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INFLUENCE OF ANTIOXIDANT-SUPPLEMENTED MEDIA ON IN VITRO MATURATION RATES AND MORPHOLOGICAL QUALITY OF SWINE OOCYTES

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Abstract: This study explores the impact of antioxidant supplementation on the in vitro maturation (IVM) of swine oocytes, focusing on morphological quality and maturation success. Oocytes were collected, classified into three quality classes, and matured in TCM-199 media supplemented with Vitamin C or rosmarinic acid. Results showed that Vitamin C significantly enhanced maturation rates, achieving 100% for high-quality (Class I) oocytes by mitigating oxidative stress and promoting cytoplasmic and nuclear maturation. In contrast, rosmarinic acid exhibited limited effectiveness, indicating the necessity for careful optimization. These findings underscore the potential of antioxidants like Vitamin C to improve IVM protocols and reproductive outcomes.

• Introduction

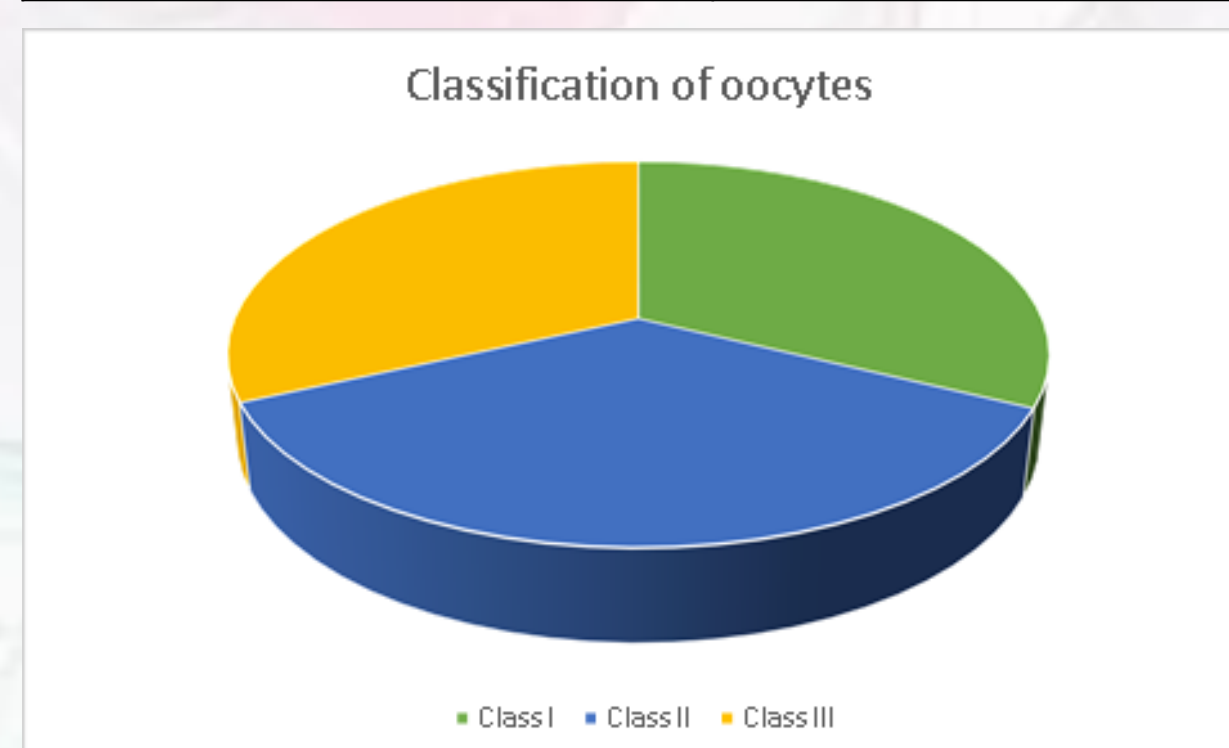
Oocyte in vitro maturation (IVM) is a critical reproductive biotechnology that can improve oocyte quality and fertility potential. However, oxidative stress is a significant challenge in IVM, as it can impair cytoplasmic and nuclear maturation, leading to reduced developmental competence. This study investigates the impact of antioxidant supplementation, specifically Vitamin C and rosmarinic acid, on the IVM rates and morphological quality of swine oocytes. The primary aim is to evaluate the effectiveness of these antioxidants in improving maturation outcomes and protecting oocytes from oxidative damage.

