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P-681 Determination of the relationship between Hippo signaling pathway and ferroptosis in cumulus cells obtained from women with diminished ovarian reserve

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Study question: Does ferroptosis, through its regulation of Hippo signaling pathway, contribute to follicular atresia, thereby leading to diminished ovarian reserve (DOR) and associated infertility?

Summary answer: Ferroptosis in cumulus cells may induce follicular atresia and causing DOR through Hippo signaling pathway.

What is known already: Ferroptosis is a novel form of regulated cell death associated with oxidative stress and lipid peroxidation. Its activation has been implicated in various pathologies, including ovarian cancer, endometriosis, polycystic ovary syndrome and primary ovarian insufficiency. The Hippo signaling pathway regulates cellular growth and apoptosis, and recent evidence suggests a potential interaction between ferroptosis and this pathway in ovarian cells. Oocytes are dependent on cumulus cells for their energy supply, with the energy necessary for oocyte maturation, fertilization and early embryonic development. However, the relationship between ferroptosis and Hippo signaling in the context of DOR has not been fully elucidated.

Study design, size, duration: This cross-sectional study was conducted in an IVF clinic between July 2023 and December 2024. 81 women aged between 18 and 39 years undergoing IVF treatment were included, divided into two groups: 46 patients diagnosed with DOR with a serum AMH level of < 1.1 ng/ml and/or ≤ 3 oocytes retrieved in prior IVF cycles. 35 with normal ovarian reserve (NOR) defined by serum AMH levels between 1.1 and 3.5 ng/ml,and 4-15 oocytes retrieved during egg collection.

Participants/materials, setting, methods: Cumulus-oocyte complexes were retrieved after ovarian stimulation with recombinant FSH and GnRH antagonist protocol and ovulation trigger with recombinant HCG. Cumulus cells were collected during egg retrieval, mechanically using a 31G insulin syringe. Ferroptosis-related (GPX4, EMP1) and Hippo pathway-related genes (MST, YAP, LATS) were analyzed by qRT-PCR. Gene expression levels were compared between DOR and NOR groups by using delta-CT values. Additionally, iron and reactive oxygen species levels in follicular fluid were measured.

Main results and the role of chance: Significantly higher expression levels of EMP1 and GPX4 were observed in the DOR group compared to the NOR group ($p = 0.024$ and $p = 0.015$, respectively), while no significant difference was found in the expression of other genes. The increase in both EMP1 and GPX4 in the DOR group suggest the activation of ferroptosis pathway in cumulus cells, which, in turn, may reflect a compensatory activation of the antioxidant system. No difference was observed in follicular fluid iron levels between the DOR and NOR groups; however, reactive oxygen species (ROS) levels were significantly elevated in the DOR group ($p = 0.008$). The increase in ROS levels may serve as an indirect indicator of the ferroptosis activation triggered by oxidative stress. Although these results support a relationship between ferroptosis and DOR, the influence of confounding factors such as age and ovarian stimulation protocols cannot be entirely excluded.

Limitations, reasons for caution: The study's limitations include its cross-sectional design and the small sample size. The findings, while promising, may not be fully generalizable due to the limited number of cases. Additionally, the complexity of cellular interactions and the lack of longitudinal follow-up limits the ability to establish causality.

Wider implications of the findings: These findings highlight the potential role of the relationship between ferroptosis and the Hippo signaling pathway in the pathophysiology of DOR. Understanding these mechanisms could

open new avenues for therapeutic strategies aimed at preserving ovarian reserve and improving fertility outcomes in women with DOR.

Trial registration number: No