

## Changes in Motility Parameters of Mouse Spermatozoa in Response to Different Doses of Progesterone during Course of Hyperactivation

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### ABSTRACT

The aim of this study was to evaluate changes that occur in motility parameters of progesterone treated mouse spermatozoa during course of hyperactivation. Mouse spermatozoa treated with different doses of progesterone were videotaped after 10 min and 90 min of incubation. For each sperm, one second of movement of the head-midpiece junction was traced from the videotape and for each tracing; seven motility parameters were studied using computer assisted image analysis. For all progesterone treated spermatozoa, motility rate differed significantly from control group after 90 min of incubation. Motility parameters for high doses of progesterone 10 and 100 µg/ml showed hyperactivation occurred during 10 min of incubation. With treatment of 1 µg/ml progesterone, hyperactivated motility pattern of spermatozoa occurred 90 min after incubation similar to the control group showing that low dose of progesterone is unable to induce hyperactivation. In conclusion, progesterone induces hyperactivation in mouse sperm and reduces the motility rate during the time. *Iran. Biomed. J. 9 (2): 73-79, 2005*

**Keywords:** Progesterone, Hyperactivation, Spermatozoa, Mouse

### INTRODUCTION

Spermatozoa are deposited in the female reproductive tract where they undergo several metabolic and structural changes, becoming fertilization-competent. These events are known as sperm capacitation and consist of several biochemical and functional modifications of sperm cells ultimately leading to changes that enable the sperm to undergo both the acrosome reaction and hyperactivation (HA).

HA is a functional change in the sperm movement pattern which usually occurs during the capacitation process [1]. This motility pattern appears to be related to the final stages or completion of capacitation [1]. Early studies described HA as a relatively progressive motion with high-amplitude flagellar bending [2]. However, later studies

referred to non-progressive motility with highly curved, 'whiplash' or 'figure-of-eight' motion [3]. Experimental evidence gathered from studying populations of mouse spermatozoa showed that HA conferred a direct mechanical advantage on spermatozoa, permitting their penetration through viscous medium [4], which could facilitate passage through oviduct and ovum investments. It has been proposed that HA may improve mammalian sperm migration through the epithelial folds of the oviduct by preventing entrapment and increasing the probability of contact with the cumulus [3].

Several studies have suggested that HA allows spermatozoa to detach from the oviductal epithelium and provides increased thrust for penetration of the cumulus [5, 6].

It has been shown that HA also facilitates penetration of spermatozoa through the oocyte zona

\*Corresponding Author; Fax: (+98-21) 694 9389; E-mail: katebi@sina.tums.ac.ir; **Abbreviations:** Hyperactivation (HA); Straight-line velocity (VSL); Curvilinear velocity (VCL); Average path velocity (VAP); Linearity (LIN); Straightness (STR); Curvilinear progressive ratio (PRC); Computer assisted sperm analysis (CASA)

pellucida [7]. Consequently, objective measures of HA can serve as biological end-points to evaluate the functional capabilities of spermatozoa.

Blazak *et al.* [8] determined that quantitative analysis of sperm motility and velocity is particularly good indicators of deleterious effects in the reproductive system. Among them, the most sensitive measures are the percentage of motile cells, mean sperm swimming speed, and linearity (LIN), as reported for rodents [9] and humans [10].

High concentrations of the steroid are present in the cumulus matrix that surrounds the oocyte which must be necessarily crossed by the sperm to reach the zona pellucida [11]. Progesterone and 17  $\alpha$ -hydroxyprogesterone present in the follicular fluid are responsible of most of the biological effects of this fluid on spermatozoa [12]. The effects of progesterone are mediated essentially by three signaling pathway: rapid increase of intracellular  $\text{Ca}^{2+}$ , efflux of  $\text{Cl}^-$ , stimulation of phospholipases and phosphorylation of sperm proteins [13].

Sperm responsiveness to progesterone is reduced in oligozoospermic and infertile patients [14], suggesting the possibility of predicting sperm fertilizing ability or enhancing fertilization by treatment of spermatozoa *in vitro* with progesterone. At present, the appropriate technique of assisted reproduction in the case of male infertility is chosen on the basis of the number of spermatozoa obtained after selection (swim up, mini-Pericoll).

In the present study, we have evaluated changes that occur in motility parameters of spermatozoa in response to different doses of progesterone during course of HA as a model to choose high quality spermatozoa.

