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Non-genomic Membrane Progesterone Receptors in Spermatozoa

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Summary

Adequate experimental data exist demonstrating the presence of a membrane bound protein that mediates rapid non-genomic mode of progesterone actions in spermatozoa to regulate acrosome reaction. Studies are needed to decipher the identity of this protein and if this receptor (protein) is the true membrane PR involved in progesterone mediated acrosome reaction. The discovery of the identity of the sperm PR would enable detailed studies to be conducted at the molecular level on the membrane receptor system, its physiological relevance and its interactions with the nuclear receptors.

Key words: Progesterone receptors and spermatozoa

Introduction

Mammalian spermatozoa must spend some time in the female genital tract or under certain conditions *in vitro* before being able to fertilize the oocyte. During this time spermatozoa undergoes a series of biochemical modifications such as capacitation which culminates with the acrosome reaction, a modified exocytotic event involving fusion followed by vesiculation of sperm head membrane (1,2).

Progesterone, secreted by cumulus oophorus cells of the ovary, is capable of initiating acrosome reaction *in vitro* in spermatozoa of several mammals, including humans. Hence, it has been postulated that this steroid acting by itself

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and/or in synergy with the zona pellucida, is a physiological initiator of the acrosome reaction in vivo (3-5). It has been well documented that progesterone induces sperm hyperactivation, stimulates acrosome reaction, increases the binding of spermatozoa to the oocyte zona pellucida and increases the sperm penetration rate into hamster oocytes (6).

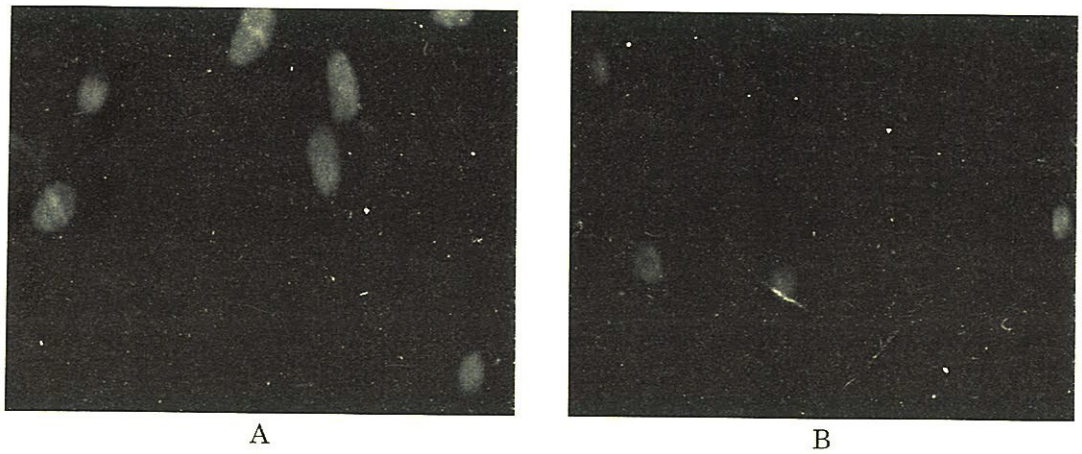
Like other steroids, progesterone acts by modulating transcriptional events mediated by an intracellular receptor belonging to the superfamily of nuclear transactivators. The intracellular receptors for progesterone are well characterized and are referred to as nuclear receptors (7,8). However, in spermatozoon, progesterone acts on almost completely transcriptionally inactive cell, with mechanisms that are not responsive to antagonists of the classical progesterone receptor (9). Furthermore, in spermatozoa progesterone mediates its action by initiating signal transduction events. It has been demonstrated that progesterone induces Ca^{++} influx and Ca^{++} dependent acrosome reaction; increase in tyrosine phosphorylation of sperm proteins in response to progesterone has also been reported (10-14). Progesterone has also been shown to induce a Ca^{++} dependent cyclic AMP increase in human spermatozoa (15). These effects of progesterone in spermatozoa are rapid and the mechanism of action of appears to be different from that of the genomic mechanism involving intracellular steroid hormone receptor and the processes of transcription and translation. These rapid effects of progesterone on spermatozoa are believed to be the nongenomic actions and it has been implicated that progesterone induces the non genomic effects via a surface receptor. In the present review we have summarized the research carried out on the characteristics of the surface receptor for progesterone binding human spermatozoa that finally lead to acrosome reaction. Rather than being comprehensive, we have focused on the recent research that has been ongoing on the identification of the membrane receptor for progesterone. For the purpose of simplicity, we refer the sperm membrane progesterone receptor as membrane PR and the classical or intracellular PR as the nuclear PR.

Biochemical properties of sperm progesterone receptor

The proof that a surface receptor for progesterone exists in spermatozoa comes from the studies demonstrating increase in intracellular calcium and induction of acrosome reaction in spermatozoa challenged with progesterone conjugated to albumin, which does not allow the steroid to cross the sperm membrane (16). Our previous studies have demonstrated that the sperm membrane PRs are masked antigens and when treated with mild detergent like digitonin (0.1%), the binding sites are exposed (17). Using a variety of detergents like SDS, CHAPS, NP40, Triton –x100, digitonin, deoxycholate etc., digitonin was found to be expose progesterone receptor (PR) on

mature human spermatozoa (18). A conjugate of fluorescein isothiocynate (FITC) - labelled bovine serum albumin (BSA) and progesterone has been used to localize PR in the sperm plasma membrane. The results of these studies show that only few percentages of spermatozoa (10~30%) binds to the hormone and the binding is marked at whole or equatorially acrosomal region of human spermatozoa (14,19). In contrast to these observations, studies carried out in our laboratory (20) have shown that after digitonin treatment more than 90% of spermatozoa show the presence of PR on the acrosomal or equatorial region (Fig 1). Our data supports the report, where image analysis technique was performed to determine the number of spermatozoa responding to progesterone, which indicated that more than 90% of spermatozoa showed the presence of PR (21). These observations suggest that PR in spermatozoa are masked antigens and treatment of spermatozoa with digitonin exposes the receptor on large number of cells. A similar experience has been recently reported in the porcine spermatozoa (22).