• Material and method

Porcine oocytes were collected from slaughterhouse-derived ovaries and transported under controlled conditions to maintain viability. Oocytes were classified into three quality classes (I, II, and III) based on cumulus-oocyte complex (COC) morphology. The oocytes were then subjected to IVM in TCM-199 media, with groups supplemented with Vitamin C (0.5 mM) or rosmarinic acid (105 µM). Morphological assessments of oocyte quality and maturation were performed using a stereomicroscope. Statistical analyses were conducted to compare the maturation rates across different treatments.

Table 1. Distribution of Oocytes Across Quality Classes

Classification	Total number of recovered oocytes	Percentage of total number
Class I	72	32.29%
Class II	80	35.87%
Class III	71	31.83%



Graphic illustration of the class distribution of recovered oocytes



Morphological classification of COC. A – class I, B – class II, C – class III



Post-Maturation Oocyte Morphology the AR batch (medium enriched with rosmarinic acid). A – oocyte class I, B – oocyte class II, C – oocyte class III

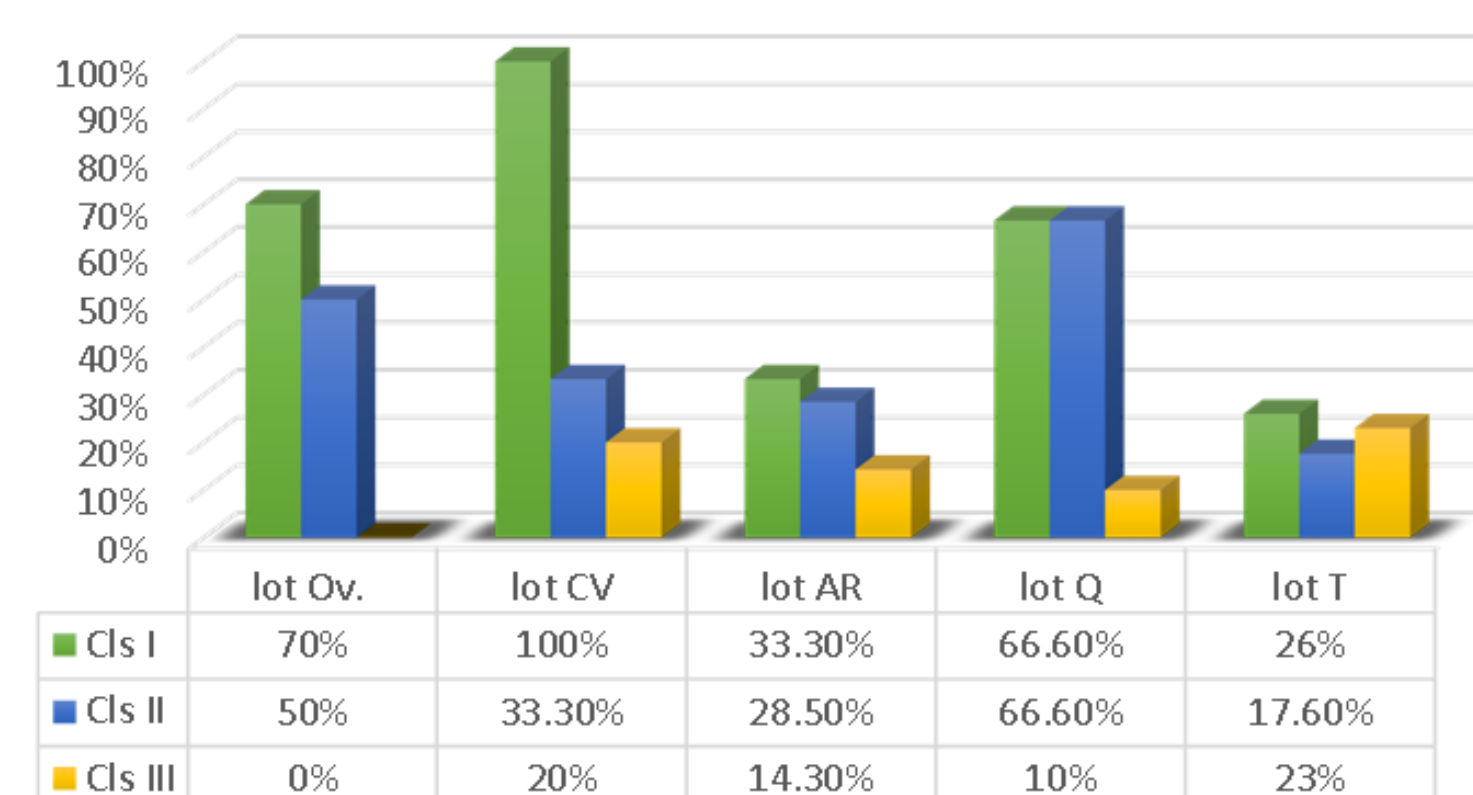
• Results and discussions

- Oocyte Classification:** Out of 354 aspirated follicles, 223 oocytes were collected and categorized into Class I (32.29%), Class II (35.87%), and Class III (31.83%).
- Maturation Outcomes:** Class I oocytes exhibited a 70% maturation rate in control media. Vitamin C supplementation increased the maturation rate to 100%, while rosmarinic acid led to a significantly lower rate of 33.33% for Class I oocytes.
- Impact of Antioxidants:** Vitamin C's robust antioxidative properties effectively neutralized ROS, enhancing both cytoplasmic and nuclear maturation. In contrast, rosmarinic acid may have had pro-oxidant effects or interfered with critical cellular mechanisms.

