# Effects of Different Doses of Hyaloronan on Human Sperm Motility, Vitality and Morphology

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**Abstract-** Important aspect of sperm function such as motility and capacitation appear to be mediated at least partially though hyaloronic acid (HA). Present study investigated effects of different doses of HA on sperm motility and vitality in human. Sperm was obtained from 20 male from IVF clinic in Imam Khomeini Hospital. Sperm motility and vitality in human semen was analyzed according to WHO criteria before and 4 hours after treatment with different doses of HA (0.750, 1000 and 1250 µg/ml). The results showed that in 1000 µg/ml the percent of stage 3 and 4 increased compare to control group. Percent of stage 1 and 2 decreased in group with 1000 µg/ml HA, there was an increase in the percentage of stage 3 and 4 and decrease in percentage of stage 1 and 2 compare to control. In the group treated with 1250 µg/ml stage 1 and 2 increased while stage 3 and 4 decreased. Vitality in all groups decreased except of the group treated with 1000 µg/ml HA. The group with 1250 µg/ml showed significantly decrease in vitality compare to fresh group ($P < 0.05$). The present study showed that the effects of HA on sperm motility and vitality is dose dependant and 1000 µg/ml HA had the effective role on sperm parameters.



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**Key words:** Human sperm, hyaloronan, sperm parameters

## Introduction

Glycosaminoglycans (GAGs) are essential components of the extracellular matrix, contributing to cell recognition, cellular adhesion and growth regulation. The GAGs can be classified by their sugar residues and other characteristics such as sulfation into four main groups of GAGs including the non-sulfated glycosaminoglycan, hyaluronic acid (HA), or hyaluronan (1). Important aspects of sperm function such as motility (2, 3) and capacitation (4, 5) appear to be mediated, at least partially, through HA. Addition of HA to fresh (6,7) and frozen–thawed (8) sperm improves sperm motility in medium. Interestingly, HA not only improves sperm motility after freezing and thawing procedures, but it further appears to stabilize spermatozoa with already declining motility (7). The enhancement of sperm motility and velocity occurred as a direct response to HA. There is an instantaneous increase in sperm velocity and tail cross-beat frequency upon HA exposure and when, after density gradient centrifugation, the HA-exposed sperm is transferred to a standard medium, the motility and velocity properties return to those of the control sperm. It can be concluded that HA effects on sperm are likely to be receptor mediated, in line with the evidence established by various laboratories for the presence of the HA receptor in human sperm (8). Concurrently, with the sperm maturation studies, in another line of experiments, scientists have investigated the effects of HA on human sperm function. Exogenous HA decreased polyspermy during

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conventional porcine IVF (4), an effect probably related to the capacity of HA to delay sperm capacitation and/or the acrosome reaction (4, 5). It has been reported that effects of hyaluronan on sperm is dose dependant (9). There isn't any report about the optimal dose of hyaluronan on human sperm motility, viability and morphology. The aim of the present study was to test the hypothesis that supplementing with different dose of HA has an important role on the viability and motility of sperm.

## Patients and Methods

This study was initially approved in the ethical and scientific committee of the Vali-e-Asr Reproductive Health Research Center, Tehran, Iran. Semen samples were obtained from 20 couples referred to Vali-e-Asr Reproductive Health Research Center for ICSI and IVF treatment. The semen samples were collected by masturbation after 3-4 days of abstinence on the day of oocyte recovery. Routine semen analysis was carried out by light microscopy according to World Health Organization (WHO) criteria. After ART treatment, the remaining samples were used for this research. Each sample was divided into five groups. The Group 1 (fresh control): These samples were analyzed freshly without any treatment. The Group 2 (control): They were maintained in HTF media (Sigma) without any hyaluronan (Sigma) supplementation for 2 h. In groups 3, 4 and 5, HA with the doses of 750, 1000 and 1250 µg/ml were added into HTF media, respectively. After 4 h incubation, the sperms were assessed for motility, vitality and morphology.

### Sperm analysis

Sperm motility was analyzed according to WHO manual. For evaluating sperm viability, we used the eosin staining. Sperm and eosin (Sigma) were mixed (3:1) and number of sperm was counted by 400 magnifications by an invert microscope rapidly. Sperm smear was prepared on the slide for morphology analyzing. After fixing with 1:1 volume of alcohol (sigma) and ether (sigma), the slides were stained with papanicolaou methods. Numbers of coiled sperm, stumped tail sperm, cytoplasmic droplet and remnant were analyzed by binocular microscope with 400 magnifications.

### Hyaluronan treatment

After evaluation of fresh sperm, the samples were treated with different doses of Hyaluronan (0, 750, 1000 and 1250µg/ml). The samples were put in incubator (37c° and 5% $Co_2$ for 4 hours). After 4 hours the different groups of sperm were analyzed again.

### Statistical analysis

Data were analyzed by One-Way ANOVA followed up with post hoc test (Tukey). Statistical significance was set at $P < 0.05$.

