J. Mamm. Ova Res. Vol. 15, 93–95, 1998

Effects of Competitive Progesterone Antagonist RU486 on In Vitro Fertilization in Hamsters

Tuyoshi Endo* and Kiichi Kanayama

Department of Veterinary Medicine, College of Bioresource Sciences, Nihon University, Kanagawa 252-8510, Japan

Abstract: *The authors examined the effects of RU486 on the In Vitro fertilization (IVF) of hamsters. Six groups of medium containing 1, 5, 10, 20, 40 and 60 μM RU486 were prepared. In the control group without RU486, the fertilization rate was 97.3%. In the 1 μM RU486 group, the fertilization rate was 94.6%, and the difference between the control and 1 μM RU486 groups was not significant. In the remaining 5, 10, 20, 40 and 60 μM RU486 groups, the fertilization rate decreased in a concentration-dependent manner, and in the 60 μM RU486 group, it was reduced to 56.3% (P<0.001). These results showed that RU486, an antagonist of progesterone, inhibited IVF of hamsters in a concentration-dependent manner.*

Key words: *Progesterone antagonist, RU486, In Vitro fertilization, Hamster.*

Progesterone plays an important role in sperm capacitation under physiological conditions in the female reproductive tract [1]. Progesterone receptors are known to be present in sperm [2]. Progesterone is considered to be closely involved in Ca^{2+} mobilization in sperm. Progesterone is reported to increase the Ca^{2+} concentration in sperm [3–5]. This increase appears to be involved in sperm capacitation and acrosome reaction [6–9].

Recently, a new anti-progesterone compound RU486 was developed. RU486 has strong binding affinity to progesterone receptors, and acts as a competitive progesterone antagonist [10]. In this study, we examined the effects of RU486 on the *In Vitro* fertilization (IVF) of hamsters.

Received: January 14, 1998
Accepted: January 23, 1998
*To whom correspondence should be addressed.

Materials and Methods

Eggs were collected from 8 to 9-week-old female golden hamsters with a regular estrus cycle at 4-day intervals. Superovulation was induced in hamsters by the following procedures according to the routine method. The day when post ovulatory discharge from the vagina was observed was regarded as Day 1, and 30 iu PMSG was subcutaneously injected into the hamsters at 10:00 a.m. on Day 1, and 55 h later 30 iu hCG was subcutaneously injected. Seventeen hours after the subcutaneous injection of hCG, the hamsters were sacrificed and celiotomized, and eggs covered with granulosa cells were collected from the ampulla of the oviduct under sterile conditions. In mature male hamsters under general anesthesia with barbital, lumps of sperm were collected from the epididymis with a needle inserted into its tail region. The sperm was allowed to swim up in a 2 ml medium in a test tube, and sperm suspensions at 10^6 sperm/ml were prepared from its supernatant. Details of the IVF procedure were described in a previous report [11].

The control group without RU486 (Mifepristone, 17β-hydroxy-11β-(4-dimethyl-aminophenyl-1)-17α-(prop-1-ynyl)-estra-4,9-diene-3-one developed by Roussel-Uclaf, Paris, France) and 6 groups with medium containing 1, 5, 10, 20, 40 and 60 μM RU486 were prepared. In all groups, sperm was incubated for 5 h at 37°C in 5% CO_2. Fertilization was examined by the previously reported method [11].

Table 1 shows the composition and methods of preparation of the medium, m-TALP-3, used in this study.

The rate of fertilization was analyzed by χ^2 test. In the analysis, differences were considered significant when *P*-values were less than 0.05.

Table 1. The composition and methods of preparation of the medium, m-TALP3, used in this experiment

Components	mM	mg/100 ml
[A]		
NaCl	101.02	590
KCL	2.68	20
$CaCl_2$ (anhydrous)	1.80	20
$MgCl_2 \cdot 6H_2O$	0.49	10
$NaH_2PO_4 \cdot H_2O$	0.36	5
$NaHCO_3$	35.70	300
[B]		
D-Glucose	4.50	81
Na-pyruvate	1.00	11
Na-lactate (60% syrup)	9.0	0.15 ml
Hypotaurine	0.5	5.5
(-) Epinephrine	0.05	0.9
NA-taurocholic acid	0.2	10.0
100mM EDTA $\cdot 2H_2O$		0.1 ml
0.1% $Na_2S_2O_5$ in saline		0.1 ml
Penicillin G		5
Streptomycin sulfate		5
Gentamicin		5
[C]		
BSA		15 mg/ml

Make 1 liter of solution [A] using double distilled water. Add phenol red as a pH indicator. This inorganic salt solution can be kept in a refrigerator for many months. Take 100 ml of solution [A] and add ingredients of [B]. [A] + [B] should be used within 3-4 days. Kept in a refrigerator. Take 10 ml of [A] + [B]. Add BSA immediately before use or within 12 h before use. For short term culture of gametes/embryos, sterilization is not necessary. For long term culture (>24 h), the medium with BSA must be sterilized with a Millipore filter (0.45 μm pores).

Results

Table 2 shows the results of this experiment. In the control group, fertilization was observed in 72 of the 74 eggs, the fertilization rate being 97.3%. In the 1, 5, 10, 20, 40 and 60 μM RU486 groups, the fertilization rates were 94.6, 81.3, 76.0, 75.3 and 56.3%, respectively. These results showed that the fertilization rate decreases with the increase in the medium RU486 concentration.

