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# Effect of mifepristone on human sperm function in vitro

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## Abstract

**Objective:** Mifepristone, as a type of well-accepted emergency contraceptive drugs, is an optional progesterone receptor modulator and widely used clinically. However, the effect of mifepristone on sperms is poorly studied. This research is intended to investigate the biological effect of mifepristone on human sperm function in vitro.
**Methods:** Spermatozoa were incubated in the culture medium containing 0.04 $\mu$M, 0.4 $\mu$M, 4 $\mu$M and 40 $\mu$M of mifepristone for one hour after capacitation. It was required to measure sperm motility, viability, DNA integrity, acrosomal reaction, sperm hyperactivation, and the concentration of free calcium ions in spermatozoa.
**Results:** After treating sperms with different concentrations of mifepristone in vitro, sperm viability, motility, morphology and DNA integrity were measured. The differences between the control group and the experimental group were of no statistical significance ($p > .05$). When the concentration of mifepristone was higher than 0.4 $\mu$M, the proportion of sperm hyperactivation was significantly reduced ($p < .01$). According to further examination on the rate of acrosomal reaction (AR rate) and the concentration of free calcium ions in sperms, it was found that acrosomal reaction was significantly inhibited with the concentration of free calcium ions in sperms decreased when the concentration of mifepristone was higher than 0.4 $\mu$M.
**Conclusions:** The in-vitro spermicidal mechanism of mifepristone is realized by inhibiting acrosomal reaction, hyperactivation and decreasing the concentration of free calcium ions.

**Key Words:** Mifepristone, Emergency contraceptive drugs, Sperm function

Emergency contraceptive drugs refer to a type of contraceptive pills that may be used to prevent pregnancy after unprotected sex or contraceptive failure. This medicine can effectively prevent women from the harm caused by abortion. However, emergency contraceptives have not fully exerted their roles and advantages at home and abroad. Exploring the mechanism of emergency contraceptives is helpful to develop acceptable contraceptive methods with few side effects. Mifepristone is not only a norethisterone derivative but also a progesterone receptor modulator that can be used as an emergency contraceptive for effective contraception after unprotected behavior.[1,2] The latest research shows that a single oral dose of 25 mg to 50 mg mifepristone can be used for unprotected behavior within 120 hours.[3] The contraceptive mechanism of mifepristone is mainly to inhibit ovulation and interfere with embryo implantation, so that the effect of emergency contraception will be better.[4] As an effective emergency contraceptive, mifepristone has attracted many researchers, who have made a great progress in basic and clinical researches. However, there are cur-

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rently few studies about the effect of mifepristone on sperms and fertilization process. Therefore, this study is intended to analyze the effect of mifepristone on sperm function by treating sperms in vitro, and explore the possibility of becoming an in-vitro contraceptive.

# 1 Data and methods

## 1.1 Collection of semen samples

From January to December in 2016, 15 cases of normal semen samples were collected from Reproductive Medicine Center of the Third Affiliated Hospital of Inner Mongolia Medical University. The relevant patients aged (35 ± 8) (mean ± *SD*), once had a history of birth or an identified female infertility, with total abstinence from sex for 2-7 d. The ejaculated semen was obtained by use of the masturbation method, and placed in a 37°C constant temperature plate for 30-minute liquefaction. The seminal routine examination was conducted according to World Health Organization (WHO, 2010) standards, the results were as follows: the number of sperms with normal morphology (percentage) > 4%, the volume of semen > 1.5 ml, the sperm concentration > 15 × $10^6$/ml, PR + NP > 40%, PR > 32%, viability (percentage) > 58%. The semen was rinsed by use of density gradient centrifugation (WHO, 2010) and suspended in G-IVF containing 10% human serum albumin (HSA). The sperm concentration was adjusted to 2 × $10^6$/ml.[5]

## 1.2 Methods

### *1.2.1 Treatment of sperms with mifepristone*

The concentration of each semen sample mentioned above was adjusted to 2 × $10^6$/ml by use of density gradient centrifugation, each case of semen sample was divided into 5 groups, with 0.5 ml in each group. Four groups out of these five were considered as the experimental group, containing 0.04 $\mu$M, 0.4 $\mu$M, 4 $\mu$M and 40 $\mu$M mifepristone (M8046, sigma) respectively; the other group was considered as the control group (containing DMSO). These samples were cultured in vitro for 1 h under the condition of 37°C and 5% $CO_2$, and rinsed out thoroughly with the scrubbing solution.