Computer-assisted image analysis has been used to characterize the motion of mouse epididymal spermatozoa and changes in motion parameters have been interpreted as indicators of effects of progesterone to identify those spermatozoa showing both increased vigour and decreased progression.

## MATERIALS AND METHODS

**Preparation of sperm.** ICR male mice (10-12 weeks) were rapidly killed by cervical dislocation. The cauda epididymis was promptly removed. After removal of blood from epididymal surface with a physiological salt solution, the distal tubules were punctured with a 27-gauge needle in three to five places, and a mass of sperm were squeezed out with forceps into a plastic Petri dish (35  $\times$  106 mm)

containing 5 ml of mWM medium prewarmed at 37°C, supplemented with 3 mg/ml bovine serum albumin (BSA; fatty acid free Fraction V, Sigma Chemical Inc., Tokyo, Japan), 100U/ml penicillin, and 0.1 mg/ml streptomycin. The spermatozoa were incubated at 37°C under 5%  $\text{CO}_2$  in air.

**Treatment of spermatozoa with progesterone.** Progesterone (Sigma Chemical Inc., Tokyo, Japan) was dissolved individually in absolute alcohol (1 mg/ml) and diluted serially in culture medium to obtain a final concentration of 1  $\mu\text{g/ml}$ , 10  $\mu\text{g/ml}$  and 100  $\mu\text{g/ml}$ , and the spermatozoa were cultured for 10 min or 90 min in a  $\text{CO}_2$  incubator.

**Sample preparation.** Each 14  $\mu\text{l}$  of sperm suspension was withdrawn to analyze the movement pattern after 10 min, 90 min of incubation. The suspension was diluted with the medium to give a final concentration of around  $2 \times 10^6$  sperm per ml. The suspension was placed on a glass slide prewarmed at 37°C and covered with an 18  $\times$  24 mm cover slip. The glass slide had been coated with 0.5% PVP (PolyvinylpyrrolidoneK-90), in order to minimize sticking of sperm to glass surfaces. As soon as the sample was prepared, photographs were taken for each analysis.

**Experimental design.** The experimental groups were as follows: 1) Control, without progesterone; 2) Exp. 1, with 1  $\mu\text{g/ml}$  progesterone; 3) Exp. 2, with 10  $\mu\text{g/ml}$  progesterone; 4) Exp. 3, with 100  $\mu\text{g/ml}$  progesterone. The groups were studied after 10 and 90 min of incubation.

**Analysis for movement pattern of sperm.** In the analysis for movement pattern of sperm, photographs were taken in 30 frames per second with FASTCAM-Net high-speed camera (PHOTRON, Tokyo) and the obtained images were recorded using Movie Ruler software (PHOTRON, Tokyo). The analysis was performed using successive 30 frames. An exposure time was 1/2000 second on the phase contrast microscope (Olympus Optical Co., Tokyo, Japan). Immotile or non-progressive spermatozoa were omitted from analysis. The sperm motility parameters in this study were as follows:

1) Straight-line velocity (VSL,  $\mu\text{m/s}$ ) is the straight-line distance between the first and last tracked points, divided by the acquisition time; 2) Curvilinear velocity (VCL,  $\mu\text{m/s}$ ) is the total distance between adjacent points, divided by the time elapsed; 3) Average path velocity (VAP,  $\mu\text{m/s}$ )

is a smoothed path constructed by averaging several neighbouring positions on the track (five points) and joining the averaged positions, which reduced the effect of lateral head displacement; 4) LIN, an index of the straightness (STR) of the path. LIN is derived from the ratio of VSL/VCL multiplied by 100; 5) STR, an index of the departure of the sperm path from a straight line. STR is derived from the ratio of VSL/VAP multiplied by 100; 6) curvilinear progressive ratio (PRC), an inverse index of lateral head displacement. PRC is derived from the ratio of VAP/VCL multiplied by 100 [11].

In order to get motility rate, the video playback option was used. At First, the total number of sperm in a static visual field was determined using the video pause function. The tape was then allowed to run, and sperm that remained in their original position were counted as immotile or non-progressive spermatozoa.

