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The association between different sperm parameters, an in vitro effect of progesterone, has not been studied satisfactorily. ln this article, the effect of progesterone on acrosome reaction (AR), plasma membrane integrity, and chromatin stability has been assessed in human spermatozoa with normal morphology and motility. Semen samples were obtained by masturbation from 25 patients. Two criteria of classification were utilized in this study: high motility group and normal morphology group incubated with progesterone. The effect of progesterone on AR, plasma membrane integrity, and chromatin stability in human spermatozoa with normal morphology and motility was realized. The results suggest that only the subpopulation of spermatozoa with normal morphology is able to undergo the progesterone-induced AR. It is possible that in the reproductive female tract it takes place a high selection of sperm with chromatin stability determined and optimal plasma membrane to undergo the AR prerequisite for the fecundation.

Keywords acrosome reaction, chromatin stability, human sperm, hypoosmotic test, progesterone

Sperm capacitation in the female reproductive tract is a complex biochemical process that includes changes in membrane lipids and proteins, ionic fluxes, increase in cyclic AMP level, and protein phosphorilation [21]. Capacitation is also associated with the ability of sperm to undergo acrosome reaction (AR), which is a prerequisite for oocyte fertilization [4]. The sperm population reaching the ovulated oocyte has been previously capacited and hyperactivated during transit through the cervical mucus. Both capacitation and hyperactivation are calcium-dependent events [17, 18].

Recent studies have demonstrated that human follicular fluid induces an influx of extracellular calcium and the AR in capacited spermatozoa. The effects of progesterone may diversify in different tissues from the same animal. Also, the same effect may take place through different mechanisms [12]. On the other hand, progesterone in human follicular fluid stimulates AR

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and hyperactive motility [1, 2, 20]. A clinical use of this steroid may represent a valuable tool in processing spermatozoa for assisted fertilization techniques [6, 8].

The sperm population reaching the oocyte has been in presence of high level of progesterone (1.0 μg/mL) during transit through the female reproductive tract [13]. Therefore, progesterone may play a role in selecting capacitated spermatozoa from the total population with fecundation capacity. Meitzel and Turner [13], reported that progesterone binds to the membrane of human sperm, suggesting the presence of a functional receptor, which could be dependent on the stability of plasma membrane and normal sperm morphology. Capacitated sperm presenting hyperactivation or high motility may not be morphologically normal. Low concentration of progesterone in cumulus ooforo might allow a selection of spermatozoa with normal morphology and ability to undergo AR. In addition, Tesarik and Mendoza [17] reported that only 10% of total spermatozoa shows membrane receptor to progesterone [3, 17].

Studies with selected human spermatozoa have not established correlation between selective effect of progesterone and sperm characteristics, such as chromatin stability, motility, and morphology [7, 12]. The association between different sperm parameters and the in vitro effect of progesterone is not clear.

In this report, we have assessed the effect of progesterone on AR, plasma membrane integrity, and chromatin stability in human spermatozoa with normal morphology and motility.

MATERIALS AND METHODS

Two criteria of classification for semen samples were used: a high-motility group when sperm motility was more than 60% and a normal morphology group when teratozoospermia was less than 40%.

Sperm Preparation

Semen samples were obtained by masturbation from 25 patients after a sexual abstinence period of 3–5 days. All semen samples were examined for volume, pH, sperm concentration, morphology, sperm motility, and viability [19]. The experiments were performed using only spermatozoa washed with HAM F-10 medium. Sperm suspension were collected and centrifuged for 10 min at 800*g*. Then sperm concentration was adjusted to 1×10^6 cells/mL. Aliquots of the sperm were supplemented with 1.0 μg/mL of progesterone dissolved in dimethyl sulfoxide (DMSO). Steroid-free solvent was added to control tubes. Aliquots of spermatozoa were incubated in HAM F-10 medium containing serum albumin (3.5 mg/mL) to induce capacitation (positive control) and HAM F-10 as negative control. Additional spermatozoa were incubated with DMSO (vehicle) for control purpose.

All samples were incubated for 3 h in an atmosphere of 5% CO_2 and 95% O_2 at 37°C. Motility and viability were determined pre- and postincubation. Triple stain (AR), hypoosmotic swelling test (membrane plasma integrity), and stability of chromatin (nuclear decondensation) were determined before and after incubations.