### *1.2.2 Seminal routine analysis*

Sperm motility and hyperactivation were evaluated according to computer-aided sperm analysis (CASA) in WHO laboratory manual for the examination and processing of human semen ($5^{th}$ ed). The parameter setting was made according to the research made by Chiu et al., at least 500 spermatozoa in a random field were chosen out to measure the percentage of hyperactivated sperms.[6] The judgment of hyperactivated sperms is made by curvilinear velocity (VCL) $\geq$ 100 $\mu$m/s, linearity (LIN) $\leq$ 60% and amplitude of lateral head displacement (ALH) $\geq$ 5.0 $\mu$m. Sperm viability was evaluated by Eosin Y staining. Sperms were stained with 0.5% Eosin Y. One drop of liquefied fresh semen was mixed with one drop of Eosin Y solution on a glass slide. Then, covered it with a cover slip. After 30 seconds, at least 200 sperms were counted under the 400 × microscope, with the percentage of live sperms counted (live sperms can not be stained, while the dead can be stained red).

### *1.2.3 Sperm morphological analysis*

The morphological analysis was made to sperms on the basis of Kruger criteria. At least 200 sperms were counted by use of the 1000 × oil lens, and the percentage of normal sperms was measured accordingly.

### *1.2.4 Detection of sperm DNA damage*

Sperm DNA damage was detected by use of comet assay (COMET).[7] After sperms were treated, alkaline single cell gel electrophoresis was performed, so that sperms with intact DNA showed the appearance of comet. After staining, the sperm cells were observed with the 200 × microscope, with tail DNA% and tail moment analyzed by the software. The calculation method is as follows: The product of the percentage of tail DNA in total DNA and the central moment between the head and the tail is used as a standard for judging the degree of sperm DNA damage. Each set of experiments was made three times, three different groups of semen samples were used in each experiment, and at least 500 sperms were observed in each experiment. The control group was treated with DMSO.

### *1.2.5 Progesterone-induced acrosomal reaction*

After the semen was fully liquefied, the concentration of semen was adjusted to 2 × $10^6$/ml by use of density gradient centrifugation. The semen samples were incubated in the incubator (37°C, 5% $CO_2$) for 3 hours to induce capacitation. The progesterone was added with the final concentration of 3.2 $\mu$M, and the solution was incubated under the condition of 37°C and 5% $CO_2$ for 60 minutes.

### *1.2.6 Determination of sperm acrosomal reaction*

Fluorescence labeling was performed by use of fluorescein isothiocyanate conjugated with pisum sativum agglutinin (FITC-PSA), in order to evaluate sperm acrosomal reaction after capacitation.[8] The concentration of sperm suspension was adjusted to 2 × $10^6$/ml by use of density gradient centrifugation. 200 $\mu$l of sperm suspension was added to

1 mg/ml Hoechst 33258 solution, mixed and stained for 10 minutes, eluted with DPBS, smeared, and then added to 30 $\mu$g/ml FITC-PSA to be incubated for 30 minutes, eluted for 10 minutes, and dried. At 450 nm to 490 nm excitation light, at least 200 sperms were randomly observed under the fluorescence microscope (× 600 oil lens). The characteristics of sperms with acrosomal reaction were as follows: Only the equatorial plate in the sperm head showed fluorescence or the entire acrosomal region showed no fluorescence. The characteristics of sperms with no acrosomal reaction were as follows: The acrosomal region showed fluorescence. Proportions of sperms with acrosomal reaction in the experimental tube and the control tube should be calculated respectively, and the subtraction of the two values was the rate of induced acrosomal reaction. Each set of experiments was made three times, three different groups of semen samples were used in each experiment.

#### *1.2.7 Determination of calcium-ion concentration in sperms*

According to the method proposed by Yang et al.,[9] the concentration of semen suspension was adjusted to $2 \times 10^6$/ml by use of density gradient centrifugation, with 5 $\mu$M Fluo-3-AM stain added, and the mixture was incubated in a lightproof place at 37°C for 40 minutes. After the incubation was finished, the mixture was rinsed out 3 times with the scrubbing solution. After being smeared and dried, the mixture was observed under the fluorescence microscope. At 485/535 nm excitation light, at least 200 sperms were randomly observed under the fluorescence microscope (× 600 oil lens). Software was used to detect fluorescence intensity and calculate the percentage of fluorescence intensity, indirectly indicating the change in $[Ca^{2+}]$ i.

