Pergamon

0960-0760(95)00046-1

J. Steroid Biochem. Molec. Biol. Vol. 53, No. 1–6, pp. 199–203, 1995

0960-0760/95 $9.50 + 0.00

Actions of Progesterone on Human Sperm: a Model of Non-genomic Effects of Steroids

Elisabetta Baldi,* Csilla Krausz, Michaela Luconi, Lorella Bonaccorsi, Mario Maggi and Gianni Forti

Unita' di Andrologia, Dipartimento di Fisiopatologia Clinica, Universita' di Firenze, 50139 Firenze, Italy

Non-genomic actions of steroids have been extensively studied in the last few years. Among these actions, the non-genomic effect of progesterone (P) on human spermatozoa appears to be very promising, in view of the dramatic effect of this steroid on intracellular calcium, activation of tyrosine kinase, and induction of acrosome reaction. We have shown that the ability of spermatozoa to respond to P increases during the process of capacitation and is not counteracted by the P-receptor antagonist RU486 nor by the $GABA_A$ antagonists bicuculline and picrotoxin. We have also shown that P increases tyrosine phosphorylation of a sperm protein of about 97 kDa, suggesting activation of tyrosine kinase(s). In addition, we found that P induces a perturbation of sperm membrane phospholipid metabolism resulting in an increase of synthesis of platelet-activating factor and liberation of arachidonic acid. Results of these biochemical studies indicate that P is able to stimulate several signal transduction pathways in human sperm. We have also investigated responsiveness to P in sperm of oligozoospermic subjects as well as of men undergoing an *in vitro* fertilization (IVF) program. Our results show that the percentage increases of intracellular calcium and acrosome reaction in response to P is significantly reduced in oligozoospermic men as well as in subjects with reduced fertilization rate. Moreover, in the latter subjects response to P is highly significantly correlated to fertilization rate of oocytes. These studies indicate that a biochemical alteration of sperm in their capacity to respond to P might be responsible for reduced fertilizing ability

J. Steroid Biochem. Molec. Biol., Vol. 53, No. 1–6, pp. 199–203, 1995

INTRODUCTION

In recent years, many studies focused on rapid, non-genomic actions of steroids on different cell types. In particular, as recently reviewed [1, 2], testosterone, progesterone, glucocorticoids, vitamin D, estrogens and aldosterone have been reported to increase intracellular free calcium concentrations ($[Ca^{2+}]_i$) as well as to exert other non-genomic actions in various cell types. The rapidity of such effects excludes the possibility of an involvement of genomic receptors. Among these effects, the non-genomic actions of progesterone (P) on male (for reviews see [3, 4]) and female [5–7] gametes have been deeply investigated. These studies indicate that besides increase of $[Ca^{2+}]_i$, P can induce perturbations of lipid membrane metabolism (due to activation of phospholipases) resulting in increases of inositol phosphates, diacylglycerol and other lipid mediators. Our group has focused on both signal transduction effects of P on mature human sperms as well as on functional involvement of sperm responsiveness to P in male pathological conditions and its relation with fertilization of oocytes. In addition, we have studied the effects of P on the synthesis of the phospholipid platelet-activating factor (PAF) by human sperm.

SIGNAL TRANSDUCTION MECHANISMS OF PROGESTERONE IN HUMAN SPERM

It is generally accepted that an increase of $[Ca^{2+}]_i$ is an obligatory step in the complex mechanisms leading to capacitation (which physiologically occurs during transit in the female genital tract) and acrosome reaction of mammalian sperms (for review see [8]). P is present in high concentrations in the follicular fluid as well as the cumuls matrix of the oocyte [9] thus being a possible physiological stimulus for the spermatozoon during transit in the female genital tract and at the

Proceedings of the IX International Congress on Hormonal Steroids, Dallas, Texas, U.S.A., 24–29 September 1994.
*Correspondence to E. Baldi.

