Research Article

Ram Sperm Motility Parameters under The Influence of Epidermal Growth Factor

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Epidermal growth factor (EGF) is one of the important cytokines that play a role in fertility. It is known that EGF affects both male and female reproduction, but its effect on sperm parameters is not fully understood. Up to the present, the effect of EGF on ram sperm motility parameters has not been published. We analyzed motility parameters of ejaculates after 24, 48, and 72 hours from the EGF addition. EGF was added to chilled ram sperm at concentrations of 0, 100, 200, and 400 ng·ml^{-1}. Analyses were realized using computer, assisted semen analyzer (CASA)—Hamilton Thorn motility analyzer (version 7). The effect of EGF was already visible after 30 min of incubation. Significant effect on ram sperm total motility and progressive movement was observed at higher EGF concentrations after 48 h of incubation. Our results show that EGF affects sperm motility parameters depending on concentration and time of exposure.

1. Introduction

Epidermal growth factor (EGF) has been shown to have a role in both male as well as female mammalian reproduction [1, 2]. EGF has been found in rat and human seminal plasma [3, 4]. Effects of EGF are probably exerted directly via EGF receptors which have been found in the acrosomal region of the spermatozoa [5] from various mammalian species including human, mouse, rabbit, bovine, and rat [6, 7]. Under *in vitro* conditions, EGF regulates capacitation, acrosome reaction, and motility in mouse, bovine, and human spermatozoa [8–11]. In *in vivo* experiments the administration of EGF improved rat epididymal sperm content and motility [12]. The removal of the submandibular gland (a source of EGF production) in mature male mice results in significant loss of plasma EGF, causing a significant decrease of spermatids in the testes and mature sperm in the epididymis [13]. These observations suggest the role of EGF in the regulation of sperm functions.

However, knowledge about EGF effect on sperm characteristics is inconsistent. Thus, Naz and Kaplan [11] in human sperm showed that EGF decreased sperm penetration rate and altered sperm motility characteristics. However, other research teams reported no effect of EGF on human sperm motility [9, 14]. This controversy may be explained by different experimental setting used by Naz and Kaplan [11], who incubated the sperm for a shorter period.

Moreover, there are no reports about EGF effects on movement characteristics of ram sperm. In our work, we focused on assessing the effect of EGF on total motility and progressive motility of ram sperm. Motility of sperm cells may be measured using both subjective and objective evaluation. Objective evaluation of sperm motility by CASA gives more accurate assessment of sperm which can better predict further fertility than visual estimation. We evaluated the dynamics of EGF action on parameters of total motility and progressive movement of ram sperm following several days of storage at cooling conditions. The assessment of ram
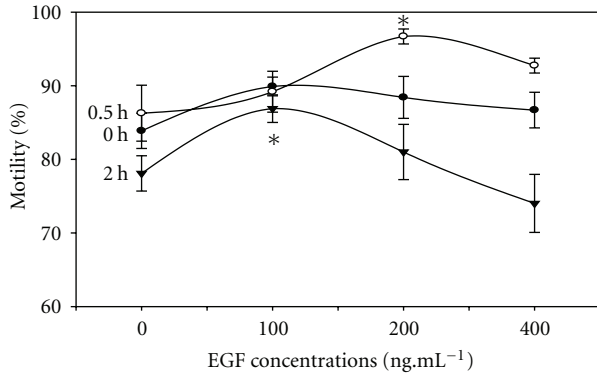
FIGURE 1: Dynamics of EGF effect on ram sperm motility. Significant difference compared to control, $*P < .05$.

FIGURE 2: Effect of cooling storage in the presence of EGF on sperm motility. Significant difference compared to control, $*P < .05$.

FIGURE 3: Effect of cooling storage in the presence of EGF on sperm progressive movement. Significant difference compared to control, $*P < .05$.

sperm motility parameters was realized using computer-assisted sperm analyzer (CASA).

