编者按："全军第十三届检验医学大会"将于 2012 年 11 月 9～13 日在昆明召开。本届检验医学大会是新成立的第九届全军医学检验专业委员会举办的第一次全国性大会，得到了全国各军区各级医院检验医学工作者的大力支持。本届大会收到具有较高学术质量的科研论文近七百篇，每篇文章都体现了作者在临床和科研工作中取得的成就，为我们了解学科发展方向起到了极大的推动作用。我们从中选择了具有一定代表性和较高学术、科研水平的论文作为本届大会优秀论文进行全文发表，旨在推动全军检验医学临床和科研工作的发展，提升军队检验医学科研事业的整体质量和水平，推动军队科学技术发展体系的建设。

本次优秀论文评选工作得到了第九届全军医学检验专业委员会各位委员的大力支持，为优秀论文筛选工作奠定了坚实的基础，在此向各位委员表示衷心的感谢！

第九届全军检验医学专业委员会主任委员　府伟灵

·临床检验基础论著(全军检验大会优秀论文)·

NGF 体外对精子活力的影响*

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摘　要：目的　观察神经生长因子(NGF)体外对精子活力的影响，确定 NGF 的最佳量效和时效关系，探索 NGF 体外对精子活力的影响以及和男性不育的关系。**方法**　分别用 0.1、1、10 μmol/L 的 NGF 对精子活力进行研究，以探讨最佳浓度。将 10 μmol/L NGF 加入精液中在 1 h 内每隔 10 min 用 CASA 检测精子的活力变化。**结果**　NGF 可显著增加精子的平均速率、直线速率、曲线速率和线性系数，差异有统计学意义($P<0.01$)；增加鞭打频率差异有统计学意义($P<0.05$)。1 μmol/L 的 NGF 和 0.1 μmol/L 的 NGF 对精子活力的促进作用相比有统计学差异，10 μmol/L 浓度组和 1 μmol/L 浓度组差异没有统计学意义。NGF 反应 30 min 后对精子活力的影响最大，一直保持到 1 h 以后。**结论**　外源性给予 NGF 可显著增加健康人和不育症患者 a 级运动精子比例，促进精子活力。

关键词：不育，男性；　神经生长因子；　精子活力

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Effect of NGF on the sperm motility of human in vitro*

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Abstract: Objective　Motility is an important physiological characteristic of a mature sperm. Nerve growth factor(NGF) is a protein essential for the development, maintenance and survival of the peripheral and central nervous systems. NGF and its receptors TrkA and p75 are widely expressed in the testis, accessory reproductive organ, and the epididymal sperms. In the present study, we investigated the role of NGF on human sperm motility. **Methods**　Use 0.1, 1 and 10 μmol/L concentrations of NGF, on sperm motility study to investigate the optimal concentration. Use CASA to detect Sperm motility changes every 10 minutes in an hour after 10 μmol/L NGF was added to the semen. **Results**　The parameters of sperm motility increased after NGF incubation had significant difference, in particular, VAP, VSL, VCL, BCF and LIN mean were significantly increased more than 32%. MAD, STR, ALH and WOB mean had no notable difference. Furthermore, NGF promotes the sperm motility in a time- and dose- dependent manner. In addition, the enhancement of NGF on sperm motility was more stronger than those of sperm culture medium. **Conclusion**　Our findings suggest that NGF plays a promoted role in human sperm motility.

Key words: infertility, male;　nerve growth factor;　sperm motility

1　Introduction

In mammals, the family of the growth factor called neurotrophins(NTs) comprises four molecules, brain-derived neurotrophic factor(BDNF), neurotrophin 3, neurotrophins 4/5 and nerve growth factor(NGF), which are involved in the nervous system development and maintenance[1]. However, the expres-

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sion of NT receptors in non-neuronal tissues and the effects strongly suggests that NTs have a broader range of actions than originally supposed. More and more data suggest a role in the regulation of non-nervous tissues of these molecules[2-3].

NGF bind to two kinds of receptors: The protein tyrosine kinase Trk receptors TrkA, act as specific high-affinity receptors. The low-affinity receptor p75, serves as a pan-neurotrophin receptor mediating pro-apoptotic or pro-survival cell programs[4]. NGF and its receptors had been reported to exist in the testis[5]. NGF was expressed in Leydig cells, primary spermatocytes and pachytene spermatocytes. TrkA only immunoreacted to elongate spermatids and p75 showed positive immunostaining in the Sertoli cells, Leydig cells, the pachytene spermatocytes and elongate spermatids[6-7]. NGF, TrkA and p75, have consistently been detected in developing and adult testicles of different mammalian species, and their expression has a hormonal regulation[8-9]. In addition, immunoreactions for NGF and its two receptors were also observed in columnar secretory epithelium lines of the seminal vesicles, prostate and coagulating gland. These results suggest that NGF is an important growth factor in male gonadal function[10-11].

