly decreased in the groups with azoospermia, oligozoospermia, oligoasthenoteratozoospermia (P≤ 0.05), and the concentration of androstenedione in the group with azoospermia (P≤ 0.05) compared to the control group. The number of semen plasma exosomes in the groups with azoospermia, oligoasthenoteratozoospermia, oligoasthenozoospermia, and asthenoteratozoospermia was reduced compared to the control group. In sperm plasma exosomes, decreased expression of miR–181a was observed in the azoospermia and oligozoospermia groups compared to the control group. ROC curve analysis for miR–181a and AMH showed sensitivity and specificity = 100%, and AUC = 1. There was no statistically significant change in miR–181b expression in any of the studied groups. Experimental studies on the Sertoli MSC–1 murine cell line showed that the expression of miR–181a was significantly increased in Sertoli cells stimulated with AMH at 10 ng / ml ** and 100 ng / ml * (* P≤ 0.05, ** P≤ 0.01) compared to the control group, however, the expression of miR–181b did not change regardless of the dose used. It was also shown that statistically AMH in the dose of 10 ng / ml significantly stimulates the proliferation of MSC–1 cells (P≤ 0.05). AMH did not affect the expression of *Amhr2*. The miR–181a inhibitor statistically decreased the proliferation of MSC–1 cells. Stimulation of MSC–1 AMH cells with the miR–181a inhibitor as well as the miR–181a activator did not affect the expression of miR–181a. The miR–181a inhibitor significantly decreased, while the miR–181a activator increased the expression of miR–181a in MSC–1 cells. AMH stimulation along with the miR–181a activator did not show any additive effect of increasing miR–181a expression.

Conclusions: 1. AMH and miRNA–181a participate in the regulation of Sertoli cell function; **2.** Determining AMH concentration, AMH content and miR–181a expression in sperm plasma can be used to diagnose the functional state of Sertoli cells; **3.** Reduced AMH concentration, AMH content and low miR–181a expression insperm plasma indicate impaired function of Sertoli cells in patients with oligozoospermia / azoospermia; **4.** Measurements of the remaining tested hormones and miRNAs cannot be used to assess the function of Sertoli cells in disorders of spermatogenesis.