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Journal of Andrology, Vol. 24, No. 3, May/June 2003 Copyright © <u>American Society of Andrology</u>

Specific Order in the Appearance of Protein Tyrosine Phosphorylation Patterns Is Functionally Coordinated With Dog Sperm Hyperactivation and Capacitation

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The aims of the present study were to characterize a slow capacitation system that records initial changes in the sperm membrane state, and, using a canine model, to order the specific protein tyrosine phosphorylation signaling in the sequence of capacitational events and to associate them with hyperactivated

motility. Dog sperm washed through Percoll were incubated in complete bicarbonate Tyrode medium for 6 hours in 5% CO₂. Capacitation was evaluated using chlortetracycline staining. Tyrosine phosphorylation patterns were assessed by immunocytochemistry. Parallel to this, a computer-assisted motility analysis was performed. Significant

changes in the percentage of capacitated and acrosome-reacted cells were first observed after 90 minutes, increasing in a linear manner during further incubation (P < .05). Changes in the percentage of capacitated cells were accompanied by motility changes. During incubation, a strictly sequential phosphorylation of sperm tail (midpiece, principal piece, and end piece) and head proteins was observed. According to an analysis of kinetics, phosphorylation of head proteins occurred after the tail became completely phosphorylated. Changes in head phosphorylation progressed at the same rates as capacitation and acrosome reaction. Sperm motility, curvilinear velocity, average path velocity, straight line velocity, and lateral head displacement were correlated positively or negatively with phosphorylation of midpiece or end piece proteins, respectively. The bicarbonate-stimulated increases in cyclic adenosine monophosphate levels and changes in protein phosphorylation kinetics of sperm proteins are potentially useful for diagnostic purposes to characterize the response of individual males to fertilizing conditions.

Key words: Kinetics, hyperactivation, membrane destabilization, sperm function, phosphorylation

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