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.

Abbreviations: 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.
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 α-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 Ca²⁺, efflux of 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-Percoll).

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 × 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% CO₂ 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 µg/ml, 10 µg/ml and 100 µg/ml, and the spermatozoa were cultured for 10 min or 90 min in a CO₂ incubator.

**Sample preparation.** Each 14 µl 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 × 10⁶ sperm per ml. The suspension was placed on a glass slide prewarmed at 37°C and covered with an 18 × 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 µg/ml progesterone; 3) Exp. 2, with 10 µg/ml progesterone; 4) Exp. 3, with 100 µg/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, µm/s) is the straight-line distance between the first and last tracked points, divided by the acquisition time; 2) Curvilinear velocity (VCL, µm/s) is the total distance between adjacent points, divided by the time elapsed; 3) Average path velocity (VAP, µm/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.

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. 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 µg /ml as control, 1 µg /ml as exp. 1, 10 µg /ml as exp. 2 and 100 µg /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 1ug/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
different 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 Ca²⁺ 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.

REFERENCES


