

# ***In vitro* effect of ulipristal acetate on human sperm parameters and function**

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**Abstract:** To observe and investigate the *in vitro* effect of ulipristal acetate (UPA) on human sperm parameters and function. The 20 patients with normal semen parameters and average age of (32.5±8.5) years old, who were treated in our hospital from January 2018 to August 2018, were selected as research objects. They were subjected to density gradient centrifugation, and then four groups were incubated for about an hour in a culture medium containing different concentrations of ulipristal acetate and the other two groups were set as blank control group and dimethylsulphoxide (DMSO) control group. Indicators including sperm motility, sperm hyperactivation and sperm concentration of free calcium ions of each group were tested. Under the ulipristal acetate concentration of 0.04 mol/L, the proportion of sperm damage was increased, the length of tail was increased, and the proportion of sperm hyperactivation was decreased,  $p < 0.05$ . In addition, the acrosome reaction was inhibited, which significantly reduced the calcium concentration in the sperm,  $p < 0.05$ . Ulipristal acetate can significantly inhibit acrosome reaction and hyperactivation of sperm *in vitro*, and can reduce the concentration of calcium ions in sperm, thus causing sperm damage.

**Keywords:** Ulipristal acetate, human sperm parameters, effect on function, *in vitro*.

## **INTRODUCTION**

In recent years, in the context of intensifying research on the mechanisms of emergency contraceptives, it is of great significance to promote the development of new measures with less adverse effects (Huang Chen and Zhang, 2017; Wang Duan and Shi, 2017). Ulipristal acetate (UPA, CDB2914) is a typical progesterone receptor modulator and an effective emergency contraceptive. Studies have shown that the drug can effectively prevent pregnancy within 120 hours after unprotected sex and has a higher success rate than levonorgestrel (Shi *et al.*, 2014; Lessey and Kim, 2017; Whitaker *et al.*, 2017). UPA can significantly inhibit acrosome reaction and hyperactivation of sperm *in vitro* and reduce the concentration of calcium ions in sperm, thus causing sperm damage. The main mechanism of UPA is to inhibit ovulation and interfere with embryo implantation. UPA has been widely recognized owe to the ideal results obtained from using it. In recent years, more progresses in the study of UPA have been obtained. This work mainly investigates the *in vitro* effect of ulipristal acetate on human sperm parameters and function (Pei, 2017; Kamal *et al.*, 2017; Zeeshan *et al.*, 2018).

## **MATERIALS AND METHODS**

### ***General design***

The 20 male patients, with averaged age of 32.5±8.5 years old, who had been treated in Zhengzhou People Hospital from January 2018 to August 2018 were selected as research objects. This paper has a rigorous structure, and

the conclusion has been approved by relevant ethics and relevant departments. This study was approved by Ethic Committee of Hospital. The selected patients realised the right to know and signed informed consent before study. All included subjects met the following criteria: having fertility history, or proved female factor infertility, abstinence for 2-6 days, average (4.6 + / 0.9 days); semen was obtained by masturbation and then subjected to liquefaction under 37°C for half an hour, then routine examination of semen was carried out according to the Guidance on Semen Analysis of World Health Organization (the 5th Edition). Semen parameters: morphological normal sperm >4%, sperm concentration > 15 × 10<sup>6</sup>/mL, semen volume >1.5 ml, sperm motility rate (PR + NP) >40% (NP represents non-forward motile sperm), sperm motility (PR) >32%, survival rate >58%. After liquefaction, semen was washed by density gradient centrifugation, and suspended in 10% human serum albumin, with sperm concentration regulated to 2 × 10<sup>6</sup>/mL.

### ***Method***

First, the sperm was treated with UPA. 20 resuspended semen samples were divided into six parts, with a specification of 0.5mL/part and finally six groups of samples (n = 20) were obtained. Among them, there were two control groups, namely blank control group and DMSO control group, and the other four groups were treated with UPA of different concentrations, i.e. 0.04µmol/L (group A), 0.4µmol/L (group B), 4µmol/L (group C) and 40µmol/L (group D). Subsequently, the samples were subjected to density gradient centrifugation, *in vitro* incubation in 5% CO<sub>2</sub> for about 1 h at 37°C, followed by liquor washing. After that, analysis of sperm