Treatment with detergents increases the permeability and fluidity of the plasma membrane by dispersal of lipids and fragmentation of the membrane and thus exposes the binding sites. It is possible that digitonin reacts with the cholesterol in the sperm membrane thus exposing the progesterone binding sites. Indeed cholesterol sulphate has been found about 20% of the acrosomal surface area (23). Since during capacitation or sperm transport in the female tract, a number of changes takes place within the plasma membrane which includes the rearrangement of lipids and proteins and unmasking of several antigens, it could be possible that treatment with digitonin



A B

Figure 1: Localization of progesterone receptors on spermatozoa using a membrane impermeable ligand P-FITC-BSA. Note the positive (green) staining in the acrosomal region (A). Negative control is shown in B.

mimics at least some of that changes that take place in the sperm plasma membrane during capacitation in the female genital tract.

Characteristics of sperm membrane PR

Based on the downstream events of progesterone action in spermatozoa atleast three different characteristics of the receptor have been postulated (Fig 2).

1) Plasma membrane Ca^{2+} channel (PR1),
2) Membrane-associated protein tyrosine kinase (PTK; PR2)
3) Plasma membrane chloride channel (PR3)

The tyrosine kinase-associated PR (PR2) seems to be the one visualized by the hormone-binding assay because those spermatozoa that bind to the BSA conjugate also increase their phosphotyrosine content and undergo acrosome reaction (13). This PTK-associated PR is probably responsible for both, the effect of progesterone on acrosome reaction and on hyperactivated motility (15). The PR responsible for the rapid opening of the Ca^{2+} channel (PR1) is active in a higher percentage (more than 90%) of spermatozoa (21), but has different ligand-binding properties than the receptor responsible for protein tyrosine kinase activation. Moreover, it is not capable of initiating the progesterone induced acrosome reaction. The third PR (PR3) is likely to be a ã-aminobutyric acid (GABA) A-receptor/chloride channel complex, and probably mediates the Cl^{2-}

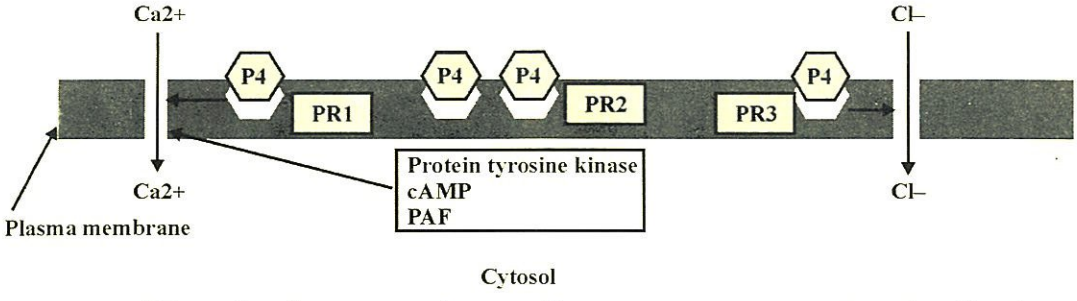


Figure 2: PR at the plasma membrane of human spermatozoa. Functionally, three types of PR exist in the plasma membrane of human spermatozoa. Progesterone (P) appears to act directly at a voltage-independent Ca^{2+} channel after binding to type 1 P receptor (PR1). The P-PR1 interaction would trigger a rapid (seconds) Ca^{2+} influx. At the same time P reacts with a second type of surface receptor (PR2). After P binding, PR2 aggregates and stimulates tyrosine kinase which leads activation of signaling cascades involving a series of intracellular messengers (box). The activation of both types of Ca^{2+} channels finally initiates exocytosis of the acrosome. A third type of surface P receptor (PR3), coupled to a Cl^{2-} channel and resembling a GABAA/Cl2 channel complex may mediates the Cl^{2-} influx which takes place during the acrosome reaction

fluxes occurring during acrosomal exocytosis (24,25). However, studies have demonstrated that GABA receptor is not involved in the progesterone induced Ca^{2+} influx in spermatozoa (26). At present it is not known whether the nongenomic effects of steroids on cells in general, appear to be mediated by multireceptor systems or these all are a component of a single type of receptor.

Biophysical characteristics of the sperm membrane PR

Extensive studies have reported that the steroid binding ability, steroid specificity and modes of progesterone actions differ in spermatozoa as compared to the nuclear PR. The basic characteristics of the sperm membrane PR and the dis-similarities with nuclear PR are reviewed previously (18) and summarized in table 1. However, the identity of the sperm membrane PR is currently unknown. Herein, we discuss the structural, biochemical and molecular nature of the putative sperm receptor that mediates the rapid non-genomic actions of progesterone.

Sperm membrane PR shows high structural specificity to progesterone

The sperm PR has specificity for steroids different from that of the nuclear PR. Using a range of progestins, binding kinetic studies have demonstrated presence of two classes of PR in spermatozoa, one that has an elevated affinity constant (in nanomolar range) and is specific for progesterone. The other class of PR has the affinity constant in micromolar range and binds equally well with other hydroxylated progesterone derivatives (27). The progesterone specific, but not the agonist binding receptor seems to be unmasked

Table 1

Comparative assessment of the characteristics of the nuclear and sperm membrane progesterone receptor (compiled from Shah et al 2003, Ambaikar et al 1998 and Puri et al unpublished data)

	Nuclear PR	**Sperm PR**
Localization	Cytoplasm and nucleus	Membrane
Isofoms	2	>2
Size	90 and 120 kDa	55-60kDa
Bmax	10nM	>22nM
KD	$2.6x10^{-9}M$	$4.07x10^{-4}M$
Affinity to agonist	High	Low
Mode of action	Transcription regulation	Signal transduction

by digitonin treatment (17); whether the agonist-binding site in spermatozoa is biologically active needs be evaluated. However, protein phosphorylation and acrosome reaction is induced only by progesterone but not other progestins, indicating that the high affinity receptor is responsible for the biological effects (27-31).

