IMPORTANCE OF OXYGEN TENSION IN HUMAN OVARIAN TISSUE IN VITRO CULTURE

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Abstract Body

Background: The primordial follicle (PMF) pool resides within the ovarian cortex, where oxygen tension is 2-8% O2. However, in vitro culture (IVC) is commonly performed at atmospheric oxygen tension (20% O2), and still shows unfavorable results.

Objective: To improve the viability of the PMF pool by modulating IVC oxygen tension.

Materials and methods: Frozen-thawed cortical fragments from 6 women were cultured for 6 days at (i) 20% O2, (ii) 5% O2, and (iii) using a hypoxic preconditioning (HPC) protocol (24 hours at 1% O2, followed by 5 days at 5% O2). Non-cultured fragments served as controls. Analyses were conducted based on follicle classification (H&E staining), follicle proliferation (Ki67 immunolabeling), follicle apoptosis (caspase-3 immunostaining), oxidative stress injury (8-hydroxy-2-deoxyguanosine immunofluorescence), DNA damage (phosphorylated histone H2AX immunofluorescence) and cellular senescence (β-galactosidase staining). Glucose and lactate medium levels were evaluated every two days for tissue metabolic activity analysis.

Results: The 20% and 5% O2 groups showed significantly ($p<0.0001$) decreased PMF proportions compared to the control group, but 5% O2 yielded significantly ($p=0.0006$) higher PMF rates than 20% O2. Percentages of apoptotic follicles ($p=0.003$), oxidative stress-associated lesions ($p<0.0001$), DNA damage ($p=0.02$), and cellular senescence ($p<0.001$) were significantly lower in the 5% O2 group than the 20% O2 group. Moreover, PMFs exposed to 20% O2 conditions exhibited a significantly ($p=0.01$) superior proliferation rate compared to 5% O2.

Apoptosis rates were 2-fold higher in the HPC group than the 5% O2 group ($p=0.0004$), probably due to insufficient oxygen supply. The former also displayed massive lactate release to the extracellular compartment.

Conclusion: IVC at 5% O2 resulted in better follicle viability than did 20% O2 or HPC conditions, mainly by protecting the PMF pool against oxidative injury and ensuing DNA damage.