moment of oocyte fertilization. As mentioned above, P and its metabolite 17-α-hydroxyprogesterone induce a rapid, dramatic, long-lasting influx of calcium in human sperm ([10–12], Fig. 1), which ultimately results in induction of acrosome reaction [9, 13]. We have shown that $[Ca^{2+}]_i$ and responsiveness to P (increase of calcium and acrosome reaction) concurrently increase in human sperm during *in vitro* capacitation [13], suggesting that the ability to respond to P is acquired during this process. In addition, we have shown [13] that such an effect is not antagonized by 1 μM concentration of the potent antiprogestin agent RU486 (Fig. 1), further substantiating the involvement of a non-genomic mechanism in P action, although this antagonist was able to decrease sperm intracellular calcium (Fig. 1). Yang *et al.* [14] have recently shown that RU486, at concentrations of 10 μM and higher, is able to inhibit the effect of P on $[Ca^{2+}]_i$ and induces a decrease of calcium in human sperm. We have also shown that the $GABA_A$ receptor antagonists bicuculline and picrotoxin, which are very potent in inhibiting non-genomic effects of P in the central nervous system [15, 16], do not inhibit P-mediated sperm calcium increase [13], nor GABA and other $GABA_A$ agonists have any effect on sperm calcium or modify response to P [13]. On the other hand, P-stimulated calcium influx in human sperm is not inhibited by antagonists of voltage-dependent calcium channels [11]. Taken together, these results suggest the possible involvement of a receptor-operated calcium channel in the non-genomic effect of P on human sperm [10, 3]. Beside an increase of $[Ca^{2+}]_i$, P activates other signal transduction pathways in human sperms, namely phospholipase C [10], and tyrosine kinase [17]. Tyrosine kinase activation of spermatozoa has been shown to play an important role at the time of oocyte fertilization at least in the mouse [18]. We have shown that an increase of protein phosphorylation in tyrosine residues occurs both during *in vitro* capacitation [Fig. 2(a)] and following stimulation of capacitated human sperm with P and PAF [Fig. 2(b)]. In particular, P and platelet-activating factor (PAF) increase tyrosine phosphorylation of two proteins, respectively of about 75 and 97 kDa [19]. Such an effect appears to be related to acrosome reaction, since two tyrosine kinase inhibitors, genistein and erbstatin, were able to antagonize both P- and PAF-stimulated acrosomal exocytosis [19].

P-MEDIATED STIMULATION OF PAF SYNTHESIS IN HUMAN SPERM

PAF is a potent pro-inflammatory lipid mediator recently detected in mammalian sperm, able to stimulate motility and other sperm functions (for review see [20]). PAF is also present in follicular fluid [21] and in the endometrium [20], thus being another possible physiological stimulus of sperm activity. The ability of sperm to synthesize PAF had not been investigated in previous studies [20] and it was not known whether PAF synthesis could be enhanced by agents able to stimulate acrosome reaction and other sperm functions. We therefore studied the effects of P and the ionophore A23187 on the remodelling pathway of PAF synthesis (measured as incorporation of [^{3}H]acetate into PAF) by human sperm, particularly focusing on two key enzymes of this route, namely lyso-PAF:acetyl-Coa-acetyltransferase and phospholipase A2 [22]. In initial

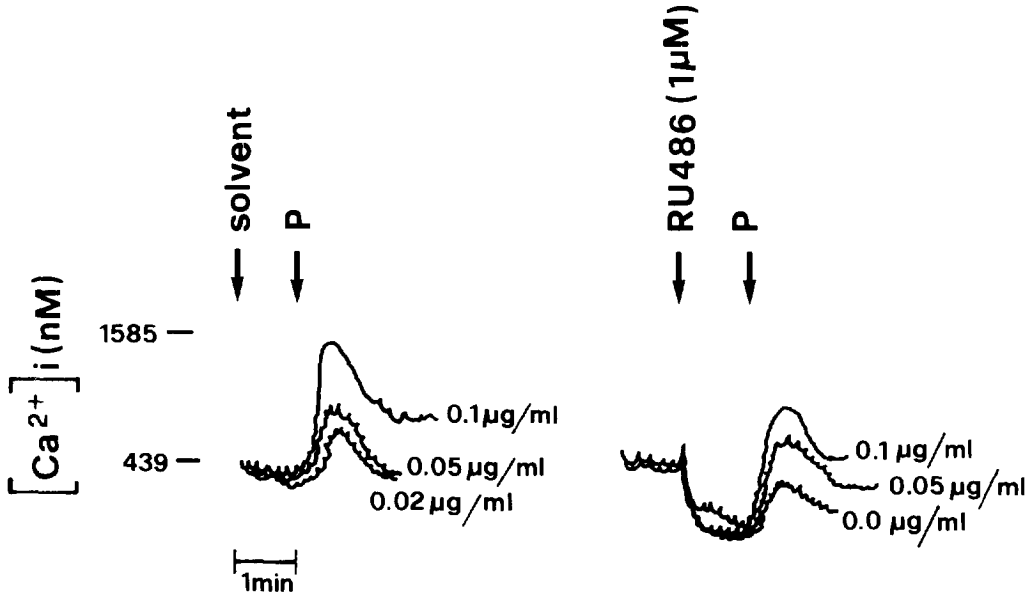
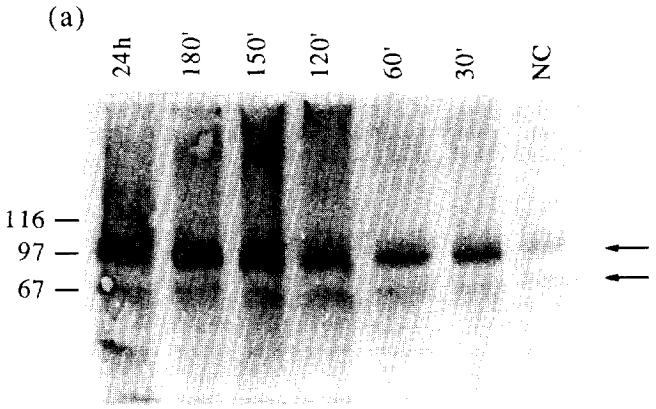