Sperm membrane PR does not interact with antiprogestins

Another striking feature of sperm membrane PR is its inability to interact with antiprogestins. Unlike the nuclear PR, sperm membrane PR does not bind to mifepristone, onapristone and other antiprogestins *in vitro* (17). It has been proposed that the failure of antiprogestins to bind the sperm membrane PR could be owing to the differences in the structure of the compounds as compared to progesterone (31,32). However, in most studies the compounds tested have substitutions in the alpha phenyl ring of the progesterone molecule (Fig 3A). In this context, we argued that compounds demonstrating modifications at sites other than the alpha phenyl ring might permit the binding of the analogs to sperm membrane PR. One such compound is the antiprogestin J867 (asoprisnil) which has substitution in the beta phenyl ring (Fig3A). This compound, at specified concentrations, has demonstrated antiprogestational activity in the conventional bioassays and has high affinity to the nuclear PR (33). However, like other antiprogestins, in a typical radio-receptor assay, a 10 to 1000 fold higher concentration of J867 failed to displace binding of labeled progesterone to digitonin treated spermatozoa (Fig 3B). These results tempt us to hypothesize that sperm membrane PR has a high structural specificity only for progesterone that may not be mimicked by similar compounds.

Molecular Characterization of Sperm Progesterone Receptor

We and others have demonstrated differences in the hormone specificity of sperm PR and uterine PR. Unlike uterine PR, sperm PR does not bind to mifepristone and other progesterone antagonists in vitro (18). These observations along with the fact that antiprogestin like mifepristone and ZK 98.299 are unable to block the progesterone-mediated increase in calcium influx in spermatozoa suggested that the sperm PR is distinct from the conventional PR.

The differences in the molecular size, mode of action, and the peculiar location of sperm PR as compared to the conventional PR prompted us to investigate

1) Whether there exist any PR-like transcripts in mature human spermatozoa
2) Whether these resemble the conventional PR in terms of its genomic organization, i.e., the presence of an N-terminal domain, DNA binding domain, and hormone binding domain.

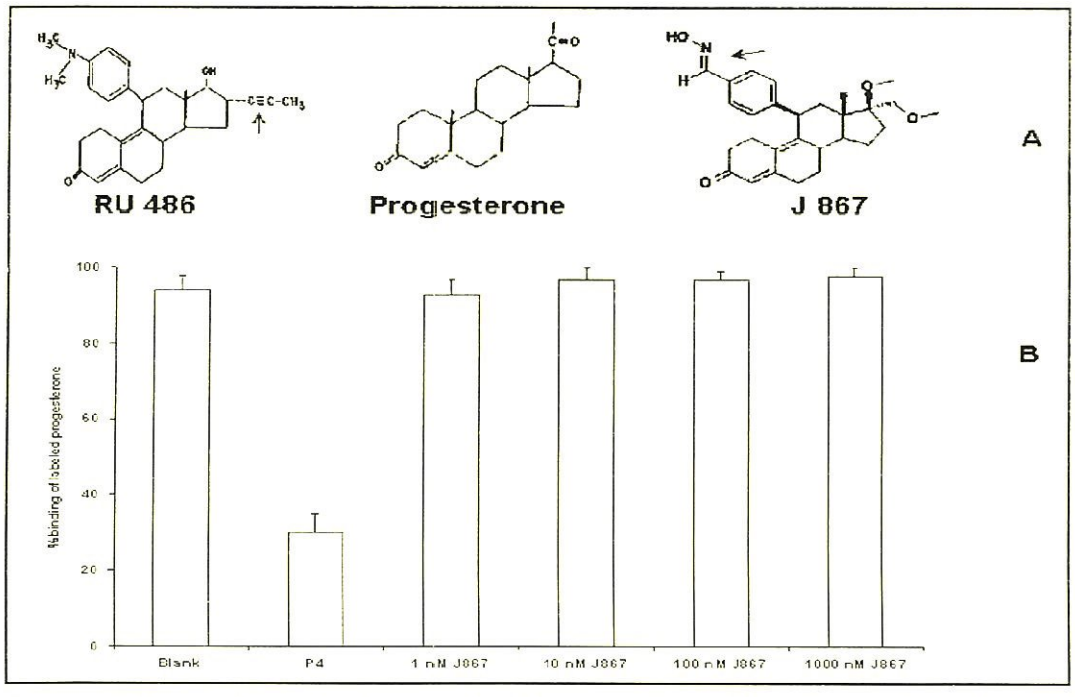


Figure 3: Sperm PR and antiprogestin (A) Comparison of the chemical structures of antiprogestin RU486 and J867 with progesterone. In the J867 compound, the modifications in the beta phenyl ring (arrow) is as opposed to that in the alpha phenyl ring (arrow) in RU486. (B) Cold progesterone significantly displaced the binding of radio labeled progesterone, J867 (1-1000nM) failed to displace progesterone binding to digitonin treated human spermatozoa. The results are expressed as mean + SD of % binding of labeled progesterone (blank was considered as 100%).

In view of the emerging concept suggesting that the progesterone- PR complex in human spermatozoa apparently does not require interaction with DNA to induce biological effects, studies on the molecular characterization of PR transcripts in spermatozoa is of great significance in understanding the nonclassical mode of action of steroids. We hypothesized that the sperm RNA contains transcripts of differentiating spermatogonia and can be successfully used to study the molecular organization of sperm PR (34).

Our study for the first time demonstrated the presence of PR transcripts in human spermatozoa (18,35-37). Using primers spanning the entire DNA and Hormone binding domains (DBD and HBD respectively) of the nuclear PR, PCR product of expected sizes were obtained in sperm cDNA (Fig 4). To

study, if this PR transcript has any mutations or deletions, we cloned and sequenced the amplified transcript and the resultant sequence was subject to homology searches using the BLAST and FASTA formats. The results revealed that the PR transcript from sperm RNA had the entire DBD and a part of HBD along with the hinge region. As compared to PR transcripts from the endometrium, no deletions, insertions or mutations were identified indicating complete homology of the sperm derived PR transcript to the conventional uterine PR transcripts. However, a study on testicular cDNA library screening has identified a novel exon in the DBD of the PR transcript (38). Contrasting these observations, our studies have failed to detect this exon by RTPCR in the sperm or testicular RNA samples (36). Interestingly, using a strategy similar to ours, Luconi *et al* (2000) (39) also found the transcripts of the DBD in the sperm cDNA population; however as compared to placental PR, the size of the DBD differs in sperm PR. At present it is unclear if the transcript detected by this group contains the additional exon reported by Hirata *et al* (2000) (38), but at other regions the transcript size of sperm PR was similar to conventional PR.