**Statistical analysis.** Data were analyzed by One-Way ANOVA followed up with post hoc test (Tukey).

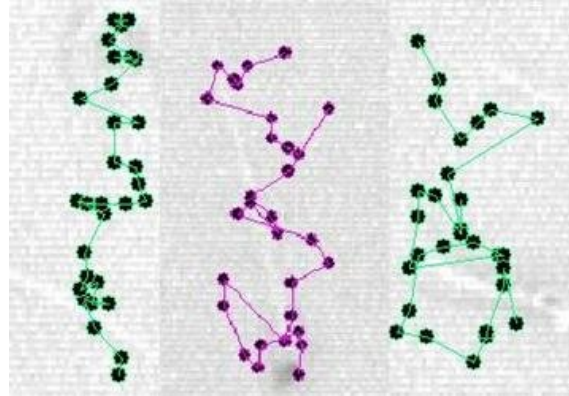
## RESULTS

The changes in the motility rate and pattern of movement of spermatozoa under the influence of progesterone were clarified by analyzing the change in movement pattern after incubation for 10 min and 90 min in different doses of progesterone. Change in the motility pattern of mouse spermatozoa during the course of HA is shown in Figure 1.

There was no significant difference in the motility rates after 10 min of incubation for control and experimental groups. But there were significant differences between control with the experimental groups after 90 min of incubation and between experimental groups ( $P < 0.05$ ).

The motility rates of the experimental groups after 90 min of incubation decreased significantly ( $P < 0.05$ ) (Fig. 3a). After 10 min of incubation, Experimental groups of exp. 2 and exp. 3 and after 90 min of incubation of groups of exp.1 and exp. 2, the track of movement of spermatozoa increased in length of lateral movement and followed a circular trajectory.

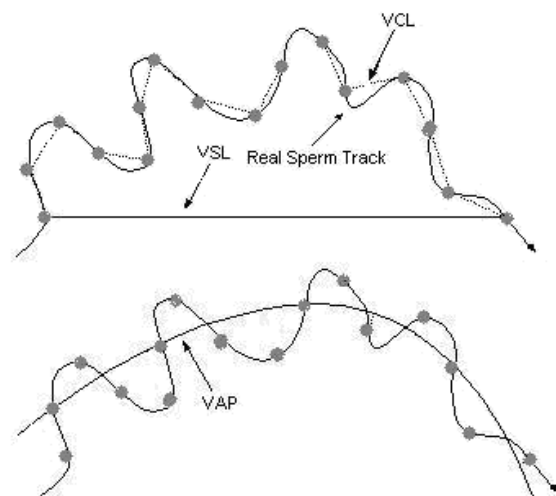
The motion parameters (VSL, VCL and VAP) which were studied are shown in Figure 2.