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motility and hyperactivation was carried out in accordance with the content of computer-assisted sperm analysis prescribed in WHO Laboratory Manual of Human Semen Testing and Treatment. At the same time, various parameters were set according to the existing studies, and sperm (500) in the random field were selected for evaluation of the percentage of super activated sperm. Moreover, the survival rate of sperm was evaluated by Y staining. Thirdly, the sperm morphology was analyzed. According to the Kruger standard and the percentage of normal sperm was obtained. In addition, sperm DNA damage was detected, and fragments formed after sperm DNA strand breaks were detected by comet experiments. After sperm treatment, the basic single-cell gel electrophoresis was carried out, the sperm with complete DNA formed the comet appearance, and the staining was performed and observed under a 200-fold microscope, and then the fluorescence percentage and tail distance of the tail and the total comet were analyzed with the software. Subsequently, detection of progesterone induced acrosomal reaction was carried out. After liquification, semen was subjected to a density gradient centrifugation to adjust the sperm concentration to  $2 \times 10^6$  /mL, followed by incubation in 5% CO<sub>2</sub> under 37°C for three hours. Then, progesterone was added, and the final concentration was set to 3.2mol /L for 1 hour of incubation. Then, the sperm acrosome reaction was determined again. The main method was to use FITC-PSA to carry out fluorescence labelling and evaluate the acrosome reaction of spermatozoa after obtaining energy. Finally, the concentration of free calcium ions in sperm was determined. The sperm suspension (after density gradient centrifugation) concentration was adjusted to  $2 \times 10^6$ /mL, then the 5  $\mu$ mol/L fluo-3-AM dye solution was added for incubation under 37 °C without light for 40 minutes, before being washed. After that, the fluorescence intensity was detected by software, and corresponding fluorescence percentage was obtained, which indirectly reflects the change of [Ca<sup>2+</sup>]<sub>i</sub> (Jeong *et al.*, 2017; Ge *et al.*, 2017).

## STATISTICAL ANALYSIS

All data were analyzed and processed by statistical analysis software SPSS21.0. Quantitative data were expressed by mean  $\pm$  average ( $\bar{x} \pm s$ ), with chi-square used for intergroup comparison; Enumeration data were expressed by natural number (n) and percentage (%), with intergroup comparison tested by t. The difference was considered statistical significant when  $p < 0.05$ .

## RESLUTS

### *Effect of UPA on sperm survival, motility and morphology*

As shown in table 1, the comparison of indicators between the blank control group and DMSO control

group showed no significant difference,  $p > 0.05$ . The comparison of the overall results between the UPA groups and the DMSO control group showed no significant difference either,  $p > 0.05$ .

### *Effect of UPA on sperm DNA damage*

As shown in table 2, there was no significant difference in the ratio of sperm damage between the two control groups,  $p > 0.05$ . Through observing the sperm damage proportion and length of tail between UPA groups and DMSO control group, the results showed significant difference,  $p < 0.05$ . See figs. 1 and 2 as follow.



**Fig. 1:** Sperm DNA damage detected by comet assay for DMSO control group



**Fig. 2:** Sperm DNA damage detected by comet assay for UPA group B

### *Effect of UPA on acrosomal reaction and hyperactivation induced by progesterone*

As shown in table 3, there were significant differences in acrosome reaction rate and the proportion of super activated sperm between DMSO control group and UPA groups,  $p < 0.05$ . See figs. 3 and 4 as below.

### *Effect of UPA on concentration of free calcium ions in sperm*

As shown in table 4, the comparison of fluorescence intensity ratio between UPA groups and DMSO control group showed significant difference,  $p < 0.05$ .

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

Indicator	Blank control group	DMSO control group	UPA group A	UPA group B	UPA group C	UPA group D
Survival rate (%)	76.0 ± 1.8	78.0 ± 1.5	76.3 ± 2.8	75.0 ± 2.8	77.5 ± 2.4	75.6 ± 3.1
Motility (%)	50.3 ± 2.1	49.4 ± 1.6	52.8 ± 1.2	49.6 ± 2.5	50.4 ± 0.8	49.6 ± 3.0
Morphology (%)	12.4 ± 2.3	12.5 ± 1.2	13.2 ± 1.1	11.4 ± 1.6	11.9 ± 1.2	11.1 ± 1.2

**Table 2:** Effect of UPA on sperm DNA damage ( $\bar{x} \pm s$ )

Indicator	Blank control group	DMSO control group	UPA group A	UPA group B	UPA group C	UPA group D
Degree of sperm damage (%)	23.3 ± 1.5	24.0 ± 1.6	22.1 ± 1.6	34.1 ± 1.8	31.8 ± 3.1	29.8 ± 1.4
The length of the tail (%)	20.1 ± 1.8	20.3 ± 1.2	21.5 ± 2.2	27.9 ± 2.2	26.5 ± 2.0	26.7 ± 2.0

**Table 3:** Effect of UPA on acrosomal reaction and hyperactivation induced by progesterone ( $\bar{x} \pm s$ )

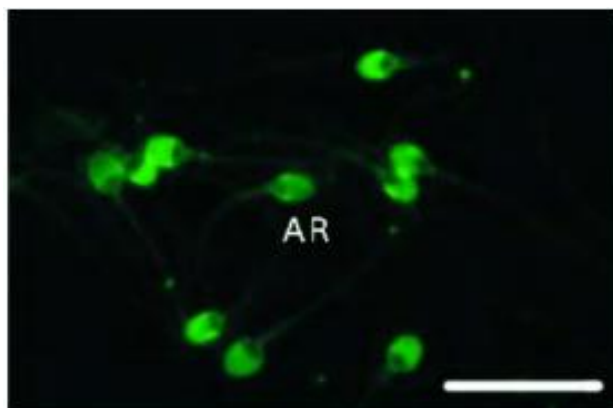
Indicator	Blank control group	DMSO control group	UPA group A	UPA group B	UPA group C	UPA group D
Acrosomal reaction rate (%)	14.5 ± 0.9	14.6 ± 1.8	12.8 ± 1.6	8.7 ± 1.3	8.4 ± 1.1	8.2 ± 1.2
Hyperactivated sperm proportion (%)	70.8 ± 2.5	70.4 ± 3.5	72.3 ± 2.0	45.8 ± 2.9	44.6 ± 3.2	44.8 ± 3.4