Table 2. Maturation Rates of Oocytes Across Experimental Conditions

	Before maturation	After maturation	Before maturation	After maturation	Before maturation	After maturation
	Class I		Class II		Class III	
bach O I	10	7 (70%)	-	-	-	-
bach O II	-	-	10	5 (50%)	-	-
bach O III	-	-	-	-	9	0 (0%)
bach CV	9	9 (100%)	12	4 (33.3%)	25	5 (20%)
bach AR	12	4 (33.3%)	14	4 (28.5%)	14	2 (14.3%)
bach Q	14	8 (66.6%)	27	18 (66.6%)	10	1 (10%)
bach T	27	7 (26%)	17	3 (17.6%)	13	3 (23%)
Total	72	35	80	34	71	11
Procent	48.61%		42.5%		15.5%	

- Morphological Changes:** Vitamin C-treated oocytes showed well-expanded cumulus cells and homogeneous ooplasm, indicative of successful maturation. Rosmarinic acid-treated oocytes displayed less pronounced morphological improvements.
- Comparison with Literature:** The observed benefits of Vitamin C align with existing studies highlighting its role in promoting glutathione synthesis and protecting oocytes from oxidative damage. The limited success of rosmarinic acid suggests that not all antioxidants are suitable at the same concentrations, necessitating further optimization.



Influence of antioxidant treatment on the maturation rate of sow oocytes

• Conclusions

Vitamin C significantly enhances the IVM rates of high-quality porcine oocytes, demonstrating its effectiveness in mitigating oxidative stress. Rosmarinic acid showed limited effectiveness, highlighting the need for careful concentration optimization. Morphological assessment remains a crucial predictor of oocyte developmental competence, reinforcing the importance of stringent quality selection. This research underscores the potential of using antioxidant-enriched media to improve reproductive outcomes in both research and commercial applications.