Evaluation of Acrosomal Status with Triple Stain

A modification of a previously described procedure [16] was used to carry out the triple stain. Spermatozoa were washed twice in HAM F-10 medium and resuspended at a concentra-

tion of 0.5×10^6 sperm/mL. Equal volume was incubated with trypan blue (2%) in PBS (pH 7.3) at 37°C for 15 min. Spermatozoa were fixed in glutaraldehyde (4%) for 40–60 min and immediately stained with Bismarck brown (0.8%, pH 1.8) at 40°C for 5 min and Bengale rose (0.8% in PBS, pH 6.0) for 30 min at 24°C. Spermatozoa were evaluated by light microscopy at 1000× magnification.

Evaluation of Plasma Membrane Integrity with HOS Test

The HOS test was carried out with 200 μL of sperm suspension (0.5×10^6 sperm) incubated in 1.0 mL of hypoosmotic solution of fructose and sodium citrate at 37ºC for 30 min as described by Smith et al. [15] and fixed in 500 μL the formalin buffer. The spermatozoa were evaluated with a phase contrast microscope at 400× magnification. Results of the HOS test were expressed as reacted (sperm with different tail swelling patterns) or not reacted sperm.

Evaluation of Nuclear Decondensation with Reducing Agents

For the decondensation test with reducing agent, 1 mL of thioglycolate (sodium thioglycolate 0.4 M, pH 9.0) was added to 200 μL of sperm suspension (0.5×10^6 sperm) and incubated at room temperature for 10 min. The reaction was stopped by a 10-fold dilution with cold phosphate-buffered saline (PBS, 0.05 M, pH 7.2) and samples were resuspended in 100 μL of PBS, spotted in slide, air dried, stained with hematoxilin/eosin, mounted with Permount, and examined under light microscope.

Statistical Analysis

The Friedman nonparametric test and multiple comparison Dunn test were utilized (GraphPad InStat TM). A level of $P < .05$ was considered significant.

RESULTS

Progesterone increased AR only on the sperm population with normal morphology (Figure 1). P showed no effect on chromatin stability in the normal morphology group, but a slight increase in decondensed chromatin in the abnormal group was observed (Figure 2). There were no significant effects of progesterone on HOS test in either population (Figure 3).

When spermatozoa were classified in the high- or low-motility groups, the effect of progesterone on AR was significant only in the group with high motility. This effect is not shown in presence of BSA (Figure 4). The low-motility group showed more decondensation than the high-motility group in presence of progesterone (Figure 5). Regarding the HOS test, differences were not significant in the high-motility group. However, in the low-motility group the response is evidenced in the presence of both progesterone and BSA (Figure 6).

DISCUSSION

Progesterone level in cumulus cells, estimated in 1 μg/mL, is believed to induce capacitation in human spermatozoa [8]. This molecular mechanism comprises an influx of calcium ions from the extracellular space [8]. The sperm population exposed to this steroid, in the cumulus matrix and human follicular fluid, is highly selective. Only sperm with high motility and vitality are present around this oocyte and are able to undergo AR before fertilization. In