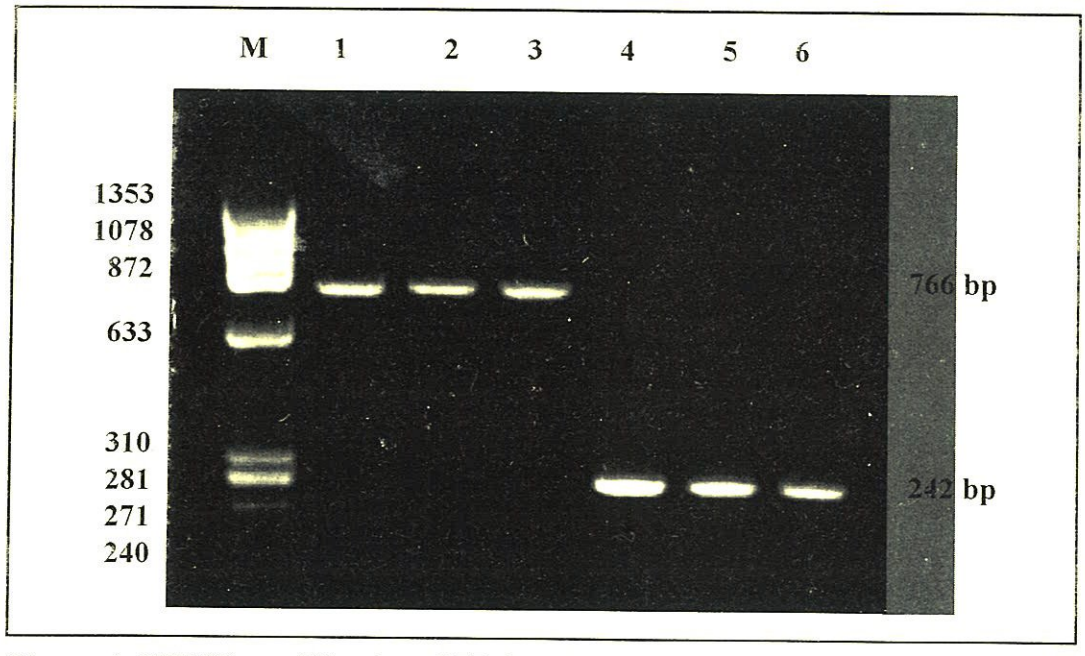


Figure 4: RTPCR amplification of PR from human testis and spermatozoa. Primers spanning the DNA and the hormone binding domain (lanes 1-3 and those unique for the region in the PR b isoform (Lanes 4-6) were used for PCR. Bands of expected sizes were generated in the testicular (lane 1 and 4) and sperm (lanes 2, 3, 5, 6) cDNA.

To rule if differences between the PR sequences at regions other than DBD and HBD, we amplified PR cDNA encoding for the extreme N terminal region to its entity (Fig 4). No differences were detected in the sequences of sperm derived PR as compared to the endometrium PR (AY382152, AY382151). These results confirm our notion that the sperm PR transcript may be identical to the conventional PR transcript. A similar conclusion has been drawn from studies on the PR transcript in xenopus oocytes. In this system, along with the nuclear PR, there exists a membrane bound PR that has non-genomic mode of action similar to that described in sperm (40). Sequence analysis of the PR transcript in these oocytes revealed its complete homology to the conventional PR (40) further substantiating the notion that at least at the transcript level, PR with non genomic mode of actions may be similar to PR having the conventional mode of action.

To summarize, our results indicate that the entire transcript for the genomic PR is present in human spermatozoa, however, in the absence of the genomic receptor in these cells suggests that no translation of PR transcripts occurs in these cells. The presence of these transcripts in mature human spermatozoa might not be an index of active synthesis, but simply reflects the transcription/translational activities during maturation processes in the testis. In the sperm possibly, post translational modification leading to a protein with a different molecular weight are operative for the non genomic actions.

Characterization of sperm membrane PR protein

Extensive studies have been carried out to determine the identity of the sperm membrane PR. Several proteins of apparently different molecular masses have been implicated as PR on human spermatozoa. Using an antibody directed against the C terminal of nuclear PR (C262 clone), a 52 kDa antigen has been found on the sperm head (41). The same antibody detected 4 bands of molecular masses of 28, 54, 57 and 66 kDa in the human sperm preparations (27). Interestingly, in the same study ligand blot assays detected only two bands of 54 and 57 kDa that could displaced by unlabelled progesterone or the C262 antibody indicating that the 54 and 57 kDa sperm membrane proteins are involved in progesterone binding. Corroborating these results, monoclonal antibodies against another epitope in the DBD or that recognizing a region within the N terminal domain of the nuclear PR identify a single band of ~55kDa in sperm lysates treated with digitonin(Fig 5A). Interestingly these antibodies block progesterone binding and kinase activation in human spermatozoa (37), indicating the presence of nuclear PR protein in human spermatozoa. Interestingly, an emerging school of thought is that the nuclear PR itself is capable of mediating rapid progesterone induced activation of signal transduction pathways in absence of gene transcription (42) (Fig 5B). The B isoform of the nuclear PR has a unique SH-3 interacting site (AF-3 domain) and is reported to be the

motif responsible for the rapid effects of progesterone (7, 43). We have recently demonstrated that PR-B mRNA is expressed in the human testis and spermatozoa (37, 44); the nuclear PR antibodies block progesterone mediated calcium influx and acrosome reaction (18,42). All these evidences are tantalizing and point towards the notion that the nuclear PR, probably the B isoform mediate the rapid non-genomic actions of progesterone in spermatozoa.