Fig. 1. Effect of increasing concentrations of P on $[Ca^{2+}]_i$ in fura 2-loaded capacitated human spermatozoa in the absence (left) and in the presence (right) of a 1 μM concentration of RU486.

studies, we partially characterized sperm lyso-PAF: acetylCoa-acetyltransferase activity, which showed similar kinetic parameters as those found in other cell types [22]. In addition we demonstrated that PAF synthesis in these cells was time-dependent, with a maximum increase at 60 min incubation [22]. Most of this newly synthesized PAF was found in the extracellular medium (Fig. 3), indicating an active release of the phospholipid by spermatozoa [22]. We also demonstrated that short-term incubation with P and A23187 enhanced synthesis and release of PAF by human sperm (Fig. 3). Moreover, we showed that P and A23187 are able to stimulate lyso-PAF:acetylCoa-acetyltransferase as well as phospholipase A2 activity (measured as release of [^{3}H]arachidonic acid from prelabeled sperm) [22], thereby demonstrating an activation of the lipid remodelling pathway by P in human sperm. In further studies from our laboratory, we found that low concentrations of PAF significantly enhanced total and progressive motility as well as acrosome reaction of human sperm [23] suggesting the possibility of an autocrine mechanism of PAF action in these cells. In addition, the increase of PAF synthesis seems to be involved, at least in part, in the mechanism of P-stimulated acrosome reaction; indeed, we have shown that the PAF receptor antagonist L659,989 inhibits this P action [23].