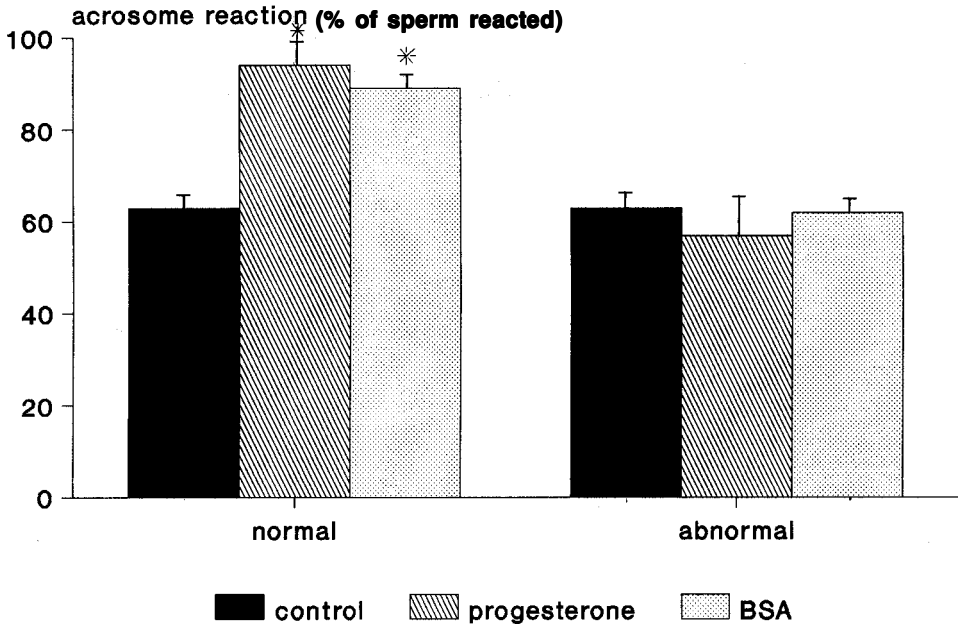

Figure 1. Effect of progesterone and BSA on the acrosome reaction in sperm with normal and abnormal morphology (* differences with $P < .05$).

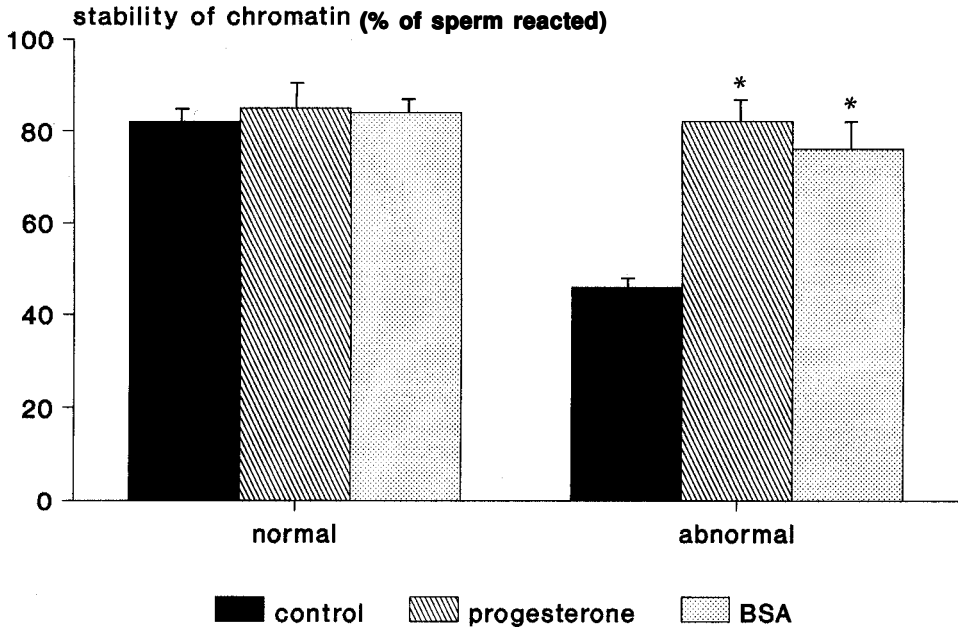

Figure 2. Effect of progesterone and BSA on the chromatin stability in sperm with normal and abnormal morphology (* differences with $P < .05$).