However it is intriguing to note that the protein recognized by various antibodies against the conventional PR in spermatozoa is of the size smaller (~55kDa) than the expected (90 or 120 kDa, Fig 5). It is possible that post transcriptional splicing of the nuclear PR may result in a truncated isoform which has conserved epitopes for antibody binding.

Alternate splicing/deletions/ insertions in the nuclear PR transcripts may lead to synthesis of a smaller protein (with conserved epitopes) that may bind to nuclear antibodies. Interestingly, several smaller forms of PR have been

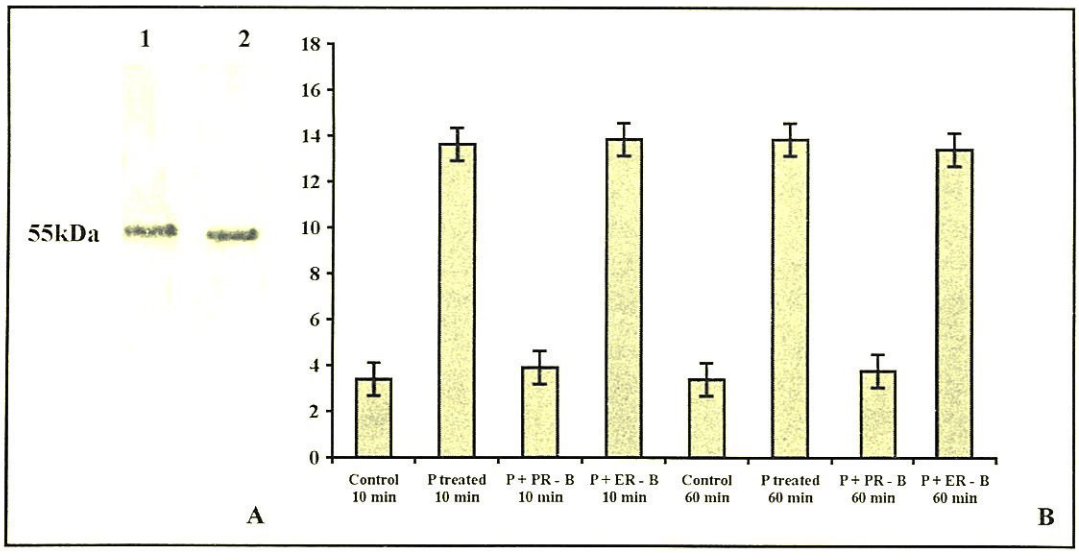


Figure 5: PR B isoform specific antibody identifies a 55kDa protein in human spermatozoa and blocks protein phosphorylation. A) Western blot of sperm lysates using PR-B specific antibody. A single band of ~55kDa was obtained (lane 1-2). B) Effect of PR-B antibody on kinase activity in human spermatozoa. Kinase activity is measured as the amount of 32P incorporated in sperm proteins within 10 or 60 min post progesterone treatment. Values are mean + SD (cpm x 1000) of 5 independent experiments. Digitonin treated sperm lysates were incubated with progesterone alone or along with PR-B or ER? antibody. The control reaction did not have progesterone (P = Progesterone).

identified in multiple tissues that are postulated to result from in-frame initiation of translation (45, 46). Many of these spliced variants generally have deletion of the DNA binding domain, the ligand-binding domain is generally conserved. Interestingly such variants are reportedly localized on the plasma membrane of cells (45). In spermatozoa, variants of nuclear PR transcripts which are predicted to translate a protein of lower molecular mass have been identified (38,47); although we have failed to confirm these findings (18,35,36,44).

Secondly, it is possible that the protein(s) recognized by the antibodies against the nuclear PR may be some other cross-reacting proteins that may only share structural motifs or immunological identity. In this context, it is interesting to note that a monoclonal antibody against the nuclear PR (c262) identified PAIRBP-1 that does not share homology with the nuclear PR (48,49). Interestingly, immunoprecpitation of sperm membrane proteins using an antibody against the conventional PR followed by 2D electrophoresis identified 3 protein spots of approximately 55kDa (data not shown). Database search of the sequence of the peptides obtained after tryptic digestion of the spots demonstrated homology to a protein unrelated to the nuclear PR (Modi, Shah and Puri unpublished data). These observations point towards the fact that the sperm PR may be an entity distinct from the nuclear PR.

Using different strategies and different model system, proteins unrealted to the nuclear PR that can be designated as the membrane PR have been identified (50-52). In search of specific steroid membrane-binding sites, two membrane progesterone- binding sites (mPR) from porcine liver microsomes with apparent *K*d values of 11 and 286 nM, have been cloned (50). Interestingly, human spermatozoa incubated with the specific antibody exhibited a significantly reduced progesterone induced calcium increase and inhibition of progesterone-mediated acrosome reaction by 62.1% (51). However, the site and size of this protein in spermatozoa is different than that speculated for the sperm membrane PR. While the sperm membrane PR is localized exclusively on the acrosome (18,20,41) the mPR antibody recognizes the protein post acrosomally and also in the midpiece (52). In Western blots, this antibody identifies a protein of approximately 40 kDa as against 50-60 kDa estimated for sperm PR. These results imply that the progesterone membrane-binding protein in spermatozoa may be closely similar but not identical to the porcine liver mPR.

Another novel class of transmembrane G protein coupled receptor (GPCR, accession no. AF 313615-AF 313620) that bind specifically to progesterone but not to antiprogestins have been identified (53,54). These proteins, identified from fish oavries, have multiple human homologues that are predicted to encode a protein with seven transmembrane domains (49). One of the isoform of GPCR activates progesterone

mediated signal in cells not responsive to progesterone (53,54). However, sperm membrane PR has not been demonstrated to be a GPCR, instead G protein inhibitors failed to block progesterone mediated acrosome reaction (42). Thus, the relevance of this class of protein in context to sperm membrane PR that mediates acrosome reaction needs to be assessed.

