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# Expression of progesterone receptor(s) during capacitation and incidence of acrosome reaction induced by progesterone and zona proteins in boar spermatozoa

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## Abstract

Sperm acrosome reaction (AR) is a prerequisite step for in vivo fertilization. In the vicinity of the oocyte, zona protein(s) (ZP) and progesterone (P4), a component of follicular fluid, are proven to be responsible for physiological AR induction. In the present study, a thorough analysis of the role of the progesterone receptor (PR) in this processing including in vitro physiological studies and biochemical isolation and characterization of the receptor protein was conducted. Following capacitation for 0, 2, 4 and 6 h, pooled fertile boar semen samples ( $n=6$ ) with >70% sperm motility were labeled with P4-BSA-FITC (100  $\mu\text{g/ml}$ ) to detect the activation of PR. Parallel sperm samples were treated with P4 (10  $\mu\text{g/ml}$ ) for 20 min to test AR inducing efficiency at different time points. To compare the ability of ZP and P4 to induce AR, spermatozoa capacitated in a modified medium supplemented with 1 mg/ml heparin for 4 h, were then treated with heat solubilized ZP (150  $\mu\text{g/ml}$ ), P4 (10  $\mu\text{g/ml}$ ) or ZP + P4 for 20 min. FITC-peanut agglutinin staining was applied to observe the disrupt acrosomal morphology. A purification protocol for crude boar sperm membrane proteins was developed based on ligand-receptor affinity chromatography procedures. The PR proteins were then identified by using mAb C262 raised against intracellular PR, combined with second antibody (SDS–PAGE, Western blotting). Their N-terminal amino acid sequence was determined. The amount

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of PR-activated spermatozoa was enhanced with time (onset:  $27 \pm 5\%$ , 2 h:  $41 \pm 4\%$ , 4 h:  $49 \pm 3\%$  and 6 h:  $52 \pm 4\%$ , mean  $\pm$  S.E.,  $n=6$ ) as evidenced by increasing percentage of spermatozoa with completed cap fluorescent staining. In parallel sperm samples, percentages of AR induced by P4 were  $9 \pm 2$ ,  $14 \pm 2$ ,  $18 \pm 2$ , and  $24 \pm 2\%$ , respectively. In solvent control at all time points, less than 10% spermatozoa had undergone AR. Capacitation for 4 h or greater time periods resulted in optimal percentage of PR-activated and acrosome-reacted spermatozoa. After sperm incubation in heparin-medium, ZP+P4 treatment induced greater amounts of AR than either P4 or ZP alone ( $13 \pm 1\%$  compared with  $8 \pm 1$  and  $10 \pm 1\%$ ,  $P < 0.01$ ). Inducing capacity of P4 was comparable to that of ZP. The molecule weights of two apparent PR molecular masses were detected to be at Mr 74 kDa and Mr 63 kDa. N-terminal amino acid sequence of 74 kDa protein was XPXNIVLIFADXLXY, which had 78% homology to arylsulfatase A and 88% homology to 72 kDa protein from boar spermatozoa. The activation of PR is associated with the capacitating process and that appears to be required for P4-induced AR. P4 and ZP appear to be equally capable of independently inducing the AR but lack synergetic or additive effects in this induction process. Both might represent alternative pathways thus resulting in alternative systems for induction of the prerequisite acrosomal exocytosis (supported by NSC 90-2313-B-005-114; 91-2313-B-005-131).

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## 1. Introduction

The sperm acrosome reaction (AR) is a calcium-dependent, exocytotic event required for mammalian fertilization. The AR facilitates the penetration of the zona pellucida by the spermatozoa and the subsequent fusion of the sperm plasma membrane with the oocyte's oolemma. Ejaculated spermatozoa require a series of preparatory changes to undergo the AR. These physiological changes are collectively termed capacitation and involve primary membrane modifications, including sperm receptor activation (Yanagimachi, 1994). Capacitation can occur either in vivo, during the passage of spermatozoa through the female genital tract, or in vitro, during incubation of washed spermatozoa under proper conditions. In vitro studies have shown that the AR can be initiated in pre-capacitated spermatozoa either spontaneously or by different physiological (zona pellucida glycoproteins, follicular fluid and progesterone) and non-physiological (calcium ionophore) inducers (Cheng et al., 1998a, 1998b; Sirivaidyapong et al., 2001). However, a biologically effective AR will depend on the presence and the activity of physiological inducers as well as on the functional capacity, in terms of availability of receptors, of spermatozoa to respond to these inducers.

Although, the zona pellucida is considered the prime physiological inducer of the AR, under in vivo conditions, other inducers (i.e., progesterone) are also present in the vicinity of oocyte complex. Questions have arisen as to how the progesterone receptor (PR) functions during the process of sperm capacitation and how the AR induction is linked to this ligand. We compared the AR inducing capacity of progesterone and heat solubilized porcine zona pellucida and determined whether these agents combined induced the AR in an additive or synergistic manner. The activation of PR during in vitro capacitation was investigated to

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