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# ABSTRACT BOOK

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## **The effect of intermittent cold exposure on the level of reactive oxygen species production in erythrocytes of rats with induced polycystic ovary syndrome**

Yevheniia A. Hromko, Maryna V. Zhulikova, Mykhailo S. Myroshnychenko,  
Oksana A. Nakonechna  
*Kharkiv National Medical University*

Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women of reproductive age, often associated with systemic oxidative stress as a key pathogenic factor. This underscores the need to identify modulatory influences on the body's redox balance. Intermittent cold exposure is one such factor, showing antioxidant effects through activation of adaptive mechanisms.

The study used blood samples from 40 female rats (28 days old), randomly divided into four equal groups. Group 1 was the intact control. Group 2 was exposed to intermittent cold for 4 hours daily over 25 days. In Group 3, PCOS was induced by subcutaneous dehydroepiandrosterone (DHEA) administration, causing a hyperandrogenic state and characteristic ovarian changes. Group 4 received both DHEA and cold exposure to assess their combined effect. For analysis, 100  $\mu$ l of venous blood was mixed with 1 ml of 0.9% NaCl and centrifuged (5 min, 1000 rpm). After plasma removal, erythrocytes were washed twice with 1% and 0.9% NaCl (1 ml per 10  $\mu$ l of cells). Then, 2  $\mu$ l of erythrocytes were incubated with 10  $\mu$ M H<sub>2</sub>DCFDA for 30 minutes in the dark. This probe is oxidized by reactive oxygen species (ROS) to fluorescent DCF. After incubation, cells were washed, resuspended, and analyzed using the FL1 channel of a BD FACSCantoII flow cytometer (BD Biosciences, USA). Fluorescence intensity was assessed by median and interquartile range [25%; 75%], and statistical analysis was performed.

The intensity of DCF fluorescence in erythrocytes was 42.77 [40.89; 44.80] arbitrary units (a.u.) in group 1, 41.52 [39.75; 42.58] a.u. in group 2, 66.08 [63.46; 68.33] a.u. in group 3, and 42.69 [39.79; 44.45] a.u. in group 4. Subsequent statistical analysis revealed no significant difference between groups 1 and 2 ( $p > 0.05$ ). However, a significant increase in fluorescence intensity was observed in rats with induced PCOS (group 3) compared to the control groups ( $p < 0.05$ ), indicating excessive production of ROS in erythrocytes. At the same time, in group 4, the fluorescence intensity was significantly lower than in group 3 ( $p < 0.05$ ) and did not differ significantly from that in groups 1 and 2 ( $p > 0.05$ ), suggesting normalization of ROS levels under the combined effect of DHEA and intermittent cold exposure.

The experiment demonstrated that induced PCOS is accompanied by excessive generation of ROS in erythrocytes, indicating a disruption of redox homeostasis and the development of oxidative stress – one of the key pathogenic mechanisms of the syndrome. Intermittent cold exposure reduces ROS production in rats with hormonally induced pathology to control levels, demonstrating its potential effectiveness in correcting oxidative imbalance in PCOS.