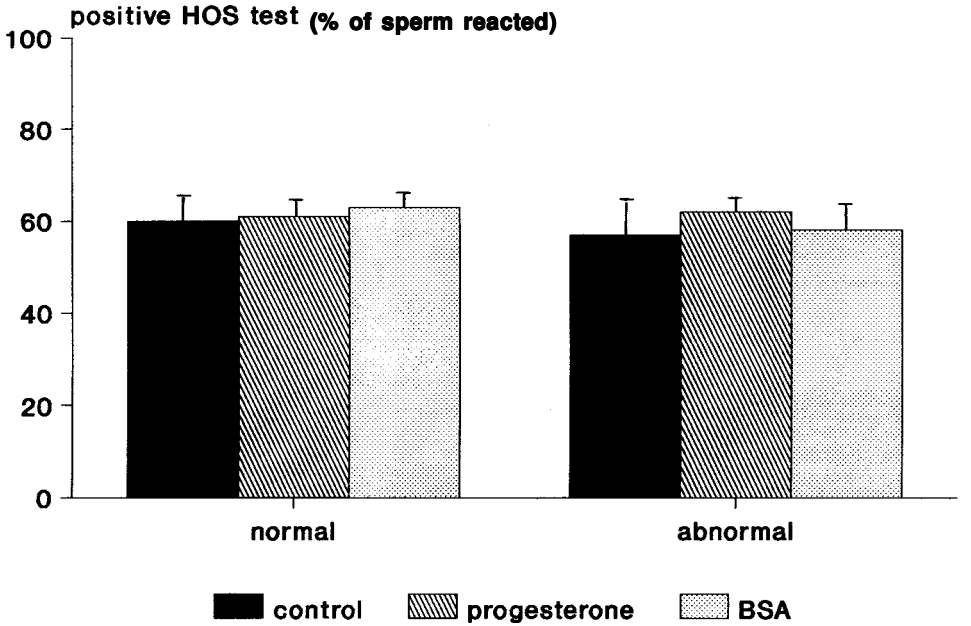

Figure 3. Effect of progesterone and BSA on the hypoosmotic swelling test in sperm with normal and abnormal morphology (* differences with $P < .05$).

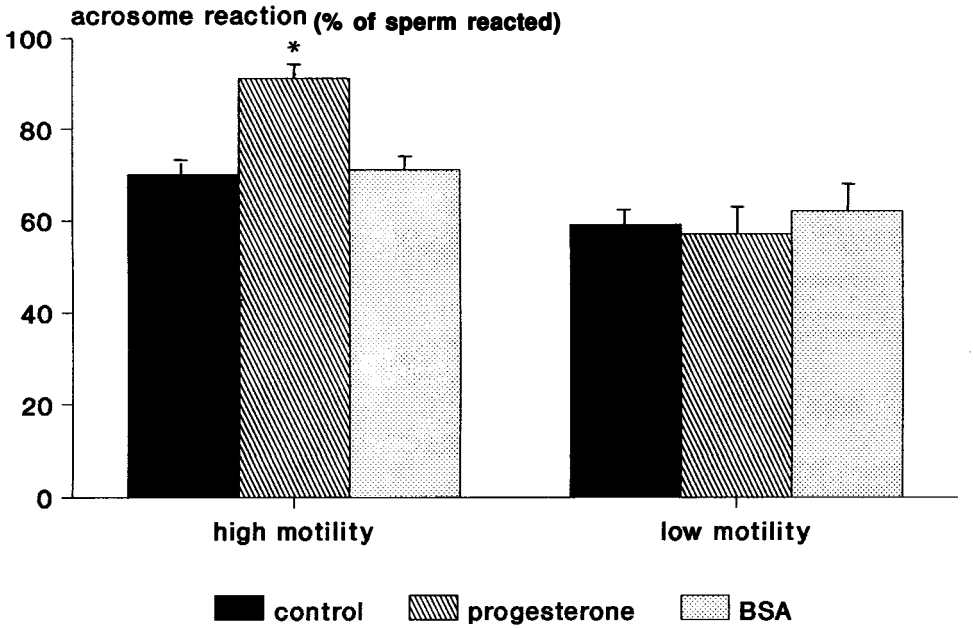

Figure 4. Effect of progesterone and BSA on the acrosome reaction in sperm with high and low motility (* differences with $P < .05$).