**Table 4:** Effect of UPA on concentration of free calcium ions in sperm ( $\bar{x} \pm s$ )

Indicator	Blank control group	DMSO control group	UPA group A	UPA group B	UPA group C	UPA group D
Fluorescence intensity ratio (%)	89.6 ± 2.2	90.8 ± 2.3	90.2 ± 2.1	66.7 ± 2.4	63.9 ± 2.8	65.0 ± 2.6

## DISCUSSION

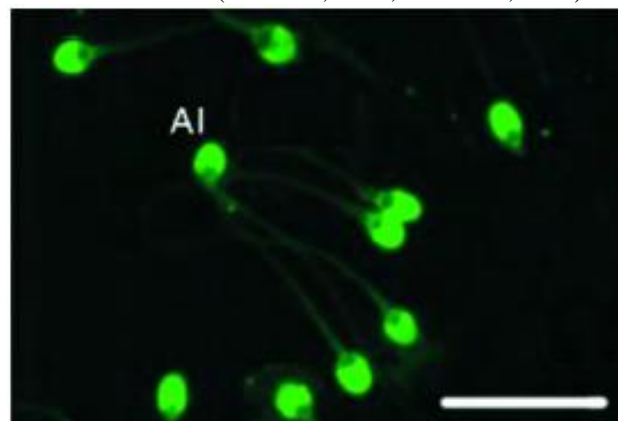
As a typical progesterone receptor modulator, UPA has a good antagonistic and exciting effect on progesterone receptor. Although studies have shown that (Rekawiecki Kowalik and Kotwica, 2017), UPA as an emergency contraceptive after unprotected sex has a higher success rate, the mechanism of its action has not yet been fully recognized.



**Fig. 3:** The acrosome reaction detected by fluorescent staining of pisum sativum agglutinin for DMSO control group.

At present, existing research results show that the addition of 1-1000 ng/mL UPA *in vitro* for sperm treatment will

not affect sperm motility and sperm protein tyrosine phosphorylation. In this study, the addition of different concentrations of UPA *in vitro* did not affect sperm motility, viability and morphology. However, when the concentration was increased to 0.4 μmol/L, the proportion of sperm DNA damage and the proportion of tail length were significantly increased. DNA Damage is closely related to fertilization, pre-implantation embryo development, and the regulator of progesterone receptors, for instance mifepristone can induce apoptosis of cells and granulocytes in the endometrium, leading to DNA fragmentation. This result is consistent with the results of relevant researches (Liu *et al.*, 2017; Gao *et al.*, 2017).



**Fig. 4:** The acrosome reaction detected by fluorescent staining of pisum sativum agglutinin for UPA group B.

The results of this study showed that the acrosome reaction ability, the proportion of super activated sperm and the concentration of free calcium ions in the sperm would be reduced if the concentration of UPA was at 0.4mb mol/L or above. Current evidence suggests that the mechanism of progesterone action in sperm may be mediated by a specific non-genomic progesterone membrane receptor or sperm cationic channel calcium channel. UPA binds to progesterone acetate receptor and exerts effects on sperm function, and inhibit  $Ca^{2+}$  from entering into sperm.

The results of this study showed that UPA reduced the calcium ion concentration in the mitochondria of sperm, which had an impact on the function of mitochondria. In the case that the function of mitochondria was damaged, the proportion of super activated sperm would be reduced. If the concentration of UPA was 0.4mol/L, the acrosome reaction rate was significantly reduced. UPA directly inhibited the calcium ions in the external environment from entering into the sperm, thereby reducing the level of free calcium ions in the sperm and avoiding acrosome reactions in the sperm.

## CONCLUSION

In conclusion, UPA can significantly inhibit acrosome reaction and hyperactivation of sperm *in vitro*, and can reduce the concentration of calcium ions in sperm. Currently, the use of contraceptives is very common, and this method can effectively avoid the damage of induced abortion. However, advantages of emergency contraceptives have not been fully displayed according to others studies. In addition, there are very limited numbers of such studies in China. UPA may avoid fertilization by increasing the proportion of sperm damage, inhibiting sperm hyper activation and acrosomal reaction *in vitro*, but its effect as an *in vitro* birth control pill is not more significant than nonbenzaldehyde. As a male contraceptive pill, more large-sample studies should be carried out in the future to observe the effect of UPA on the fertilization mechanism.

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