However, the key role of NGF on the human sperm motility remains unclear. In the present study, we attempt to elucidate the physciological role of NGF on human sperm motility in vitro.

2 Materials and methods

2.1 Subjects　20 normal male subjects with fertility were investigated between January and June 2011 at Air Force General Hospital. The sperm samples have motility higher than 60% and count over 20 millions/mL. The subjects provided written consent after being given detailed explanations of the proposed study.

2.2 Preparation of semen samples　Fresh semen samples were collected by masturbation into sterile plastic containers after 7 days of sexual abstinence and were allowed to liquefy for 30 minutes at room temperature[12]. Semen analysis was performed according to WHO protocols. Samples were processed according to the routine wash and swim-up procedure in HEPES-HTF (Santa Ana, CA, USA) medium supplemented with 10% serum substitute supplement (Santa Ana, CA, USA).

2.3 Determination of sperm motility parameters　10 mL of sperm suspension was placed on a Makler chamber and sperm motility parameters were analyzed by CASA, following incubation for 1 hour at 37 in air. The semen analyzer used was the Hamilton Thorne Research semen analyzer (IVOS, Version 10.8x. Hamilton Thorne, Beverly, USA). The standard parameter settings employed for analysis were as follows: frame acquired, 30; frame rate, 60 Hz; minimum contrast, 80; minimum cell size, 3; static head size limits, 1.00－2.90; static head intensity limits, 0.60－1.40; static elongation limits, 0－80 and temperature, 37 ℃. At least 10 fields were examined.

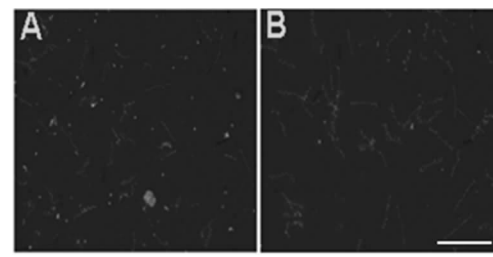
The post swim-up sperm suspensions were used to assess the sperm motility, as it was proposed that the post swim-up motility is a better predictor of fertilizing ability of spermatozoa than fresh semen. The following motility parameters were measured: concentration (CON, Million/mL), rate of motility (%MOT) and rate of rapid progression (%PMOT). For those spermatozoa that exhibit an average path velocity (VAP) greater than 25 mm/s, the following parameters were determined: average path velocity (VAP, mm/s), straight line velocity VSL, mm/s), curvilinear velocity (VCL, mm/s), amplitude of lateral head displacement (ALH, mm), beat-cross frequency (BCF, Hz), Straightness (STR, %); VSL/VAP and Linearity (LIN, %).

A more specified measure is motility grade, where the motility of sperm are divided into four different grades. Grade a: sperm with progressive motility. These are the strongest and swim fast in a straight line. Sometimes it is also denoted motility Ⅰ. Grade b (non-linear motility): These also move forward but tend to travel in a curved or crooked motion, and sometimes also denoted motility Ⅱ. Grade c: these have non-progressive motility because they do not move forward despite the fact that they move their tails, and sometimes also denoted motility Ⅲ. Grade d: These are immotile and fail to move at all. Sometimes also denoted motility Ⅳ.

2.4 Statistical analyses　For statistical analysis, a standard software package (SPSS for Windows 10.1) was used. All data were given as $\bar{x}\pm s$. Differences between groups were compared by using a one-way analysis of variance (ANOVA). $P<0.05$ were considered significant.

3 Results

3.1 Effects of NGF on sperm motility　The parameters of sperm motility increased after NGF incubation for 30 min at the concentration of 10 μmol/L. In particular, the means of VAP, VSL, VCL, BCF and LIN were significantly increased more than 32%. The means of MAD, STR, ALH and WOB had no notable difference (Fig. 1, Table 1).



A: In NGF absence; B: In NGF presence. The bar represents 50 μm.

Fig. 1　Effect of NGF on sperm motility of human at the concentration of 10 μmol/L for 30 min.

