PROTEASOMAL PROTEOLOYSIS DURING PORCINE FERTILIZATION

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ABSTRACT

Protein degradation is essential for maintaining a healthy cellular state. One such protein degradation pathway is the Ubiquitin Proteasome System (UPS) that consists of a small chaperone protein ubiquitin, a proteolytic holoenzyme known as the 26S proteasome and three key ubiquitin-activating and ubiquitin-conjugating enzymes (E1, E2, E3). The UPS participates in multiple reproductive processes in various species including spermatogenesis, oocyte maturation and fertilization. Based on this knowledge, the first objective of this thesis was to determine if the UPS was active during the penetration of fertilizing boar spermatozoa through the porcine egg coat, zona pellucida (ZP). The second objective was to determine if the UPS was actively targeting acrosomal proteins for degradation during the zona induced acrosome reaction. Activity of the UPS was determined based on the protection of both zona and acrosomal proteins from degradation by the 26S proteasome, via addition of specific proteasomal inhibitors. It was concluded that the UPS is active during ZP penetration of porcine oocytes and acrosome reaction of fertilizing boar spermatozoa. Furthermore, it was shown that by adding specific proteasomal inhibitors, these proteins could be protected from degradation by the UPS. While attempting to determine which proteins were being protected from degradation, a member of the UBR box family of E3 enzymes, known as the, the ubiquitin ligase UBR7 was identified in the boar testis and spermatozoa. Collectively, these results provide a unique window by which to gain a better understanding of the activity of the UPS during mammalian fertilization.