Another candidate membrane PR has been identified in rat granulosa cells. Referred as PAI-1 mRNA binding protein 1 (PAIRBP1; accession number XM 216160), is reported to be localized on the cell membrane and overexpression of PAIRBP1 in granulosa cells increases progesterone binding and its responsiveness (51). In addition, an antibody against PAIRBP1 ablates progesterone mediated biological actions in granulosa cells. However, nothing is known about this protein in humans and its relevance to sperm membrane PR is not explored.

Although, the above studies demonstrate the existence of the membrane PR which is distinct from the nuclear PR, in all these cases the definition of the role of this molecule as the modulator of progesterone actions awaits experimental investigations using transgenic and knockout approaches. However, the differences in the experimental systems used, precludes us from making strong conclusions on the identity of the membrane PR that is responsible for the rapid actions of progesterone in spermatozoa studies are needed to identify the membrane PR that mediate the non genomic action and regulate acrosome reaction in sperm.

Clinical relevance of sperm progesterone receptors

Considering the facilitatory role of progesterone in sperm functions, specifically acrosome reaction, it is conceivable that defective progesterone binding or function in spermatozoa might account for some cases of male infertility particularly those associated with acrosome reaction defects (55,56). Indeed, an association between defective progesterone activity and reduced fertility has been demonstrated. Subnormal response of progesterone on calcium influx and acrosome reaction has been demonstrated in spermatozoa from oligozoospermic men and in spermatozoa from male partners of unexplained infertility (57-61). Along with defective responsiveness, the binding of progesterone on sperm membrane is also altered in men with abnormal spermiograms. As compared to fertile men, significant decrease in the number of progesterone binding spermatozoa was observed in men suffering from oligozoospermia, asthenozoospermia, oligoasthenozoospermia and teratozoospermia (20). A weak positive correlation was observed between the percentage of progesterone binding spermatozoa and the traditional semen parameters like sperm motility and morphology. Interestingly, a strong positive correlation was observed between the number of progesterone binding spermatozoa and the percentage of hypo-

osmotic swelling test positive and acrosome reacting spermatozoa from normozoospermic males and also in men with abnormal spermiograms (20). These observations suggest that assessment of progesterone binding and progesterone-mediated calcium influx in spermatozoa can serve as markers to diagnose men with dysfunctional acrosome reaction. The priming effect of progesterone on ZP-mediated acrosome reaction (4) as well as the enhancing effect of progesterone on oocyte penetration in infertile patients (59) are further evidences pointing towards the role of progesterone in fertilization.

Summary and future directions

Adequate experimental data exist demonstrating the presence of a membrane bound protein that mediates rapid non-genomic mode of progesterone actions in spermatozoa to regulate acrosome reaction. Studies are needed to decipher the identity of this protein and if this receptor (protein) is the true membrane PR involved in progesterone mediated acrosome reaction. The discovery of the identity of the sperm PR would enable detailed studies to be conducted at the molecular level on the membrane receptor system, its physiological relevance and its interactions with the nuclear receptors.

The understanding of the nongenomic signaling pathways for PR and also other steroid hormone receptor would represent a highly promising and exciting new scientific horizon. Non-genomic signaling mechanism would represent the system through which steroids would rapidly activate cellular functionality needs to accommodate dynamic changes in the surrounding milieu. These mechanisms would rapidly enable the cells to use the tools, which are already present and get functionally activated or repressed. The effects through this pathway and would be determinants of the immediate and long-lasting modification of cell program.

However, the non-genomic steroid hormone receptor signaling is still a conundrum. Although, the work in recent years has highlighted the molecular basis of some of these actions, a large number of rapid effects exerted in different tissues and cell types still have to be fully characterized. The rapid actions of steroid hormones pose number of important questions that needs to be addressed. It needs to be determined whether conditions exist where modifications of the local steroid concentrations could trigger the nongenomic signaling *in vivo*. It is presumable that in some ways these tissues could be sensitive to either general or local hormonal variations, which would lead to the non-genomic effects. In this context, a possibility of activating or repressing some steroid hormone receptor actions would open newer avenues for designing drugs which could differentially recruit transcriptional and non-transcriptional effects on selected tissues. Thus understanding the molecular mechanism through which the nongenomic actions represent an

important frontier in engineering newer pharmacological tools for prevention and treatment of a wide range of diseases including infertility. These molecules in reverse could be also used as contraceptives.

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References

1. Wassarman PM, Jovine L, Qi H, Williams Z, Darie C and Litscher ES (2005). Recent aspects of mammalian fertilization research *Molecular and Cellular Endocrinology* **234:** 95-103.
2. Kopf GS and Gerton GL (1991). The mammalian sperm acrosome and the acrosome reaction. *Elements of mammalian fertilization,* 153-203
3. Baldi E, Krausz C, Luconi M, Bonaccorsi L, Maggi M and Forti G (1995). Actions of progesterone on human sperm: a model of non-genomic effects of steroids. *Journal of Steroid Biochemistry and Molecular Biology* **53**: 199–203
4. Roldan ER, Murase T and Shi QX (1994). Exocytosis in spermatozoa in response to progesterone and zona pellucid. *Science* **266**: 1578– 1581
5. Osman RA, Andria ML, Jones AD and Meizel S (1989). Steroid induced exocytosis: the human sperm acrosome reaction. *Biochemical Biophysical Research Communication* **160:** 828-833
6. Calogero AE, Burrello N, Barone N, Palermo I, Grasso U and D'Agata R (2000). Effects of progesterone on sperm function: mechanism of action. *Human Reproduction* **15**: 28-45
7. Leonhardt SA, Boonyaratnakornkit V and Edwards D (2003) Progesterone receptor transcription and non transcription signaling mechanisms. *Steroids* **68**: 761-770
8. Losel R and Wehling M (2003). Nongenomic actions of steroid hormones. *Nature (Molecular and Cell Biology)* **4**: 46-56
9. Losel RM, Falkenstein E, Feuring M, Schultz A, Tillmann HC, Rossol-Haseroth K and Wehling M (2003). Nongenomic steroid action: controversies, questions, and answers. *Physiological Reviews* **83:** 965-1016
10. Harper CV and Publicover SJ (2005). Reassessing the role of progesterone in fertilization-compartmentalized calcium signaling in human spermatozoa? *Human Reproduction* **20**: 2675-2680
11. Kirkman-Brown JC, Bray C, Stewart PP, Barratt CLR and Publicover SJ (2000). Biphasic elevation of [Ca2+] in individual human spermatozoa