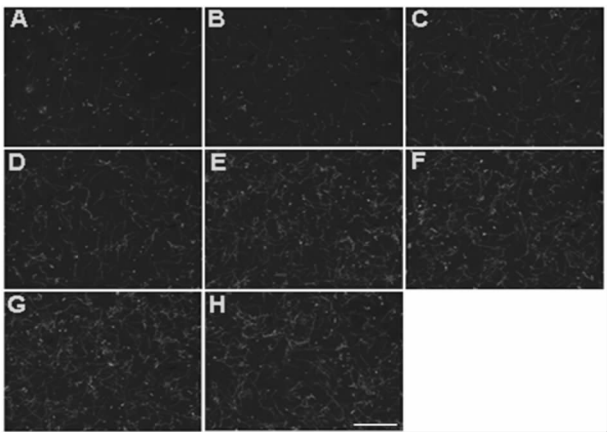
3.2 Time-dependent effect of NGF on sperm motility　The mean percentage of motile sperm were assessed by CASA after 10 μmol/L NGF for 2, 10, 20, 30, 40, 50 and 60 min incubation, respectively. As compared with the control, the mean percentage of a and b grade sperm significantly increased and the mean percentage of c and d grade sperm declined after NGF incuba-

tion(Fig. 2, 3). As compared with the control, the mean percentage of a grade sperm significantly increased and the mean percentage of c and d grade sperm declined after NGF incubation(Fig. 3).

Table 1　Effects of 10 μmol/L NGF on the human sperm motionparameters($\bar{x}\pm s$, $n=20$)

Sperm motion parameters	NGF absence	NGF presence
VCL(μm/s)	2.80±1.03	3.70±1.33*
VAP(μm/s)	44.59±17.26	59.50±24.57*
LIN(%)	17.91±8.77	25.53±11.46*
BCF(Hz)	26.21±11.64	36.06±15.80*
MAD	29.33±10.03	32.71±10.57
VSL(μm/s)	61.97±10.62	64.70±9.40*
STR(%)	57.39±10.49	60.17±9.14
ALH(μm)	18.17±2.38	18.71±1.44
WOB(%)	10.57±1.70	9.91±1.70

*: $P<0.05$, vs. NGF absence.



A: control; B: 2 min; C: 10 min; D: 20 min; E: 30 min; F: 40 min; G: 50 min; H: 60 min. The bar represents 50 μm.

Fig. 2　Effects of 10 μmol/L NGF on the sperm motility in time-dependent manner.

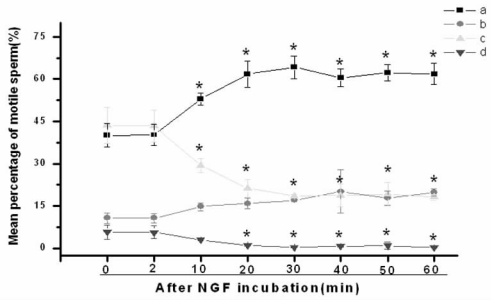
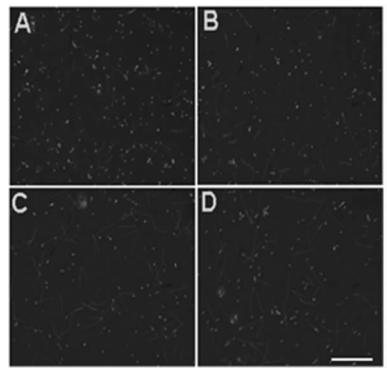


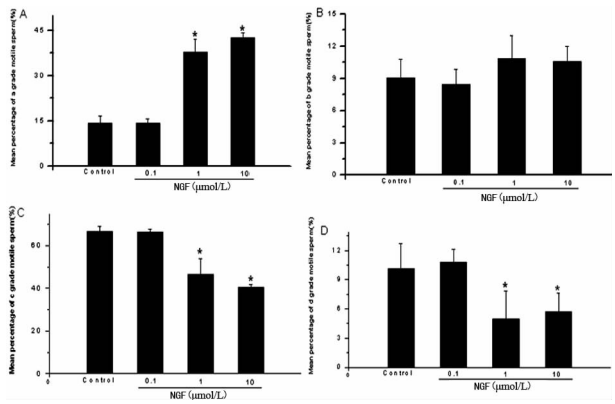
Fig. 3　Effects of 10 μmol/L NGF on the mean percentage of motile sperm of a, b, c and d grades in time-dependent manner($\bar{x}\pm s$, $n=10$).

3.3　Dose-dependent effect of NGF on sperm motility　As compared with the control, a significant dose-dependent improvement in sperm motility was noticed in the experimental fractions supplemented with 0.1, 1 and 10 μmol/L concentrations of NGF for 30 min. NGF at the concentration of 1 and 10 μmol/L produced an increment of the mean percentage of a grade sperm, meanwhile, NGF produced an reduction of the mean percentage of c and d grade sperm(Fig. 4, 5).