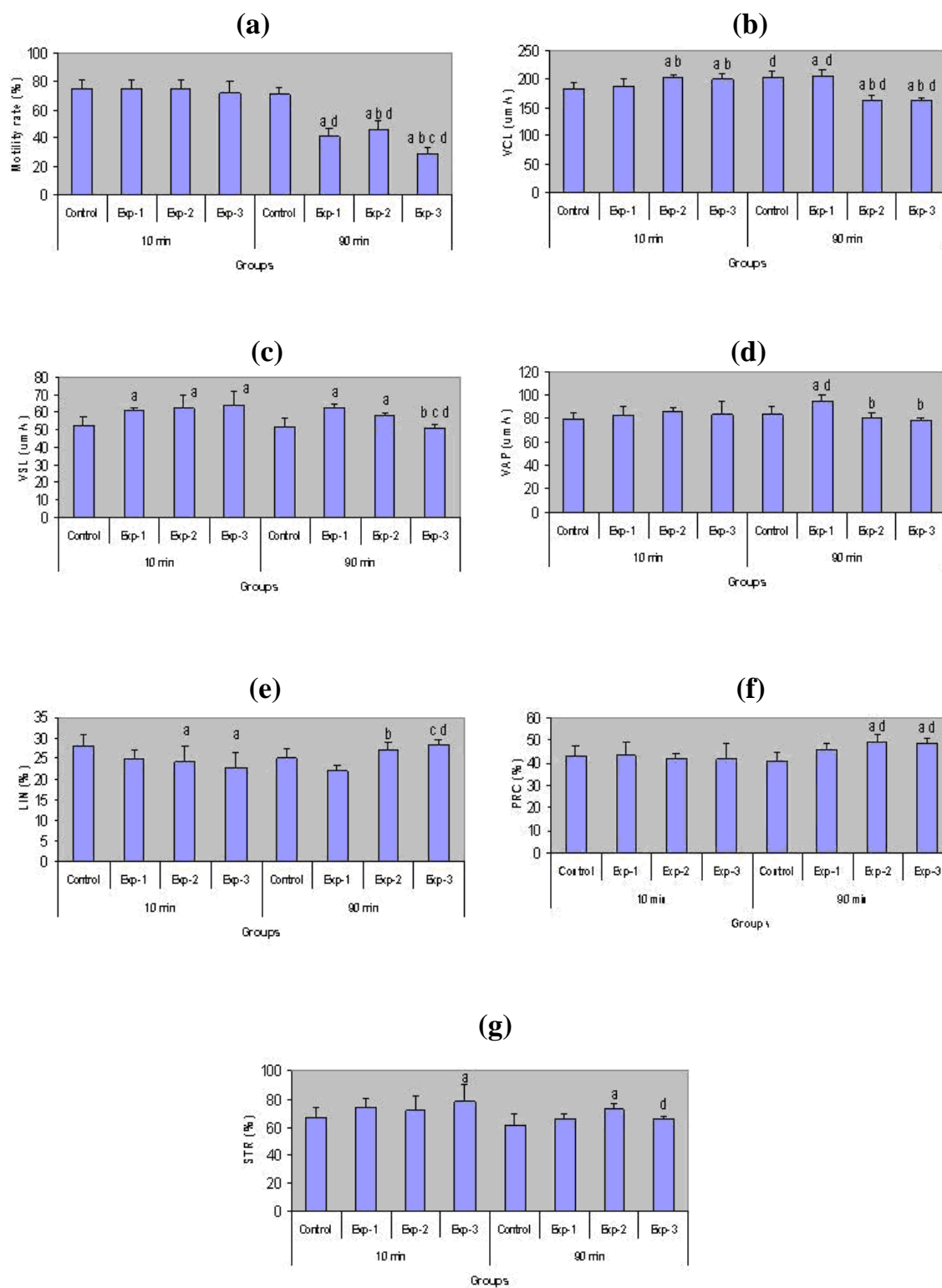
**Fig. 1.** Definition of changes in motility pattern during capacitation of mouse spermatozoa. Left, incapacitated or fresh sperm; Middle, intermediate sperm; Right, hyperactivated sperm.

The track of lateral movement also changed in length with time. These characteristics of the changes in movement pattern were also shown quantitatively by measuring the parameters such as: VCL, VSL, VAP, LIN, PRC and STR. VCL, an index of swimming speed, increased in the control and exp.1 after 90 min. It means that HA occurred with delay but in the exp.2 and exp.3 HA happened at 10 min and decreased after that (Fig. 3b).

As it was expected VSL, distance between the first and the last tracked points, decreased slightly during 90 min of incubation except the exp.1 which increased insignificantly (Fig. 3c).



**Fig. 2.** Definition of sperm tracks and related parameters. VCL, curvilinear velocity; VSL, straight-line velocity; VAP, average path velocity. The gray circles show the position of the sperm head in each frame.



**Fig. 3.** Comparison of motility parameters including motility rate (a), VCL (b), VSL (c), VAP (d), LIN (e), PRC (f), STR (g) after treatment of progesterone (0  $\mu\text{g}/\text{ml}$  as control, 1  $\mu\text{g}/\text{ml}$  as exp. 1, 10  $\mu\text{g}/\text{ml}$  as exp. 2 and 100  $\mu\text{g}/\text{ml}$  as exp. 3) within time (10 and 90 min). a, significant difference versus control; b, significant difference versus exp. 1; c, significant difference versus exp. 2; d, significant difference versus same group during 10 min.

VAP, obtained by measuring the smooth path of the average of successive 5 points, to reduce the effect of lateral head displacement, increased in the control and exp.1 groups but just significant difference in the exp.1 (Fig. 3d). LIN, an index of path STR, decreased in the control and exp.1 with time, reflecting the time dependent decrease in the radius of the circular swimming path or the increase in the lateral head displacement.

In the exp.2 and 3 as HA accrued during 10 min. LIN increased after 90 min of incubation indicating that progesterone treated spermatozoa loses HA during the 90 min of observation (Fig. 3e).