## Results

### Sperm motility

Table 1 indicates the various motility stages of sperm in different groups. As shown in this table, percentage of stage 1 in group 4 (1000 µg/ml) was decreased significantly compared to control groups ($P<0.05$) but in group 5 (1250 µg/ml) the stage 1 significantly increased. The percentage of stage 2 decreased in group 4 compared to other groups. In group 4, stage 4 motility significantly increased compared to all off other groups. In group 5 there was significant decrease in stages 4 motility ($P < 0.05$).

### Sperm viability

As shown in figure 1, viability in all groups after treatment with HA was decreased except for group 4. In group 5 significant decrease ($P < 0.05$) in percentage of viability compared to fresh group was observed.

**Table 1.** Various stages of motility in different groups

| Groups / Stages | Fresh control | Control after 4 hr | 750 µg/ml HA | 1000 µg/ml HA | 1250 µg/ml HA |
|---|---|---|---|---|---|
| 1 | 14% | 15% | 16% | 12% | 21% |
| 2 | 16% | 17% | 17% | 10% | 25% |
| 3 | 25% | 28% | 29% | 33% | 28% |
| 4 | 45%* | 40% | 38% | 45% | 26% |

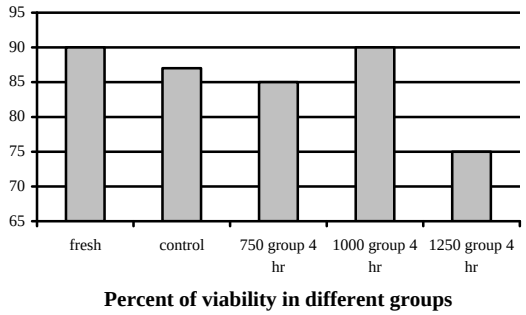


**Figure 1.** Percent of viability in different groups

## Discussion

Hyaluronan (HA) is a linear polysaccharide which is a mediator of cell locomotion (9, 10). HA also occurs physiologically in the reproductive system in cervical mucus, the cumulus and the follicular and seminal fluids. The effects of HA on human spermatozoa have been explored in other clinical and research applications (12-15)**.** There are some reports regarding the effect of different doses of HA on sperm function(8,9). Sbracia *et al*. (8) showed that addition of 250µg/ml HA provided a significant motility increase for at least 4 h after the Percoll purification of spermatozoa. However, what remains to be elucidated is determining the optimum doses of HA on sperm function. Therefore, we used different doses of HA were applied on sperm to evaluate their effect on motility, viability and morphology. The results of this study showed that effect of HA on sperm motility were dose dependant and 1000 µg/ml was optimal dose to improve the fast motility (stages 3 and 4). Furthermore, results showed that 1250µg/ml had a toxic effect on sperm and motility was decreased significantly. The mechanisms behind a HA-induced increase in sperm motility remains to be determined. A HA-receptor has been localized along the tail, mid piece, and head of human spermatozoa (16). Blocking this receptor with mono-specific antibodies resulted in inhibition of sperm motility. Moreover, enhanced phosphorylation of the 34 kDa HA-binding protein (HABP) has been observed in response to HA supplementation, suggesting an important role of this glycosaminoglycan in initiating the transduction of signals controlling important processes of sperm physiology, such as motility (17, 18) that may result in an increase of the ATP levels and improved flagellar function(8). Bakhtiari *et al*. showed that, the hyaluronan may have an influence on motility, viability and fertilizing rate of mouse sperm and the dose of 750 µg/ml had a significant effect on these factors. The results of this study, in addition, showed that viability in hyaluronan group after four hours decreased except for 1000 µg/ml. Sbracia *et al*. (8) showed that viability of incubated with HA increased. These results are in agreement with those of other research (8, 9). This effect could be related to protective role of HA on sperm membrane. According to our study it seems that 1000 µg/ml is the optimal dose that affects motility and vitality of human sperm. In this investigation different doses didn't have an effect on sperm morphology. Previous studies have shown that sperm morphology is controlled by spermiogenic events such as acrosome formation and chromatin rearrangement. Protamine deficiency and aberrant P1/P2 ratios are negatively related to abnormal morphology (19). After ejaculation sperm morphology isn't affected by HA but it is possibly affected by osmolarity of sperm medium preparation. It seems that HA has a substantial advantage over other sperm motility enhancers, which have a temporary effect. Unlike other motility enhancers, some of which cause short-term maintenance of improvement after removal of the agent, HA preserves motility in spermatozoa and therefore it can be useful in assisted reproduction technique (ART) in utilizing, especially when thawed semen samples are used. Also, when preparing sperm for IUI, IVF, application of HA which leads to extended motility will increase fertilizing efficiency, while in intracytoplasmic sperm injection (ICSI) the improved sperm motility will enhance the selection of viable spermatozoa.

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