exposed to progesterone. *Developmental Biology* **222**: 326-335

12. Meizel S, Turner KO and Nuccitelli R (1997). Progesterone triggers a wave of increased free calcium during the human sperm acrosome reaction. *Developmental Biology* **182**: 67-75
13. Tesarik J and Mendoza C (1993). Insights into the functions of a sperm surface progesterone evidence of ligand–induced receptor aggregation and the proteolysis. *Experimental Cell Research* **205:** 111-117
14. Tesarik J, Moos J and Mendoza C (1993). Stimulation of protein tyrosine phosphorylation by a progesterone receptor on the cell surface of human sperm. *Endocrinology* **133:** 328-335
15. Parinaud J and Milhet P (1996). Progesterone induces Ca11-dependent 39, 59-cyclic adenosine monophosphate increase in human sperm. *Journal of Clinical Endocrinology and Metabolism* **81:** 1357–1360
16. Meizel S and Turner KO (1991). Progesterone acts at the plasma membrane of human sperm. *Molecular and Cellular Endocrinology* **77:** R1–R5
17. Ambhaikar MB and Puri CP (1998). Cell surface binding sites for progesterone on human spermatozoa. *Molecular Human Reproduction* **4:** 413-421
18. Shah CA, Modi D, Gadkar S, Sachdeva G and Puri CP (2003). Progesterone receptors on human spermatozoa. *Indian Journal of Experimental Biology* **41:** 773-780
19. Blackmore PF and Lattanzio F (1991). Cell surface localization of a novel non-genomic progesterone receptor on the head of human sperm. *Biochemical and Biophysical Research Communication* **181:** 331–336
20. Gadkar S, Shah CA, Sachdeva G, Samant U and Puri CP (2002). Progesterone receptor as an indicator of sperm function. *Biology of Reproduction* **67:** 1327-1336
21. Plant A, McLaughlin EA and Ford WCL (1995). Intracellular calcium measurements in individual human sperm demonstrate that the majority can respond to progesterone. *Fertility and Sterility* **64:** 1213–1215
22. Losel R, Dom-Beineke A, Falkenstein, F and Wehling, M. (2004). Porcine spermatozoa contain more than one membrane progesterone receptor. *International Journal of Biochemistry and Cell Biology* **38:** 1532-1541
23. Langlais J. Kan F W K, Granger L, Bleau G, Roberts K D (1987). Identification of sterol receptors that stimulate cholesterol efflux from human spermatozoa during *in vitro* capacition. *Gamete Res.* **20:** 185-189
24. Calogero AE, Burrello N, Barone N, Palermo I, Grasso U and D'Agata R. (1999). γ–Aminobutyric acid (GABA) A and B receptors mediate the

stimulatory effects of GABA on the human sperm acrosome reaction: interaction with progesterone. *Fertility and Sterility* **71:** 930-936

25. Wistrom AC and Meizel S (1993). Evidence suggesting involvement of a unique human sperm steroid receptor/Cl2 channel complex in the progesterone-initiated acrosome reaction. *Developmental Biology* **159:** 679–690

26. Baldi E, Luconi M, Bonaccorsi L, Krausz C and Forti G (1996). Human sperm activation during capacitation and acrosome reaction: role of calcium, protein phosphorylation and lipid remodeling pathways. *Frontiers in Bioscience* **1**: 189-205

27. Luconi M, Bonnaccorsi L, Maggi M, Pecchioli P, Krausz, C, Forti G and Baldi E (1998). Identification and characterization of functional nongenomic progesterone receptors on human sperm membrane. *Journal of Clinical Endocrinology and Metabolism* **83:** 877-885

28. Baldi E, Casano R, Falsetti C, Krausz C, Maggi M and Forti G (1991). Intracellular calcium accumulation and responsiveness to progesterone in capacitating human spermatozoa. *Journal of Andrology* **12:** 323–330

29. Revelli A, Massobrio M and Tesarik J (1998). Nongenomic actions of steroid hormones in reproductive tissues. *Endocrine Reviews* **19:** 3-17

30. Harper CV, Kirkman –Brown JC, Barratt CL and Publicover SJ (2003). Encoding of progesterone stimulus intensity by intracellular (Ca2+) and (Ca2+)ions in human spermatozoa. *Biochemical Journal* **372:** 407-417

31. Alexander NJ, Kim HK, Blye RR and Blackmore PF (1996). Steroid specificity of the human sperm membrane progesterone receptor. *Steroids* **61:** 116-125

32. Yang J, Serres C, Philbert D, Robel P, Baulieu EE and Jouanner P (1994). Progesterone and RU486: Opposing effects on human sperm. *Proceedings of National Academy of Science USA* **91:** 529-533

33. DeManno D, Elger W, Garg R, Lee R, Schneider B, Hess-Stumpp H, Schubert G and Chwalisz K. (2003). Asoprisnil (J867): A selective progesterone receptor modulator for gynecological therapy. *Steroids* **68:** 1019-32

34. Modi D, Shah CA, Sachdeva G, Gadkar S, Bhartiya D and Puri C (2005). Ontogeny and cellular localization of SRY transcripts in the human testes and its detection in spermatozoa. *Reproduction* **130:** 603-613

35. Sachdeva G, Shah CA, Kholkute SD and Puri CP (2000). Detection of progesterone receptor transcript in human spermatozoa. *Biology of Reproduction* **62:** 1610-1614

36. Sachdeva G, Gadkar S, Shah CA, Kholkute SD and Puri CP (2005). Characterization of a critical region

in the hormone binding domain of sperm progesterone receptor. *International Journal of Andrology* **28**, 120-124