The difference between the control and 1 μM RU486 groups in the fertilization rate was not significant, but the fertilization rate in the remaining RU486 groups was significantly lower than that in the control group. In the 10, 20, and 40 μM RU 486 groups, $P<0.01$, and in the 60 μM RU group, $P<0.001$. This study demonstrated that RU486 inhibited IVF of hamsters in a concentration-dependent manner.

Table 2. Effects of RU486 on the *in vitro* fertilization in hamsters

RU486 concentration (μM)	No. of eggs examined	No. of eggs fertilized	% fertilized*
0 (control)	74	72	97.3[a]
1	74	70	94.6[a, b]
5	74	64	86.5[b, c]
10	75	61	81.3[c]
20	75	57	76.0[c]
40	73	55	75.3[c]
60	71	40	56.3[d]

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

Discussion

Ca^{2+} was shown to be essential for sperm capacitation [12]. Progesterone plays an important role in sperm capacitation [6, 7, 13] and acrosome reaction [6, 7, 14–16]. Acrosome reaction is considered to be caused by an influx of extracellular Ca^{2+} into sperm induced by progesterone [3–5]. RU486 is reported to reduce the Ca^{2+} concentration in sperm [4]. RU486 antagonizes progesterone in Ca^{2+} dynamics in sperm. The reduction of the fertilization rate observed in the RU486 addition groups of this study is most likely due to the inhibition of Ca^{2+} mobilization in sperm caused by RU486.

The binding of RU486 to progesterone receptors is stronger than that of progesterone [5, 10]. From this, the authors initially thought that inhibition of fertilization caused by RU486 would be an all-or-none phenomenon. This study, however, showed that the fertilization rate decreases with the increase in the medium RU486 concentration. This result agrees with the study by Juneja and Dodson [17] on the effects of RU486 on IVF in mice.

Acknowledgments

The authors thank Dr. M. Sakuma for her excellent technical help. We also thank Dr. Baulieu E. E., Hospital Cochin, Universite Paris V, France, for providing us, through Dr. R. Yanagimachi, the University of Hawaii, the RU486 used in this research. We are grateful to Dr. R. Yanagimachi for suggesting the present experiments.

References

1) Margalioth, E.J., Bronson, R.A., Cooper, G.W. and Rosenfeld, D.L. (1988): Luteal phase and progester-

one enhance sperm penetration in the hamster egg assay. Fertil. Steril., 50, 117–122.
2) Revelli, A., Modotti, M., Piffaretti-Yanez, A., Massobrio, M. and Balerna, M.(1994): Steroid receptors in human spermatozoa. Hum. Reprod., 9, 760–766.
3) Thomas, P. and Meizel, S. (1989): Phosphatidylinositol 4,5-bishosphate hydrolysis in human sperm stimulated with follicular fluid or progesterone is dependent upon Ca^{2+} influx. Biochem. J., 264, 539–546.
4) Blackmore, P.F., Beebe, S.J., Danforth, D.R. and Alexander, N. (1990): Progesterone and 17α-Hydroxyprogesterone; Novel stimulators of calcium influx in human sperm. J. Biol. Chem., 265, 1376–1380.
5) Yang, J., Srttes, C., Philibert, D., Robel, P., Baulieu, E.E. and Jouannet, P. (1994): Progesterone and RU486: Opposing effects on human sperm. Proc. Natl. Acad. Sci. U.S.A., 91, 529–533.
6) 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. J. Androl., 12, 323–330.
7) Uhler, M.L., Leung, A., Chan, S.Y.W. and Wang, C. (1992): Direct effects of progesterone and antiprogesterone on human sperm hyperactivated motility and acrosome reaction. Fertil. Steril., 58, 1191–1198.
8) Thomas, P. and Meizel, S. (1988): An influx of extracellular calcium is required for initiation of the human sperm acrosome reaction induced by human follicular fluid. Gamete. Res., 20, 397–411.
9) Yudin, A., Gottlieb, W. and Meizel, S. (1988): Ultrastructural studies of the early events of the human sperm acrosome reaction as initiated by human follicular fluid. Gamete. Res., 20, 11–24.
10) Schreiber, J.R., Hsueh, A.F. and Baulieu, E.E. (1983): Binding of the antiprogestin RU-486 to rat ovary steroid receptors. Contraception, 28, 77–85.
11) Yanagimachi, R. (1970): *In vitro* capacitation of golden hamster spermatozoa by homologous and heterologous blood sera. Biol. Reprod., 3, 147–153.
12) Yanagimachi, R. and Usui, N. (1974): Calcium dependence of the acrosome reaction and activation of guinea pig spermatozoa. Exp. Cell. Res., 89, 161–174.
13) Foresta, C., Rossato, M., Moini, R. and Zorzi, M. (1992): Progesterone induces capacitation in human spermatozoa. Andrologia, 24, 33–35.
14) Llanos, M.N., Morales, P.J. and Anabalon, M.C. (1993): Characterization of the progesterone induced hamster sperm acrosome reaction. Biol. Reprod., 48 (suppl. 1), 106.
15) Osman, R., Andria, M.L., Jones, A.D. and Meizel, S. (1989): Steroid induced excytosis; the human sperm acrosome reaction. Biochem. Biophys. Res. Commum., 160, 828–833.
16) Tesarik, J., Mendoza, C., Moos, J., Fenichel, P. and Fehlmanm, M. (1992): Progesterone action through agglutination of receptor on sperm plasma membrane. FEBS (Letters), 308, 116–120.
17) Juneja, S.C. and Dodson, M.G. (1990): *In vitro* effect of RU486 on sperm-egg interaction in mice. Am. J. Obste. Gynecol., 163, 216–221.