A: control; B: 0.1 μmol/L NGF; C: 1 μmol/L NGF; D: 10 μmol/L NGF. The bar represents 50 μm.

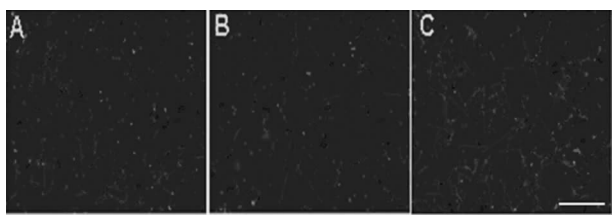
Fig. 4　Effects of NGF on the sperm motility in dose-dependent manner.



Vertical bars represent $\bar{x}\pm s$. *: $P<0.05$, **: $P<0.01$ vs. control. $n=10$.

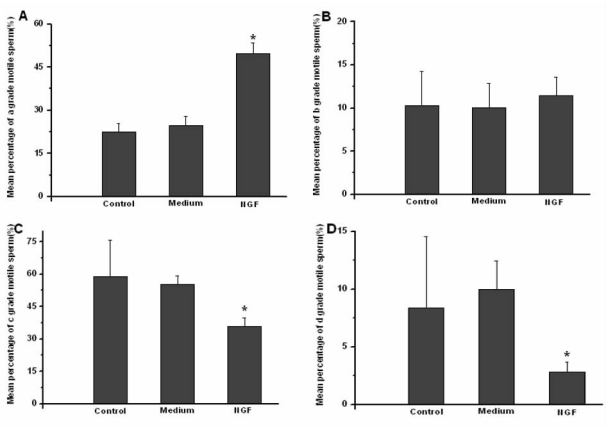
Fig. 5　Effects of NGF on the mean percentage of motile sperm of a, b, c and d grades in dose-dependent manner.

3.4　Comparison of sperm culture medium and NGF on sperm motility　As compared with the control, the mean percentage of a and b grade sperm significantly increased and the mean percentage of c and d grade sperm declined after NGF incubation. The role of NGF was significantly stronger than those of the sperm culture medium(Fig. 6, 7).



A: control; B: sperm cultyre medium; C: 10 μmol/L NGF. The bar represents 50 μmol/L.

Fig. 6　Effects of NGF and sperm culture medium on human sperm motility.



Vertical bars represent $\overline{x} \pm s$. *: $P<0.05$, **: $P<0.01$, vs. control, $n=10$.

Fig. 7　Effects of sperm culture medium and 10 μmol NGF on the mean percentage of motile sperm of a, b, c and d grades.

4 Discussion

In the current study, we revealed an evidence that demonstrate a direct role of NGF on human sperm motility. NGF treatment could enhance and maintain sperm motility in dose- and time dependent manner. furthermore, the role of NGF were more stronger than sperm culture medium.

A positive immunoreactivity for NGF has been reported in the adult mouse testis. This immunoreactivity appeared to be localized to the cells of the germ line. it was positive from primary spermatocytes to mature sperm. The presence of NGF mRNA and protein in adult rodent testis has also been demonstrated. NGF mRNA was detected in spermatocytes and early spermatids of adult mouse and rat testis[13,14]. The presence of NGF-like substance in a testicular extract was also confirmed by a bioassay. An analysis of the stage-specific expression of NGF during the cycle of the seminiferous epithelium in the rat revealed NGF mRNA and protein at all stages of the cycle. A quantitative determination by immunoassay of NGF in human testis revealed the presence of 5. 44 ng of NGF per g wet weight[15-16].

NGF remains associated with spermatozoa throughout their maturation in the epididymis, and further peptides may be absorbed to the sperm surface from sources in the epididymal epithelium. The presence of NGF in spermatozoa and in prostatic secretions raises important questions concerning the role of this protein in sperm motility, particularly since NGF is a known chemoattractant for motile leucocytes. The possible relationship between NGF and the "forward motility protein" also needs to be addressed carefully[17].

In this study we also compared the sperm motility incubated in a culture medium versus NGF. NGF resulted in a greater percentage of motile spermatozoa available and perhaps appears to have been advantageous in terms of fertilization rates, with a resulting increase in the proportion of better motile sperm.

In summary, NGF have critical role in human sperm motility. These results may promote the application of NGF in assisted reproductive technology in clinic.

