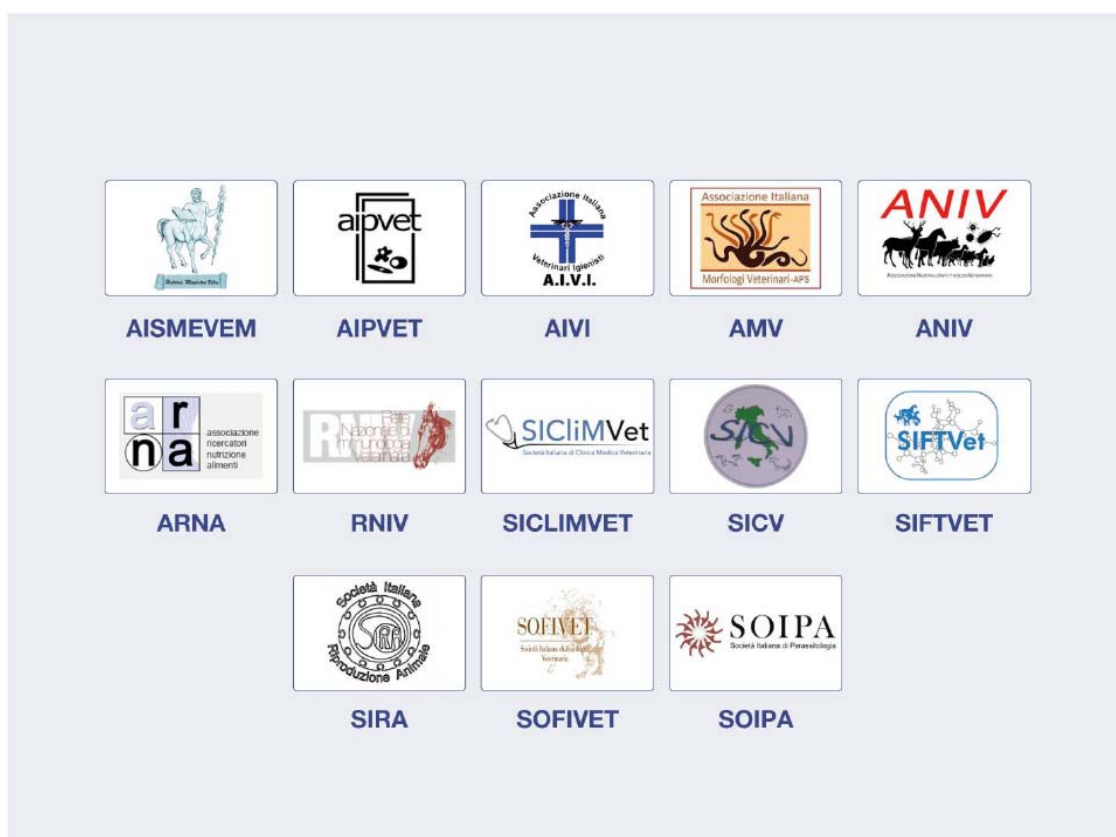




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EXPRESSION OF HEAT SHOCK PROTEINS 60, 70, 90 IN EQUINE GRANULOSA CELLS.

Maria Albrizio (1), Salvatore Desantis (1), Antonio Ciro Guaricci (1), Mario Cinone (1)

(1) Department of Precision and Regenerative Medicine and Ionian Area (DiMePre-J), University of Bari Aldo Moro, Italy

Corresponding Author: **Maria Albrizio** - maria.albrizio@uniba.it

It is well known that heat shock proteins (HSPs) are not only mediators of heat stress but also regulators of cell proliferation (1), differentiation (2) and apoptosis (3). During the equine reproductive cycle, the ovary experiences all these events. The aim of our study was to investigate on the quantitative presence of HSPs (60, 70, 90) in granulosa cells from follicles of small (6-10mm), medium (11-20mm) and large (>20mm) size (4). Granulosa cells were recovered from ovaries obtained from slaughtered females. Proteins were extracted and run on polyacrylamide gel, transferred to PVDF membranes and hybridized to monoclonal mouse primary antibodies (anti-HSP 60 cat. n. SC-13115, Santa Cruz Biotechnology; anti-HSP 70 cat. n. SC-7298, Santa Cruz Biotechnology and anti-HSP 90 cat. n. SMC-107, StressMarq Biosciences). For normalization anti-actin primary antibody was utilized (cat. n. A2103, SIGMA-Aldrich). A negative control was obtained omitting each primary antibody. Revelation was performed by the Vectastain elite system (Vector-Laboratories) and positive signals quantified by Quantity one software (BioRad). The values, expressed as arbitrary units, were calculated as the ratio of the specific protein to the corresponding beta-actin optical density. Results evidenced that granulosa cells from all different kind of follicles do not express HSP60. On the contrary, the expression level of the HSP70 is about six times higher than that of HSP90 despite the size of the follicle. Granulosa cells from large follicles show the highest HSP70 expression level compared to medium and small follicles ($P<0.05$), while HSP90 is more expressed in granulosa cells from medium follicles than that from small and large respectively ($P<0.05$). This work evidenced for the first time in the equine species the expression of HSP70 and 90 in follicular granulosa cells. Investigations on the relation about HSPs expression and the follicular size will allow establishing the involvement of these proteins in all phases of the mare reproductive cycle.

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