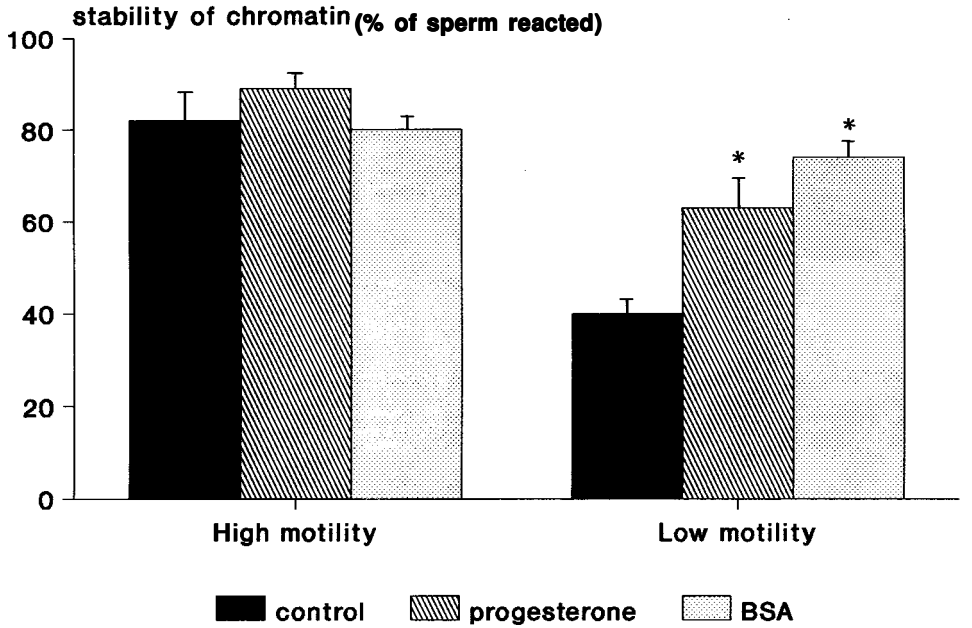

Figure 5. Effect of progesterone and BSA on the chromatin stability in sperm with high and low motility (* differences with $P < .05$).

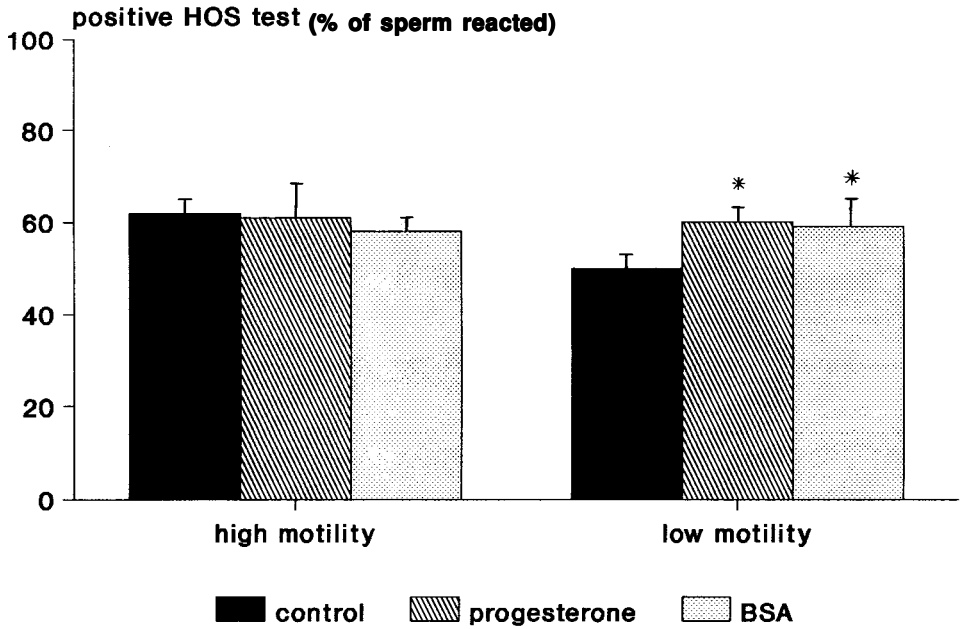

Figure 6. Effect of progesterone and BSA on the hypoosmotic swelling test in sperm with high and low motility (* differences with $P < .05$).

addition, spermatozoa with normal morphology and plasma membrane integrity are needed for the events involved in the fecundation process [14].

The results obtained in this study suggest that only the subpopulation of spermatozoa with normal morphology are able to undergo progesterone-induced capacitation. Previous observations from Katz et al. [9] showed motility differences between morphologically normal and abnormal human seminal spermatozoa. Indeed, only normal spermatozoa with plasma membrane integrity are able to acquire fertilizing capacity [9, 10]. Probably, the sperm population reaching the fertilization site showed these characteristics. How the female tract may actively select a population of spermatozoa and transport it to the site of fertilization is not yet clarified.

The mechanism of progesterone on human spermatozoa involved changes at the intracellular level that take place in different types of spermatozoa. These changes are not necessarily associated with the parameters analyzed in this study. The question of whether the spermatozoa showing normal morphology are the same as those presenting DNA stability or plasma membrane integrity is not answered.

In the total sperm population the effect of the progesterone nongenomic level was proposed by Blackmore [2]. In one spermatozoon the progesterone interacts with a cell surface steroid receptor, causing an immediate increase in [Ca], but in other sperm their action was on the membrane phospholipids not inducing AR [2]. Several reports have shown the capacitating effect of progesterone on ejaculated spermatozoa obtained with two layers of Percoll, but those effects cannot be addressed to other sperm parameters as DNA stability or plasma membrane integrity [4, 5].