### 1.3 Statistical treatment

SPSS20.0 statistical software was applied to the statistical analysis, the quantitative data fitted to normal distribution were represented by mean ± standard deviation ($\bar{x} \pm s$), analysis of variance was used in the comparison among groups, and the comparison between two groups was made by use of *LSD-t*. The difference $p < .05$ was of statistical significance.

## 2 Results

### 2.1 Effect of mifepristone on sperm viability, motility and morphology

After sperms were treated with different concentrations (0.04 $\mu$M, 0.4 $\mu$M, 4 $\mu$M, 40 $\mu$M) of mifepristone, sperm viability, motility and morphology were examined accordingly. The results showed that, after the sperms were treated with different concentrations of mifepristone, sperm viability, motility and morphology were not significantly different from those in the control group ($p > .05$). See Table 1 for detailed results.

### 2.2 Effect of mifepristone on sperm DNA damage

The effect of different concentrations of mifepristone on sperm DNA damage in the experimental group was not significantly different from those in the control group ($p > .05$). See Table 2 for detailed results.

**Table 1:** Effect of mifepristone on sperm viability, motility and morphology (%, $\bar{x} \pm$ s)

| Item | Experimental group | | | | Control group |
|---|---|---|---|---|---|
| | 0.04 μM | 0.4 μM | 4 μM | 40 μM | |
| Viability | 80.6 ±1.53 | 81.7 ±1.53 | 80.3 ±3.21 | 79.0 ±2.65 | 82.7 ±2.52 |
| Motility | 53.7 ±1.53 | 50.3 ±2.31 | 51.7 ±0.55 | 50.3 ±3.21 | 50.0 ±2.00 |
| Morphology | 14.0 ±1.00 | 11.7 ±2.08 | 12.7 ±1.15 | 11.0 ±1.00 | 13.3 ±1.53 |

**Table 2:** Effect of different concentrations of mifepristone on sperm DNA damage (%, $\bar{x} \pm$ s)

| Item | Experimental group | | | | Control group |
|---|---|---|---|---|---|
| | 0.04 μM | 0.4 μM | 4 μM | 40 μM | |
| Proportion of damaged sperms | 20.6 ±1.5 | 25.2 ±1.3 | 22.6 ±3.1 | 21.0 ±1.2 | 24.6 ±1.5 |
| Tail length | 22.4 ±1.4 | 23.3 ±2.0 | 20.3 ±1.1 | 19.2 ±1.2 | 21.2 ±1.3 |

### 2.3 Effect of mifepristone on progesterone-induced acrosomal reaction and hyperactivation

A research was conducted to investigate whether mifepristone could affect progesterone-induced acrosomal reaction and hyperactivation. The results showed that when the concentration of mifepristone in the experimental group was $\geq 0.04$ $\mu$M, the ability of acrosomal reaction and the proportion of hyperactivated sperms were significantly decreased in comparison with the control group ($p < .01$); when the concentration of mifepristone in the experimental group was 0.4 $\mu$M, AR rate and the proportion of hyperactivated sperms were decreased to (9.9 ± 1.2) and (29.4 ± 3.4) respectively. See Table 3 for detailed results.

**Table 3:** Effect of different concentrations of mifepristone on progesterone-induced acrosomal reaction and hyperactivation (%, $\bar{x}$ ± s)

| Item | Experimental group | | | | Control group |
|---|---|---|---|---|---|
| | 0.04 μM | 0.4 μM | 4 μM | 40 μM | |
| AR rate | 14.0 ±1.6 | 9.9 ±1.2* | 9.2 ±1.2* | 10.1 ±1.2* | 15.7 ±1.5 |
| Proportion of hyperactivated sperms | 71.1 ±3.8 | 29.4 ±3.4* | 24.8 ±3.7* | 24.9 ±3.1* | 70.8 ±3.7 |