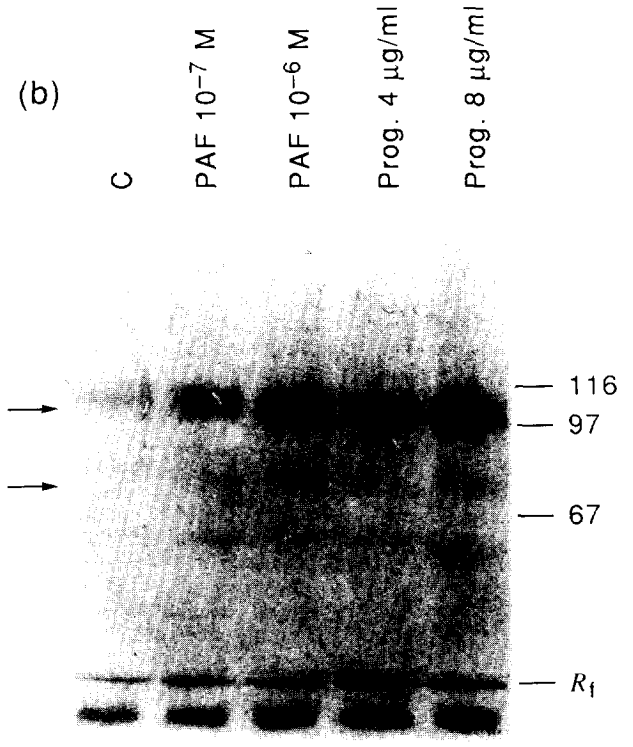


Fig. 2. (a) Western blot analysis of the reactivity of anti-phosphotyrosine antibody with human sperm proteins at various times of *in vitro* capacitation. NC indicates non-capacitated sample. (b) Effect of PAF (0.1 and 1 µM, 5 min incubation) and progesterone (Prog, 4 and 8 µg/ml, 2 min incubation) on tyrosine phosphorylation of human sperm proteins. C indicates unstimulated sample. The antibody reactivity was followed by incubation with alkaline phosphatase conjugated goat anti-rabbit antibody and stained with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as substrate. Molecular weight markers ($\times 10^3$) are indicated to the left (a) and to the right (b) of the blots. The arrows indicate the 97 and the 75 kDa phosphoproteins.

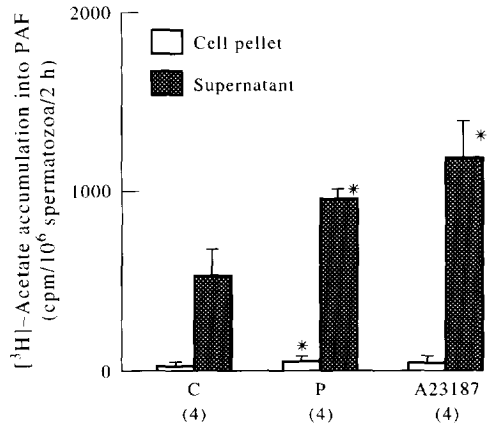


Fig. 3. Remodelling pathway of PAF synthesis ([^{3}H]acetate incorporation into PAF) in human spermatozoa in basal conditions (C) and following 2 h stimulation with progesterone (P, 10 µg/ml) and A23187 (10 µg/ml). After incubation, lipids were separately extracted from cell pellet and supernatant and the amount of radioactive PAF evaluated in both compartments. Reproduced from Baldi *et al.*, *Biochem. J.*, 292 (1993) 209–213, with permission.

SPERM RESPONSIVENESS TO P IN PATHOLOGICAL CONDITIONS AND ITS RELATION WITH *IN VITRO* FERTILIZATION OF OOCYTES

Reduced fertilization of human oocytes has often been associated with oligo-asthenozoospermia [24]. Although the use of modern techniques to select motile spermatozoa (Percoll gradients, swim up) in *in vitro* fertilization (IVF) programs should overcome problems related to poor number or motility of individual sperm samples, this does not appear to be the case [25], and about 25% of poor IVF outcome is due to male factor (astheno-oligozoospermia and unexplained infertility) [25]. In this light, we hypothesized that

a defective sperm function (rather than sperm number) could be responsible for poor fertilization in these subjects. In particular, we investigated whether responsiveness to P (increase of $[Ca^{2+}]_i$ and acrosome reaction) could be impaired in these subjects. In addition, we studied the relationship between response to P and *in vitro* fertilization of oocytes.

We first studied responsiveness to P in a group of 17 oligozoospermic subjects with reduced sperm motility, compared to 19 normozoospermic men. The average percentage increase of $[Ca^{2+}]_i$ in response to P was significantly lower in oligozoospermic men (138.7 ± 8.2 vs 263.3 ± 39.7 in normospermic, $P < 0.001$) [26]. The increase of calcium induced by P was significantly correlated with sperm motility, concentration and percentage of normal forms [26]. In addition, we found that the ability of P to induce acrosome reaction was severely impaired in oligozoospermic men when compared to normospermic, whereas similar responses were obtained when the ionophore A23187 was used to stimulate sperms [26]. These results have been also confirmed by other authors [27, 28] and indicate that sperm from oligozoospermic men are characterized by a reduced ability to initiate the cascade of events which ultimately results in acrosome reaction, in response to a physiological stimulus, such as P, and might contribute in explaining the reduced fertilizing ability of these subjects. Whether this reduced responsiveness to P is due to reduced sperm receptor concentration or to altered signal transduction pathway has not been addressed by these studies and awaits further investigation. We next studied responsiveness to P in a group of 22 unselected subjects admitted to the IVF program, and compared $[Ca^{2+}]_i$ and acrosome reaction increases in response to P to percentage fertilization rate (%FR) of these men. We found a significant correlation between %FR and $[Ca^{2+}]_i$ ($r = 0.78$) as well as acrosome reaction ($r = 0.79$) increases in response to P [29]. Moreover, the two parameters were significantly higher in subjects with %FR > 50 (Table 1) [29]. These results indicate that responsiveness to P is of functional importance with respect to fertilization of the oocyte, and prospect the use of $[Ca^{2+}]_i$ or acrosome reaction in response to P as predictive tests in IVF programs.