PRC, an inverse index of lateral head displacement, increased in exp.2 and 3 after 90 min. incubation showing that lateral head displacement is reducing after 90 min. incubation in high doses of progesterone (Fig. 3f). STR, an index of the departure of the sperm path from a straight line, increased in exp.3 after 10 min of incubation (Fig. 3g).

## DISCUSSION

Our results showed that the motility rates of mouse spermatozoa treated with 1 µg/ml, 10 µg/ml and 100 µg/ml progesterone after 90 min of incubation decreased significantly ( $P<0.05$ ). Other authors [15] reported low cytotoxic effect of progesterone in human spermatozoa at much higher (3-300 µg/ml) concentrations. Thérien *et al.* [16] found that concentrations of progesterone higher than 2 µg/ml provoke a significant decrease of viability in ejaculated bovine spermatozoa at long incubation. Sperm motility appears to be a sensitive parameter of sperm function [17, 18].

The motility of hyperactivated spermatozoa has been analyzed using computer assisted sperm analysis (CASA) in various species, for example mice [19], humans [20], rats [21] and hamster [22]. In CASA analysis, these patterns have been compared in freshly collected and hyperactivated spermatozoa, but have not been examined over time, as HA appears to occur abruptly. However, in elucidating the mechanism of HA, it is important to examine the time-dependent changes in these parameters.

Our study showed that mouse sperm treatment with 10 µg/ml and 100 µg/ml progesterone can induce HA after 10 min incubation. However, treatment of sperm with 1 µg/ml progesterone did not induce HA suggesting that P affects the spermatozoa of mouse in a dose-dependent manner.

The high doses caused HA soon after treatment and the same results obtained by the others [23]. Other studies also failed to show a stimulation of HA by progesterone across a wide range of progesterone concentrations [24]. Kay *et al.* [25] demonstrated that no effect upon HA was detected on exposure of fresh or cryopreserved human spermatozoa to 1 µg/ml progesterone. This result indicates that progesterone treated sperm had significantly lower mean straight-line velocities than non-treated sperm ( $P<0.05$ ); this difference was reversed after 90 min of incubation. This is very similar to the results previously obtained by Suarez *et al.* [26]. They were compared swimming velocities of sperm incubated for 60 min with those of fresh sperm. Their results showed that hyperactivated sperm had significantly lower mean straight-line velocities than fresh sperm. Our results showed that progesterone at 10 and 100 µg/ml after 10 min incubation, significantly increased VCL than non-treated sperm ( $P<0.05$ ).

After 90 min of incubation, curvilinear progressiveness ratio was increased significantly than non-treated sperm ( $P<0.05$ ). Suarez *et al.* [27] were measured HA of mouse spermatozoa from videotape recordings and compared with those of epididymal sperm incubated for 90 min under capacitating conditions. Their results showed higher VCL and wider-amplitude head movements as measured by PRC. Our result showed higher VCL of untreated spermatozoa after 90 min of incubation than 10 min incubation but there was no significant difference in PRC of control groups. The PRC of spermatozoa after 90 min of incubation with 10 µg/ml and 100 µg/ml progesterone was increased significantly ( $P<0.05$ ). Indicating that progesterone treated spermatozoa loses the motility pattern of fresh caudal epididymal sperm during the 90 min of observation.

Kobori *et al.* [28] indicated that prolonged responses required higher doses of P and their occurrence was enhanced significantly by pre-incubation for 2-4 h as compared with transient responses. Our results showed that with prolongation of time, P treated sperm lost its hyperactivated pattern which was measured using motion parameters and the others have the same results [29]. The reason could be different types of study as Kobori *et al.* [28] studied the features of intracellular  $Ca^{2+}$  and we studied the motility parameters. Furthermore, spermatozoa may switch back and forth between transitional and whiplash motion [30] and probably they show HA pattern again in the longer time.

Standardization of methods, such as the one described here, allow accurate and objective identification of hyperactivated spermatozoa. In conclusion, progesterone stimulated HA of mouse spermatozoa in a dose-dependent manner providing a rationale to conduct studies utilizing human spermatozoa to find if the change in motility pattern of spermatozoa in response to progesterone has predicting value of sperm quality. It is necessary to evaluate the correlation between HA pattern of progesterone treated spermatozoa with its fertilization ability during IVF and ICSI until we can have an accurate judgment about HA pattern study as a model to choose high quality spermatozoa.

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