*Note.* *$p < .01$ in comparison with the control group

### 2.4 Effect of mifepristone on calcium fluxes

Both acrosomal reaction and hyperactivation are calcium-dependent events. Therefore, the calcium-ion specific dye Fluo-3-Am can be used to detect the concentration of free calcium ions in sperms. The results showed that the percentage of fluorescence intensity in the control group was (89.3 ± 1.2). When the concentration of mifepristone in the experimental group reached 0.04 $\mu$M, the percentage of fluorescence intensity was (90.9 ± 1.8). In comparison to the control group, the difference was not statistically significant ($p > .05$). However, when the concentration of mifepristone reached 0.4 $\mu$M, the percentage of fluorescence intensity was decreased to (76.7 ± 2.4), reflecting that mifepristone significantly depressed the concentration of calcium ions. In comparison to the control group, the difference was statistically significant ($p < .01$). When the concentration reached 4 $\mu$M and 40 $\mu$M, the percentages of fluorescence intensity were (73.7 ± 2.6) and (75.4 ± 2.6) respectively, which were lower than those in the control group. The difference was statistically significant ($p < .01$).

## 3 Discussion

Mifepristone is a novel antiprogestin that has a strong affinity with progesterone receptors and glucocorticoid receptors. Studies have shown that low-dose mifepristone can be used for emergency contraception among women, with the advantage of few side effects.[3] A single oral dose of 10 mg mifepristone for emergency contraception can make the maximum concentration in serum reached up to (1.14 ± 0.31) $\mu$M.[10] The doses used in this study cover this range. After ejaculation, sperms are exposed to high levels of progesterone secreted by cumulus cells and corpus luteum by passing through the female reproductive tract.[11] The concentration of progesterone in the extracellular matrix of cumulus cells can reach up to 3.2-32 $\mu$M,[12] which can induce calcium-ion fluxes to pass through the CatSper channel into sperms and trigger multiple $Ca^{2+}$-dependent physiological responses such as acrosomal reaction and hyperactivation to complete fertilization.[13] Progesterone has been identified to be effective on enhancing acrosomal reaction and associated with the fertilization ability.[14] We supposed that mifepristone could reduce the incidence of acrosomal reaction by impairing sperm function, thereby preventing fertilization. The results of this study indicate that treating sperms with different concentrations of mifepristone in vitro does not affect sperm viability, motility and morphology. Studies have shown that giving a dose of 200 mg mifepristone to volunteers respectively can not affect sperm motility,[15] which is consistent with the results of this study. Detection of DNA integrity has revealed that treating sperms can not damage sperm DNA. DNA damage in human sperms is associated with fertilization and preimplantation embryo development.[16] Progesterone receptor modulators, including mifepristone, have been identified to induce endometrial apoptosis and increase cellular DNA fragments.[17] Compared to somatic cells, this study does not detect the effect of progesterone receptor modulators on sperm DNA integrity, it is probably because sperm DNA is denser and more resistant to division.[18]

The research was conducted to investigate whether treating sperms with mifepristone in vitro could affect acrosomal reaction, sperm hyperactivation and the concentration of free calcium ions in sperms. The results have shown that when the concentration of mifepristone is $\geq 0.04$ $\mu$M, the ability of acrosomal reaction, the proportion of hyperactivated sperms and the concentration of free calcium ions in sperms are significantly decreased accordingly. We speculate that mifepristone can lower the concentration of calcium ions in sperm mitochondria by decreasing the concentration of calcium ions, further affecting mitochondrial function. Mitochondrial dysfunction can probably lead to the decrease in the proportion of hyperactivated sperms ($p < .01$). Previ-

ous researches suggest that mifepristone can not rival acrosomal reaction induced by progesterone,[19] however, these researches show opposite results sometimes. Yang et al. considered[9] that mifepristone could prevent acrosomal reaction by depressing the inflow of calcium ions caused by progesterone. The results of this study showed that when the concentration of mifepristone reached up to 0.04 $\mu$M, acrosomal reaction rate was significantly decreased ($p < .01$). We consider that mifepristone directly inhibits the process of the external calcium ions transporting across cell membranes into sperms, which leads to the decrease in the level of free calcium ions in sperms to prevent them from acrosomal reaction.

In summary, mifepristone can prevent the incidence of fertilization by inhibiting sperm hyperactivation and acrosomal reaction in vitro. However, as a type of in-vitro contraceptive drugs, the effect of mifepristone is not more significant than that of nonoxynol. Therefore, it is necessary to further investigate the effect of mifepristone on the fertilization mechanism as a male contraceptive drug.

## Conflicts of Interest Disclosure

The authors have no conflicts of interest related to this article.

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