In conclusion, non-genomic actions of P on spermatozoa seem to play an important role in activation of these cells during fertilization, and can be considered a good marker of fertilizing ability of human sperm.

Table 1. Percentage $[Ca^{2+}]_i$ increase and acrosome reaction in response to progesterone (0.1 μg/ml for calcium and 4 μg/ml for acrosome reaction) in IVF subjects with a fertilization rate (FR) > or < 50%

	% increase $[Ca^{2+}]_i$	ARPC*
FR > 50%	227.1 ± 40.7 ($n = 9$)	15.7 ± 2.9 ($n = 6$)
FR < 50%	43 ± 22.8† ($n = 7$)	5.8 ± 1.6† ($n = 10$)

*Acrosome reaction following progesterone challenge (percentage acrosome reaction following progesterone minus basal acrosome reaction).

†$P < 0.005$ vs FR > 50%; data are expressed as percentage ± SEM.

Acknowledgements—This work was supported by Grants from Universita' di Firenze, Ministero dell'Universita' e della Ricerca Scientifica e Tecnologica, and from Consiglio Nazionale delle Ricerche (CNR, Rome, Targeted Project: FATMA, contract No. 92.00097-PF41). We are greatly indebted to Professor Mario Serio (Unita' di Endocrinologia, Universita' di Firenze), for his precious help in developing this project.

REFERENCES

1. Nemere I. and Norman A. W.: Steroid hormone actions at the plasma membrane: induced calcium uptake and exocytotic events. *Molec. Cell. Endocr.* **80** (1991) C165–C169.
2. Griffing G. T.: Dinosaurs and steroids. *J. Clin. Endocr. Metab.* **77** (1993) 1450–1451.
3. Blackmore P. F. Rapid non-genomic actions of progesterone stimulate Ca^{2+} influx and the acrosome reaction in human sperm. *Cell. Signal* **5** (1993) 531–538.
4. Revelli A., Modotti M., Piffaretti-Yanez A., Massobrio M. and Balerna M.: Steroid receptors in human spermatozoa. *Human Reprod.* **9** (1994) 760–766.
5. Chien E. J., Kostellow A. B. and Morrill G. A.: Progesterone induction of phospholipid methylation and arachidonic acid turnover during the first meiotic division in amphibian oocytes. *Life Sci.* **39** (1986) 1501–1508.
6. Chien E. J., Morrill G. A. and Kostellow A. B.: Progesterone-induced second messengers at the onset of meiotic maturation in the amphibian oocyte: interrelationships between phopholipid N-methylation, calcium and diacylglycerol release, and inositol phospholipid turnover. *Molec. Cell. Endocr.* **81** (1991) 53–67.
7. Stith B. J., Kirkwood A. J. and Wohnlich E.: Insulin-like growth factor 1, insulin, and progesterone induce early and late increases in *Xenopus* oocyte sn-1,2-diacylglycerol levels before meiotic cell division. *J. Cell, Physiol.* **149** (1991) 252–259.
8. Yanagimachi R.: Mammalian fertilization. In *The Physiology of Reproduction* (Edited by E. Knobil and J. P. Neill). Raven Press, NY (1994) pp. 135–185.
9. Osman R. A., Andria M. L., Jones A. D. and Meizel S.: Steroids induced exocytosis: the human sperm acrosome reaction. *Biochem. Biophys. Res. Commun.* **160** (1989) 829–833.
10. Thomas P. and Meizel S.: Phosphatidylinositol 4,5-bisphosphate hydrolysis in human sperm stimulated with follicular fluid or progesterone is dependent upon calcium influx. *Biochem. J.* **264** (1989) 539–546.
11. Blackmore P. F., Beebe S. J., Danforth D. R. and Alexander N.: Progesterone and 17alpha-hydroxyprogesterone: novel stimulators of calcium influx in human sperm. *J. Biol. Chem.* **265** (1990) 1367–1380.
12. Blackmore P. F., Neulen J., Lattanzio F. and Beebe S. J.: Cell surface-binding sites for progesterone mediate calcium uptake in human sperm. *J. Biol. Chem.* **266** (1991) 18,655–18,659.
13. Baldi E., Casano R., Falsetti C., Krausz Cs., Maggi M. and Forti G.: Intracellular calcium accumulation and responsiveness to progesterone in capacitating human spermotazoa. *J. Androl.* **12** (1991) 323–330.
14. Yang J., Serres C., Philibert D., Robel P., Baulieu E-E. and Jouannet P.: Progesterone and Ru486: opposing effects on human sperm. *Proc. Natn. Acad. Sci. U.S.A.* **91** (1994) 529–533.
15. Brann D. W., Putnam C. D. and Mahesh V. B.: Gamma-aminobutyric acid receptors mediate 3alpha-hydroxy-5alpha-pregnan-20-one induced gonadotropin secretion. *Endocrinology* **126** (1990) 1854–1859.
16. Im W. B., Blakeman D. P., Davis J. P. and Ayer D. E.: Studies on the mechanism of interaction between anesthetic steroids and

