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Assessment of Motility and Survival Rate of Asthenospermic Men's Sperm Cultured in Media Plus Leukemia Inhibitor Factor

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Abstract: As our knowledge no report was given about effect of leukemia inhibitor factor on sperm motility and survival rate of asthenospermic infertile men. That's why this study was decided to review the effects of Leukemia Inhibitor Factor (LIF) with different concentrations of 0, 3, 5, 10, 50 ng mL⁻¹ on motility and survival rate of asthenospermic infertile men. Semen samples of 15 asthenospermic men who referred to IVF unit of Imam Khomeini Hospital, Ahvaz, Iran were collected and put in incubator under condition 5% CO₂ in air at 37°C for 45-30 min then total sperm count was done first, then calculate the motile sperm with a degree a and b. In this study, we evaluated only samples that had sperm motility percent of more than 30%. After that time from every sample about 10 µL removed and culture in different media. Every drop was evaluated 6, 24, 48 h after cultured of sperm in it for motility and survival rate of sperm. Statistical analysis shows that the forward motility of sperm cultured for 6 h in media with and without of LIF is not significant (p>0.05) but after 24 h the forward motility and survival rate of sperm cultured in media with 10 and 50 ng mL⁻¹ of LIF significantly increased (p<0.05) and after 48 h the forward motility and as well as survival rate of sperm cultured in media with 50 ng mL⁻¹ of LIF significantly increased (p<0.05). The conclusion from this study is that certain concentrations of leukemia inhibitor factor can increase the motility and survival rate of sperm.

Key words: LIF, sperm motility, survival rate, asthenospermia

INTRODUCTION

Infertility is defined as the failure of a couple to achieve a pregnancy after at least one year despite having unprotected sexual intercourse. In the United States about 10% of couples are affected by infertility (Philippov *et al.*, 1998; Bhattacharya *et al.*, 2009). According to the American Society for Reproductive Medicine, around 33% of the time the diagnosis is due to female infertility, 33% of the time it is linked to male infertility and the rest 33% is due to a combination of factors from both partners. (Philippov *et al.*, 1998).

Causes of male infertility can be related to the reduction in the number, morphology abnormal sperm and poor motility of sperm (Schlegel, 2009; O'Flynn *et al.*, 2010).

Currently not possible to improve sperm count and morphology *in vitro* while it is possible to improve the quality of sperm motility by using drugs. Many trying to improve sperm movement were performed in medium that can be used, including the platelet-activating factor (Ripps *et al.*, 1993), progesterone (Blackmore *et al.*, 1990) follicular fluid (Ravnik *et al.*, 1990) and pentoxifylline (Yovich *et al.*, 1990).

This material could improve sperm movement *in-vitro*. Leukemia Inhibitory Factor (LIF) is a member of a cytokine family that also includes IL-6, IL-11, oncostatin M, ciliary neurotrophic factor and cardiotrophin 1 (Hilton *et al.*, 1988; Cork *et al.*, 2002). The LIF is a secreted glycoprotein with a range of molecular weight forms, from 38 to 67 Kd, resulting from differential glycosylation of protein of approximately 20 Kd (Hilton *et al.*, 1988). The LIF has the four α helix cytokine structure that contains six cysteine residues, already known to occur in many hematopoietic factors (Akita *et al.*, 1996; Auernhammer *et al.*, 1998). Leukemia inhibitory factor plays an important role in the embryo development (Tsai *et al.*, 1999; Dimitriadis *et al.*, 2010). The LIF has been shown to enhance *in vitro* blastocyst development in mice (Stewart *et al.*, 1992; Michell *et al.*, 1994), *in vitro* blastocyst hatching in sheep and increased pregnancy rates for *in vitro* cultured embryos transferred back into recipient ewes (Fukui *et al.*, 1994). It has been reported that the expression of LIF in the endometrium is absolutely essential for mouse embryo implantation (Bhatt *et al.*, 1991; Marwood *et al.*, 2009). In humans the peak expression of LIF and its receptor (LIF-R) in the endometrium at the mid secretory phase of the menstrual

cycle also suggests a potential autocrine role for LIF in regulatory human implantation (Cullian *et al.*, 1996). However, the target of LIF may not be restricted to the endometrium, because there is a report revealing the expression of LIF in the epithelium of ampullary portion of fallopian tube (Keltz *et al.*, 1996). Because the fertilization takes place in the ampullary portion of fallopian tube and LIF was expressed in the fallopian tube, suggesting that LIF may play a role in fertilization (Saki *et al.*, 2006). Attar *et al.* (2003) announced that Leukemia Inhibitory Factor (LIF) improved the sperm motility and as well as the survival rate of normal sperm when used in *in-vitro*. As our knowledge no report was given about effect of leukemia inhibitor factor on sperm motility and survival rate of asthenospermic infertile men. That's why this study was decided to review the effects of leukemia inhibitor factor with different concentrations of 0, 3, 5, 10, 50 ng mL⁻¹ on motility and survival rate of asthenospermic infertile men.