2. Experimental Procedures

All the experiments were carried out with fresh ram spermatozoa. The semen was collected from East-Friesian (VF) and Lacaune (Lc) rams using artificial vagina. The rams were kept at a local farm under uniform nutritional conditions. Volume, concentration, and sperm activity where assessed shortly after collection. Ejaculates from all rams were pooled together to make heterospermia in order to avoid individual influence of ram and were used for the assay. Ejaculates were diluted in Triladyl (Minitüb, Tiefenbach, Germany) containing 20% egg yolk, lactose, and 6% glycerol. Semen was cooled at 5–7°C, transported to the laboratory, and kept in a fridge for one week. Samples were divided into four groups, with 1 mL of ejaculates in each, and subsequently EGF (recombinant; Sigma-Aldrich Ltd, Bratislava, Slovakia) was added at concentrations of 100, 200, and 400 ng·mL^{-1}, whereas control group did not contain EGF (0 ng·mL^{-1}). We analyzed motility parameters of ejaculates after 24, 48, and 72 hours from the EGF addition. Analyses were realized using computer-assisted semen analyzer (CASA)–Hamilton Thorn motility analyzer (version 7). We analyzed, effect of various concentrations of EGF on ram sperm motility and progressive movement, as well as the dynamics of the effect of EGF after its addition for different time periods (0, 0.5, and 2 h).

Experiments have been done in three replications. The results were statistically evaluated by two-way ANOVA test and graphically processed using SigmaPlot graphic software (version 9.01 for windows).

3. Results

EGF affected observed parameters of sperm motility following 0.5 hours of incubation. The more expressed effect of EGF at this time point was observed at the concentration of 200 ng·mL^{-1}, where total motility was increased from 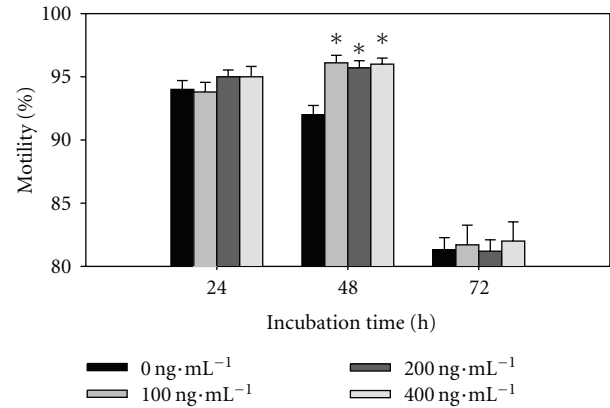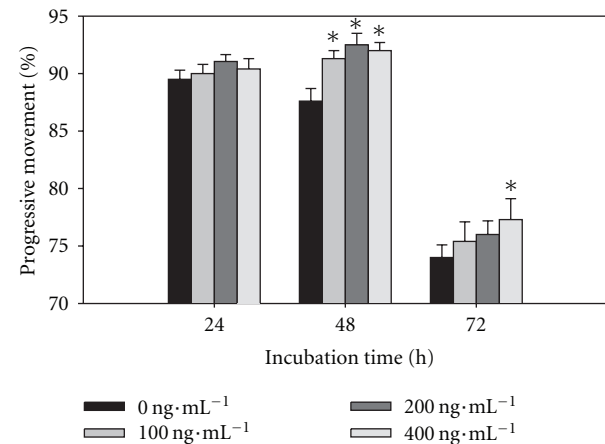86.3% (control group) to 96.7%. After 2 hours of incubation, the stimulating effect of EGF was visible at the concentration of 100 ng·mL^{-1}. Further elevation of EGF concentration above 200 ng·mL^{-1} was not effective at any time interval of sperm incubation in the presence of EGF (Figure 1).

The effect of EGF on the motility of cooling-stored sperm after 24, 48, or 72 hours is shown in Figure 2. Slight but not significant increase in total motility following 24 hours was observed at concentrations of 200 and 400 ng·mL^{-1}. Significantly higher motility at all concentrations of EGF was observed after 48 hours of sperm storage, although sperm motility in the control group was reduced when compared to the 24 h interval of sperm storage. Following 72 h of cooling storage, total sperm motility was dramatically reduced compared to the 24 or 48 h interval, and no effect of EGF at all concentrations was observed (Figure 2).