gamma-aminobutyric acid$_A$ receptors. *Molec. Pharmac.* **37** (1990) 429–434.
17. Tesarik J., Moos J. and Mendoza C.: Stimulation of protein tyrosine phosphorylation by a progesterone receptor on the cell surface of human sperm. *Endocrinology* **133** (1993) 328–335,
18. Dean J.: Biology of mammalian fertilization: role of the zona pellucida. *J. Clin. Invest.* **89** (1992) 1055–1059.
19. Luconi M., Bonaccorsi L., Krausz Cs., Gervasi G., Forti G. and Baldi E.: Stimulation of protein tyrosine phosphorylation by platelet-activating factor and progesterone in human spermatozoa. *Proceedings of 8th European Workshop on Molec. Cell. Endocr.* **108** (1995) 35–42
20. Harper M. J. K.: Platelet-activating factor: a paracrine factor in preimplantation stages of reproduction? *Biol. Reprod.* **40** (1989) 907–913.
21. Amiel M. L., Testart J. and Benveniste J.: Platelet-activating factor is a component of human follicular fluid. *Fertil. Steril.* **56** (1991) 62–65.
22. Baldi E., Falsetti C., Krausz Cs., Gervasi G., Carloni V., Casano R. and Forti G.: Stimulation of platelet-activating factor synthesis by progesterone and A23187 in human spermatozoa. *Biochem. J.* **292** (1993) 209–216.
23. Krausz Cs., Gervasi G., Forti G. and Baldi E.: Effect of platelet-activating factor on motility and acrosome reaction of human spermatozoa. *Human Reprod.* **9** (1994) 471–478.
24. Matson P. L., Troup S. A., Lowe B., Ibrahim Z. H. Z., Burslem R. W. and Lieberman B. A.: Fertilization of human oocytes *in vitro* by spermatozoa from oligozoospermic and normospermic men. *Int. J. Androl.* **12** (1989) 117–123.
25. Wiston R. M. and Handyside A. H.: New challenges in human *in vitro* fertilization. *Science* **260** (1993) 932–936.
26. Falsetti C., Baldi E., Krausz Cs., Casano R., Failli P. and Forti G.: Decreased responsiveness to progesterone of spermatozoa in oligozoospermic subjects. *J. Androl.* **14** (1993) 17–22.
27. Tesarik J. and Mendoza C.: Defective function on a non-genomic progesterone receptor as a sole sperm anomaly in infertile patients. *Fertil. Steril.* **58** (1993) 328–335.
28. Oehninger S., Blackmore P., Morshedi M., Sueldo C., Acosta A. A. and Alexander N.: Defective calcium influx and acrosome reaction (spontaneous and progesterone-induced) in spermatozoa of infertile men with severe teratozoospermia. *Fertil. Steril.* **61** (1994) 828–833.
29. Krausz Cs., Bonaccorsi L., Luconi M., Fuzzi B., Criscuoli L., Pellegrini S., Forti G. and Baldi E.: Intracellular calcium increase and acrosome reaction in response to progesterone in human spermatozoa correlates with *in vitro* fertilization. *Human Reprod.* **10** (1995) 120–125.