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Effects of Competitive Progesterone Antagonist RU486 on the Penetration of Zona-Free Hamster Eggs by Human Sperm

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Abstract: *Progesterone plays an important role in sperm capacitation and acrosome reaction. In this experiment we examined the effect of a progesterone antagonist, RU486, on the penetration of zona-free hamster eggs by human sperm. Media containing 10, 15, 20, 25 or 30 μM RU486 were prepared. In the control medium without RU486, the sperm penetration rate was 80.7%. In the medium with 10 μM RU486, the sperm penetration rate was 67.0%. The rate further decreased as the level of RU486 increased. In the medium with 30 μM RU486, the sperm penetration rate was reduced to 4.8%. This study demonstrates that RU486 inhibits penetration of zona-free hamster eggs by human sperm in a dose-dependent manner.*

Key words: *Progesterone antagonist, RU486, Zona-free hamster eggs, Sperm penetration.*

Progesterone receptors are present in human sperm [1]. It has been also shown that progesterone plays an important role in sperm capacitation [2–4] and acrosome reaction [2, 3, 5–7]. Yanagimachi *et al.* [8] reported in 1976 that capacitated human sperm could penetrate zona-free hamster eggs. Since only sperm in which capacitation and acrosome reaction have been completed can penetrate zona-free hamster eggs, these reactions can be indirectly confirmed by using the experimental system of Yanagimachi *et al.* [8].

Recently, a new anti-progesterone compound RU486 has been developed. RU486 has a strong binding affinity for progesterone receptors. It acts as a competitive progesterone antagonist [9].

Although there have been a number of reports which describe an inhibitory action of RU486 in implantation [10] and pregnancy [11] in this decade, only one paper [12] reported that RU486 inhibits human sperm entry into zona-free hamster eggs.

Here we report that RU486 inhibits human sperm penetration of zona-free hamster eggs in a dose-dependent manner.

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Materials and Methods

The animals used were 8-week-old female golden hamsters. They were purchased from a commercial breeder (Japan SLC Inc., Hamamatsu, Japan) and fed commercial pellets (Labo MR Stock; Nihon Nosan Kogyo Co., Yokohama, Japan) with tap water *ad libitum*.

The animal room was air-conditioned at 23 ± 2°C, 55 ± 5% relative humidity, with 15 air changes per hour, and illuminated for 14 h (05:00-19:00) per day with 300 lx day-light fluorescent lamps.

Eggs were collected from hamsters with regular 4-day estrus cycles. To induce superovulation, PMSG (30 IU) and hCG (30 IU) were administered to these hamsters at a 56 hr interval by subcutaneous injection. The animals were euthanized with an overdose of sodium pentobarbital (Nembutal; Abbott Laboratories, North Chicago, IL, USA). Eggs were collected from the ampulla of the oviduct under sterile conditions. Cumulus cells and *zonae pellucidae* were removed as previously described [8].

Human semen was obtained from a healthy adult volunteer. Sperm were incubated as previously described [8]. Table 1 shows the composition of the medium (HCM) used for preparation of the sperm suspension. The concentration of sperm was adjusted to approximately 10^7/ml.

In this study, control medium without RU486 (Mifepristone, developed by Roussel-Uclaf, Paris, France) and five media with 10, 15, 20, 25 or 30 μM RU486 were prepared. Sperm suspensions (200 μl)

Table 1. The composition of the medium (HCM) used in this experiment

Components	mM	mg/100 ml
NaCl	117.5	686
KCl	8.6	64
$CaCl_2$ (anhyd.)	2.5	28
$MgCl_2 \cdot 6H_2O$	0.49	10
$NaH_2PO_4 \cdot H_2O$	0.36	5
$NaHCO_3$	25.0	210
EDTA · $2H_2O$	100 (in H_2O)	0.1 ml
Na-lactate (60% syrup)	19.0	0.3 ml
Glucoase	2.0	36
Penicillin G		7
Streptomycin sulf.		5
BSA		26 mg

were covered with mineral oil, and pre-incubated for 15–16 h at 37°C under 5% CO_2 in air. RU486 was added to the sperm suspensions and adjusted to the above concentrations.

Ten minutes later a 2 μl progesterone solution (100 μg/1 ml of 10% DMSO) was added to the sperm suspension. Then ten minutes after the addition of progesterone, zona-free hamster eggs were placed in the sperm suspension and incubated at 37°C under 5% CO_2 in air. After three hours of incubation, the eggs were placed on a glass slide with a small amount of the medium, covered with a coverslip, and gently pressed between the slide and coverslip to make the egg preparation.

With these preparations, penetration of eggs by sperm was examined with a phase-contrast microscope. Penetration by sperm was considered positive when more than one swollen sperm head or male pronuclei with their tails were observed. The number of eggs penetrated by sperm was counted to determine the rate of sperm penetration.

The rate of penetration of eggs by sperm was analysed using by χ^2 test. In the analysis, differences were considered significant when *P*-values were less than 0.05.

Results

Table 2 summarizes the results of the experiments. For 135 eggs in the control medium, the sperm penetration rate was 80.7%. The sperm penetration rate was lower in all RU486-containing media than that in the control medium in a dose-dependent manner. It was 60.7, 47.9, 35.2, 27.0 and 4.8% in the 10, 15, 20, 25 and 30 μM RU486 groups respectively. These rates were all significantly lower than the penetration rate in the control group. The penetration rate was the lowest (4.8%) in the medium containing 30 μM RU486.

These results showed that the rate of penetration by sperm decreased with the increase in the RU486 concentration in the media.

Discussion

Yanagimachi and Usui [13] showed that Ca^{2+} was essential for acrosome reaction in the guinea pig. Divalent metal cation, ionophore A23187 is known to induce sperm acrosome reaction [14]. This is considered to be caused by alkalization of the sperm cell and membrane fusion induced by Ca^{2+} which reaches acrosomes through the cell membrane and external acrosomal membrane by changes in its conductivity [15].

In human sperm, progesterone is believed to induce an influx of extracellular Ca^{2+} through Ca^{2+} channels [16–19]. Progesterone is therefore closely involved in Ca^{2+} mobilization within sperm cells. This is considered to be the mechanism by which progesterone induces the acrosome reaction of sperm.

In this study, we added progesterone 10 minutes

Table 2. Effects of RU 486 on penetration of zona-free hamster eggs by human sperm

RU 486 concentration (μM)	No. of eggs examined	No. of eggs sperm-penetrated	% penetration*
0 (control)	135	109	80.7[a]
10	106	71	67.0[b]
15	121	58	47.9[c]
20	105	37	35.0[c,d]
25	111	30	27.0[d]
30	83	4	4.8[e]

*Values with different superscripts are significantly different (P<0.05).

after the addition of RU486 to sperm suspensions. RU486 inhibited the penetration of eggs by sperm in a concentration-dependent manner, suggesting that RU486 is a strong antagonist of progesterone at the receptor level. This study demonstrated that the rate of sperm penetration of zona-free hamster eggs is noticeably reduced by RU486. The results of our study almost entirely agree with those by Yang *et al.* [12] who have already studied the effects of RU486 on the penetration of zona-free hamster eggs by human sperm.

RU486 is known to reduce the Ca^{2+} concentration in human sperm [20], so that reduction of the rate of sperm penetration of zona-free hamster eggs by increasing the RU486 concentration is perhaps due to the inhibition of the acrosome reaction.

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