No significant increase in progressive movement among all tested groups was observed following 24 h storage of ram sperm in the presence of EGF (Figure 3). Significant increase ($P < .05$) in progressive movement at all tested concentrations of EGF in comparison to the control group
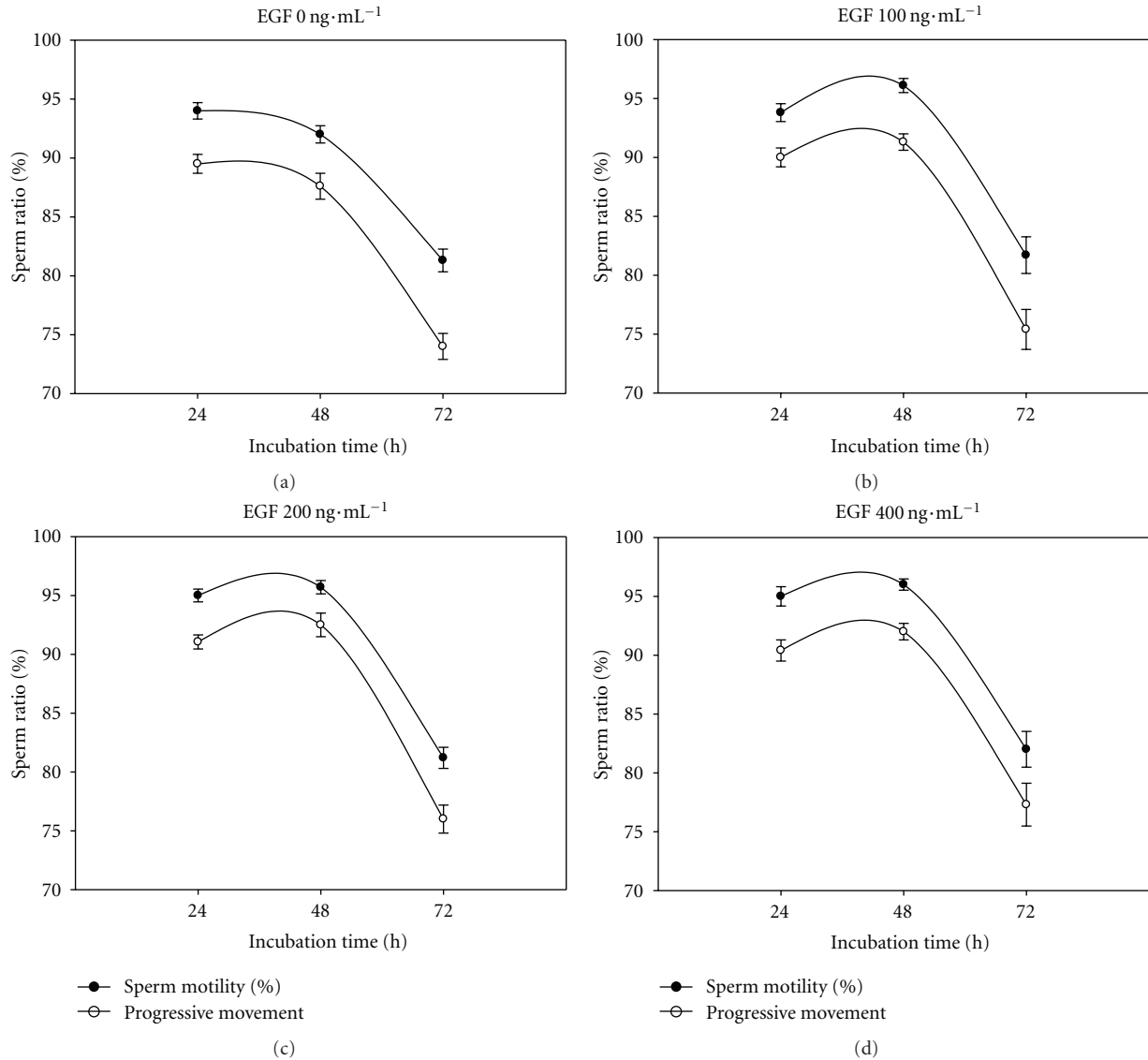
(a)

(b)

(c)

(d)

Figure 4: Interrelationships between total motility and progressive movements depended upon EGF concentrations and length of cooling storage.

was observed following 48 h storage of ram sperm. Following 72 h of storage, significant increase in progressive motility was observed only when EGF at the highest concentration (400 ng·mL^{-1}) was applied.

Progressive movement values were lower than total sperm motility; nevertheless, the similar pattern of curves for both the total motility and the progressive movement was visible in control group. However, such an equal character of both curves was not noted in either group with EGF. At concentrations of 200 and 400 ng·mL^{-1} EGF, values of progressive movement were situated close to the percentage of total motility beginning from 48 hour of cooling storage (Figure 4).