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·临床检验基础论著(全军检验大会优秀论文)·

红细胞体积分布宽度对急性冠状动脉综合征患者心力衰竭的诊断价值*

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摘　要:目的　评估红细胞体积分布宽度(RDW)对急性冠状动脉综合征(ACS)患者心力衰竭的临床应用价值。**方法**　连续纳入 380 例因 ACS 入院的患者,按照左室射血分数(LVEF)进行分类,分析其临床基线数据特征。利用受试者操作特征(ROC)曲线分析研究 RDW 对 ACS 患者是否存在心力衰竭的诊断价值。**结果**　不同 LVEF 的各组 ACS 患者,其年龄、血肌酐、白细胞和 RDW 均存在统计学差异($P<0.01$)。ROC 曲线分析可知,RDW 对 ACS 患者心力衰竭的诊断准确性可达 0.653($P<0.001$),以 13.2%作为临界点,其诊断灵敏度为 61.0%,特异度为 61.4%。**结论**　作为一个检测便捷的指标,RDW 对 ACS 患者出现的心力衰竭有一定的诊断价值。

关键词:红细胞体积分布宽度;　急性冠状动脉综合征;　心力衰竭
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Clinical usefulness of measuring red cell volume distribution width to diagnosis heart failure in acute coronary syndrome patients*

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Abstract: Objective　To evaluate the clinical value of red cell volume distribution width(RDW) to diagnosis heart failure in acute coronary syndrome(ACS) patients. **Methods**　We analyzed the baseline clinical data of 380 ACS patients consecutively admitted into our hospital, according to the classification of left ventricular ejection fraction(LVEF). Receiver operating characteristic (ROC) curve analysis was used to evaluate the clinical usefulness of RDW. **Results**　There were significant differences of the age, plasma creatinine, white cell count and RDW among different LVEF groups($P<0.01$). ROC analysis results showed that the accuracy of RDW was 0.653($P<0.001$). At the cut-off value of 13.2%, the clinical sensitivity and specificity of RDW in ACS patients were 61.0% and 61.4% respectively. **Conclusion**　As an item that can be tested rapidly and easily, RDW is useful to diagnose heart failure in ACS patients.

Key words: red cell volume distribution width;　acute coronary syndrome;　heart failure

急性冠状动脉综合征(ACS)包括不稳定型心绞痛和急性心肌梗死,是一组临床上常见的急性危重综合征。ACS 患者的心功能是评估其预后的重要指标。红细胞体积分布宽度(RDW)是利用全自动血细胞分析仪根据循环中红细胞大小不同而对其体积不均一性进行的定量测量,可以利用 RDW 对贫血进行辅助诊断和分类。国外有报道指出,RDW 与冠状动脉疾病患者的预后有较密切的联系,RDW 较高的患者,其发生心力衰竭的危险、心血管事件的发生率以及患者的全因死亡率均明显增加,这大大拓展了 RDW 在临床上的应用潜力[1]。由于 RDW 的检测十分便捷廉价,其应用前景十分广泛。本文的目的是探讨 RDW 对于 ACS 患者出现心力衰竭的临床诊断应用价值。

1　资料与方法

1.1　一般资料　连续纳入 2009 年 1～8 月在武警医学院附属医院心血管内科住院治疗的 ACS 患者(年龄大于或等于 22 岁),共 380 例。入选标准:ACS 诊断按美国心脏病学会、美国心脏病协会(ACC/AHA)制定的标准[2]。排除标准:临床资料不齐(如缺少血常规、生化检测指标等);恶性肿瘤、放疗、化疗;器官移植;严重肝病;严重肾衰(血肌酐大于 2.0 mg/dL);失血性贫血;近 1 月内有严重感染等。

1.2　实验室指标　收集患者一般情况、既往史、心脏彩色多普勒超声结果、血常规以及血糖、血脂等生化指标,均选取患者入院后首次检测结果。取患者静脉血约 5 mL,用 EDTA-K_2 或肝素进行抗凝。使用全自动五分类血球分析仪 XT-1800(Sysmex,日本)对 RDW 等血常规指标进行检测,使用全自动生化分析仪 7180(日立公司,日本)对血生化指标进行检测。

1.3　LVEF 检测　采用彩色超声心动图仪 iE33(飞利浦公司,美国),用 Simpson 双平面法测量左心室的射血分数。

1.4　统计学处理　测定结果以 $\bar{x}\pm s$ 或例数(百分比)表示,应用 SPSS18.0 软件,连续变量组间比较选用 t 检验或秩和检验,计数资料组间比较使用 χ^2 检验,并利用受试者操作特征(ROC)曲线进行分析。

2　结　　果

连续纳入研究的 380 例 ACS 患者的一般临床特征、生化及血常规等指标见表 1。LVEF 的数值范围为 22%～75%(四分位数 1:2%～50%;四分位数 2:51%～57%;四分位数 3:

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