Considering that the progesterone effect was established in only 10% of the sperm population with binding to progesterone [18], it is possible that in the reproductive female tract it takes place a high selection of sperm with potential to fecundation. Al1 characteristics separately determined in this study may be present in the spermatozoon physiologically selected for fecundation.

REFERENCES

1. Blackmore P, Neulen J, Lattanzio F, Beebe S (1986): Cell surface-binding sites for progesterone mediate calcium uptake in human sperm. J Biol Chem 266:18655–18659.
2. Blackmore P (1993): Rapid non-genomic actions of progesterone stimulate Ca^{+2} influx and the acrosome reaction in human sperm. Cell Signaling 5:5321–5328.
3. Blackmore P, Fisher J, Spilman C, Bleasdale J (1996): Unusual steroid specificity of the cell surface progesterone receptor on human sperm. Mol Pharmacol 49:727–739.
4. Cross N, Razy-Faulkner P (1997): Control of human sperm intracellular pH by cholesterol and its relationship to the response of the acrosome to progesterone. Biol Reprod 56:1169–1174.
5. Emiliozzi C, Fenichel P (1997): Protein tyrosine phosphorilation is associated with capacitation of human sperm in vitro but is not sufficient for its completation. Biol Reprod 56:674–679.
6. Emiliozzi C, Cordonier J, Guerin J, Ciapa B, Benchaib M, Fenichel P (1996): Effects of progesterone on human spermatozoa prepared for in vitro fertilization. Int J Androl 19:39–47.
7. Evenson D, Darzynkiewicz Z, Melamed M (1980): Relation of mammalian sperm chromatin heterogeneity to fertility. Science 210:1131–1133.
8. Foresta C, Rossato M, Mioni A, Zorzi M (1992): Progesterone induces capacitation in human spermatozoa. Andrologia 24:33–35.
9. Katz D, Diel L, Ovestreet J (1982): Differences in the movement of morphologically normal and abnormal human seminal spermatozoa. Biol Reprod 26:566–570.

10. Kruger T, Menkaveld R, Stander F, Lombard C, Van der Merwe J, Van Zyl J, Smith K (1986): Sperm morphology as a prognostic factor in in vitro fertilization. Fertil Steril 46:1118–1126.
11 Kruger T, Haque A, Acosta A, Pleban P, Swanson K, Simmonds K, Matta J, Morshedi M, Oehninger S (1988): Correlation between sperm morphology: acrosin, and fertilization in an IVF program. Arch Androl 20:237–241.
12. Mahesh V, Brann D, Hendry L (1996): Diverse modes of action of progesterone and its metabolites. J Steroid Biochem Mol Biol 56:209–219.
13. Meitzel S, Turner K (1991): Progesterone acts at the plasma membrane of human sperm. Mol Cell Endocrinol 11:R1–R5.
14. Ramirez J, Carreras A, Mendoza C (1992): Sperm plasma membrane integrity in fertile and infertile men. Andrologia 24:141–144.
15. Smith R, Madariaga M, Bustos-Obregón E (1992): Reappraisal of the hypoosmotic swelling test to improve assessment of seminal fertility status. Int J Androl 15:5–13.
16. Talbot P, Chacon R (1981): A triple stain technique for evaluating normal acrosome reactions of human sperm. J Exp Zool 215:201–210.
17. Tesarik J, Mendoza C (1992): Defective function of a nongenomic progesterone receptor as a sole sperm anomaly in infertile patients. Fertil Steril 58:793–797.
18. Tesarik J, Moos J, Mendoza C (1993): Stimulation of protein tyrosine phosphorilation by a progesterone receptor on the cell surface of human sperm Endocrinology 133:328–335.
19. World Health Organization (1987): WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction. Cambridge, UK: The Press Syndicate of the University of Cambridge, pp 27–33.
20. Yang J, Serre C, Philibert D, Roberl P, Baulieu E, Jouannet P (1994): Progesterone and RU 486 on human sperm: inverse effect. Proc Natl Acad Sci USA 91:529–533.
21. Zaneveld L, De Jonge C, Anderson R, Marck S (1991): Human sperm capacitation and the acrosome reaction. Hum Reprod 6:1265–1274.