4. Discussion

Effect of EGF on sperm has not been fully elucidated yet. There is a report stating that EGF given at concentrations

about 100 ng·mL^{-1} did not affect several parameters of spermatozoa like acrosomal status, membrane integrity, or motility patterns [7]. On the other hand, Naz and Kaplan [11] suggested that EGF, given at higher concentrations, may inhibit capacitation and/or the acrosome reaction of human sperm. Oliva-Hernández and Peréz-Gutiérrez [7] observed that endogenous EGF produced in the reproductive tract may increase the quality of boar sperm movement after acrosome reaction.

Results of our work confirm that EGF affects sperm motility parameters depending on the concentration and time of exposure to EGF. The highest effect on ram sperm motility was observed at higher EGF concentrations. The effect of EGF in our study was exhibited already after 30 min of incubation. These results are consistent with the previous study of Naz and Kaplan [11], who showed that EGF did not affect the motility of human sperm at concentrations below 100 ng·mL^{-1}, whilst concentrations above

$100 \, \text{ng} \cdot \text{mL}^{-1}$ significantly affected all motility parameters, such as velocity, linearity, beat frequency, and amplitude of lateral head displacement.

The importance of sperm motility during the fertilization process has attracted considerable attention over the past decades. Several researchers have reported the relationship between fertility potential *in vitro* and sperm motility parameters measured with CASA [15, 16]. Some studies [17–21] suggested that the characteristics of progressive motility of the spermatozoa were related to their fertilizing capacity and the sperm motility was dependent on mitochondrial function. When the sperm mitochondrial membrane potential increases, sperm motility parameters and fertility potential will also increase [16].

It is well known that premature capacitation occurs during the processing of semen samples, ultimately leading to a reduced fertility in comparison to fresh semen samples [22]. The high percentage of motile spermatozoa in processed semen samples in our tests may indicate that these spermatozoa have not been damaged by the process of dilution and storage. Our results indicate that EGF also affects the progressive movement, important for fertilization ability of sperm. The importance of the effect of EGF is also in the retention of motility of cooling-stored sperm for a longer period (72 h).

The assessment of quality (speed) of progressive motility is very important because it is an essential prognostic fertility factor, especially when the proportion of motile spermatozoa is below 40% [23]. Objective analysis of sperm motility parameters resulted in significant correlations between the value of lateral head displacement (ALH) [24], curvilinear velocity (VCL) [25–27], average path velocity (VAP) [28], linearity (LIN) [26], and the *in vitro* fertilization rates. In addition to VCL and VAP, sperm hyperactivation has been shown to be an important marker of fertilizing ability in the *in vitro* situation [27, 29–31].

Sperm motility is commonly believed to be one of the most important characteristics for evaluating the fertility potential of ejaculated spermatozoa. However, in bulls, no significant correlation between the percentage of motile spermatozoa evaluated by CASA and the 59-day NRR (nonreturn rate) has been found, whereas highly significant correlations were detected when CASA parameters describing the velocity of motile spermatozoa or the trajectory line of motile spermatozoa were included [32]. In earlier studies on boar, no relationship between motility parameters evaluated by CASA and fertility of boars was found [33, 34]. More recently, results of a fertility trial demonstrated a correlation between objectively measured boar sperm motility parameters and the outcomes of on-farm inseminations [35]. In the study of Hirai et al. [36], a significant difference in the percentage of motile spermatozoa between boars with high or low litter size was demonstrated.

The high percentage of motile spermatozoa in processed semen samples may indicate that these spermatozoa have not been damaged by the process of dilution and storage. It is well known that premature capacitation occurs during the processing of semen samples, ultimately leading to a reduced fertility in comparison to fresh semen samples [22].

Optimal value of sperm motility and progressive movement are important factors for successful fertilization. EGF affects sperm motility parameters depending on concentration and time of exposure to EGF. The effect of EGF addition on cooling-stored ram sperm was visible after 30 min of incubation, and the more expressed effect was observed at the concentration of $200 \, \text{ng} \cdot \text{mL}^{-1}$. The higher concentration of EGF (100, 200, and $400 \, \text{ng} \cdot \text{mL}^{-1}$) significantly helped in the retention of motility and progressive movement of cooling-stored sperm for a longer period (48 h).

Acknowledgment

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