37. Shah CA, Modi D, Sachdeva G, Gadkar-Sable S, D'Souza S and Puri, CP (2005a). N-terminal region of progesterone receptor B isoform in human spermatozoa. *International Journal of Andrology* **28:** 360-371

38. Hirata S, Shoda T, Kato J and Hoshi K (2000). The novel isoform of the progesterone receptor cDNA in the human testis and detection of its mRNA in the human uterine endometrium. *Oncology* **39:** 39-44

39. Luconi M, Bonaccorsi L, Bini L, Liberatori S, Pallini V, Forti G and Baldi E (2002). Characterization of membrane nongenomic receptor for progesterone in human spermatozoa. *Steroids* **67:** 505-509

40. Bayaa M, Booth RA, Sheng Y, Liu XJ (2000). The classical progesterone receptor mediates Xenopus oocyte maturation through a nongenomic mechanism. *Proc Natl Acad Sci USA* **97**: 12607-12.

41. Sabeur K, Edwards DP and Meizel S (1996). Human sperm plasma membrane progesterone receptor(s) and the acrosome reaction. *Biology of Reproduction* **54:** 993-1001

42. Luconi M, Carloni V, Marra F, Ferruzzi P, Forti G and Baldi E (2004). Increased phosphorylation of AKAP by inhibition of phosphatidylinositol 3-kinase enhances human sperm motility through tail requirement of protein kinase. *American Journal of Cell Science* **117:** 1235-1246

43. Boonyaratnakornkit V, Scott MP, Ribbion V, Sherman L, Anderson SM and Miller WT (2001). Progesterone receptor contains proline rich motif that directly interacts with SH3 domains and activates c-Src family tyrosine kinase. *Molecular Cell* **8:** 269-280

44. Shah CA, Modi D, Sachdeva G, Gadkar S and Puri CP (2005b). Coexistence of nuclear and membrane bound progesterone receptor in human testis. *Journal of Clinical Endocrinology and Metabolism* **90:** 474-483

45. Saner KJ, Welter BH, Zhang F, Hansen E, Dupont B, Wei Y and Price TM (2003). Cloning and expression of a novel, truncated, progesterone receptor. *Molecular and Cellular Endocrinology* **200:** 155-163

46. Wei LL and Minner R (1994). Evidence for the presence of third progesterone protein in human breast cancer cell line T47D. *Cancer Research* **54:** 340-343

47. Hirata S, Shoda T, Kato J and Hoshi K (2003). Novel isoform of the mRNA for human female sex steroid hormone receptors. *Journal of Steroid Biochemistry and Molecular Biology* **83:** 25-30

48. Peluso JJ (2004). Rapid actions of progesterone on granulosa cells. *Steroids* 69: 579-583

49. Peluso JJ, Pappalardo, A, Fernandez G and Wu CA (2004). Involvement of an unnamed protein, RDA288 in the mechanism through which progesterone mediates its antiapoptotic actions in spontaneously immortalized granulosa cells. *Endocrinology* **145:** 3014-3022

50. Falkenstein E, Meyer C, Eisen C, Scriba PC and Wehling M (1996). Full length cDNA sequence of a progesterone membrane-binding protein from porcine vascular smooth muscle cells. *Biochemical Biophysical Research Communication* **229:** 86-89

51. Peluso JJ, Pappalardo A, Losel R and Wehling M (2005). Expression and function of PAIRBP1 within gonadotropin primed immature rat ovaries: PAIRBP1 regulation of granulosa cells and luteal cell viability. *Biology of Reproduction* **73:** 261-270

52. Buddhikot M, Falkenstein E, Wehling M and Meizel S (1999). Recognition of a human surface protein involved in the progesterone initiated acrosome reaction by antisera against an endomembrane progesterone binding protein from porcine liver. *Molecular and Cellular Endocrinology* **158:** 187-193

53. Zhu Y, Bond J and Thomas P (2003a). Identification, classification and partial characterization of genes in humans and other vertebrates homologues to a fish membrane progestin receptor. *Proceedings of National Academy of Science USA* **100:** 2237-2242

54. Zhu Y, Rice CD Pang Y Pace M and Thomas P (2003b). Cloning expression and characterization of a membrane progestin receptor and evidence it is an intermediary in meiotic maturation in fish oocytes. *Proceedings of National Academy of Science USA* **100:** 2331-2363

55. Forti G , Baldi E, Krausz C, Luconi M, Bonaccorsi L, Maggi M, Bassi F and Scarselli G (1999). Effect of progesterone on human spermatozoa: clinical implications. *Annals of Endocrinology (Paris)* **60:** 107-110

56. Benoff S, Barcia M, Hurley IR, Cooper GW, Mandel FS, Heyner BR and Hershlag A (1996). Classification of male factor infertility relevant to in-vitro fertilization insemination strategies using mannos ligands, acrosome status and anti-cytoskeletal antibodies. *Human Reproduction* **11:** 1905-1991

57. Tesarik J and Mendoza C (1992). Defective function of a nongenomic progesterone receptor as a sole sperm anomaly in infertile patients. *Fertility and Sterility* **58:** 793–797

58. Falsetti C, Baldi E, Krausz C, Casono R, Failli P and Forti G (1993). Decreased responsiveness to progesterone of spermatozoa in

oligozoospermic patients. *Journal of Andrology* **14:** 17-22

59. Oehninger S, Blackmore PF, Morshedi Ml, Sueldo C, Acosta A and Alexander NJ (1994). Defective Calcium influx and acrosome reaction in spermatozoa of infertile men with severe teratozoospermia. *Fertility and Sterility* **61:** 349-354

60. Baldi E, Luconi M, Bonaccorsi L and Forti G (1998). Nongenomic effects of progesterone on spermatozoa: mechanisms of signal transduction and clinical implications. *Froniers in Bioscience* **3**: d1051-d1059

61. Giojalas LC, Iribaren P, Molina R, Ravasio RA, and Estifan D (2004). Determination of human sperm calcium uptake mediated by progesterone may be useful for evaluating unexplained sterility. *Fertility and Sterility* **82:** 738-740