MATERIALS AND METHODS

This study was conducted from March 2008 to September 2009. Semen samples of 15 asthenospermic men who referred to IVF unit of Imam Khomeini hospital, Ahvaz, Iran were collected. It should be mentioned that the people who were studied before their consent was acquired. In order to complete liquefaction, the sample put in incubator under condition 5% CO₂ in air at 37°C for 45-30 min then drop of semen was removed and placed on the lower part of Makler Chamber. Total sperm count was done first, then calculate the motile sperm with a degree (a) and (b). This process was done in order to determine astheno-spermic samples. In this study we evaluated only samples that had sperm motility percent of more than 30%.

Preparation of samples: In this study, in order to preparation of samples the non-sequential of pure sperm was used. This method was first put, 2 mL solution of pure sperm (80%) in 12 mL tube. Then the equal volume of pure sperm solution (40%) was added then 1.5 to 2 mL of semen was added slowly and the solution was centrifuged (350 g) for 15 min. After centrifugation, pellet was removed from the bottom of tube, then 2 mL of medium was added and centrifuged (200 g) for 5 min and then

upper solution was removed and added about 0.5 mL of medium to sediment Sperm. Solution obtained was incubated in the incubator for 1 h and under the terms 5% CO₂ in air at 37°C for 1 h.

Drops investment: After the preparation medium (without LIF and with 3, 5, 10, 50 ng mL⁻¹ of LIF), about 10-12 h before collecting sample, the drops investment were done in three 35 mm Petri dishes, then in order to prevent evaporation, acidity and micro-organisms influence, the droplets were covered by mineral oil. The samples were evaluated within 6, 24, 48 h after culture. In this assessment first, motile sperm were counted using makler chamber. Number of motile sperm was shown as percentage. Hypo-Osmoting Swelling Test (HOST) was used to evaluate sperm survival (Fukui *et al.*, 1994; Bhatt *et al.*, 1991; Liu *et al.*, 1997).

In this method 0.9 saline was mixed with saline with equal volume of distilled water then prepared sample was mixed with this solution. After half an hour sperm were investigated under a microscope and deflection created by different patterns of sperm tail considered as the HOS positive test.

Statistical method: Percentage of motile and survival sperm cultured in medium without and with different concentration of leukemia inhibitor factor and during different time periods of culture collected and evaluated by ANOVA test and using version 13 SPSS statistical program. The p value less than 0.05 was considered significant.

RESULTS

Mean±standard deviation of asthenospermic men's age that participants in this study was 8/6±28. Table 1 has all the information related to sperm motility and also their survival after culture in different concentrations of leukemia inhibitor factor.

Sperm motility and survival rate of asthenospermic men 6 h after cultured in medium containing 0 to 50 ng mL⁻¹ of leukemia inhibitor factor.

Mean±standard deviation of forward motility of sperm which 6 h cultured in control medium and medium with 3, 5, 10, 50 ng mL⁻¹ of leukemia inhibitor factor was 47±10.62, 49.07±10.49, 47.87±9.94, 51.40±10.54

Table 1: Sperm motility and survival rate of asthenospermic men 6 h after cultured in different concentration of leukemia inhibitor factor

Variables (%)	Media without LIF	Media+3 ng mL ⁻¹	Media+5 ng mL ⁻¹ LIF	Media+10 ng mL ⁻¹ of LIF	Media +50 ng mL ⁻¹ of LIF
Progress motility	47.00±10.62	49.07±10.49	47.87±9.94	51.40±10.54	52.40±10.13
Survival rate	88.53±5.86	87.25±4.58	86.40±6.04	86.60±5.87	87.20±5.34

Values are expressed as Mean±SD

Table 2: Sperm motility and survival rate of asthenospermic men 24 h after cultured in different concentration of leukemia inhibitor factor

Variable (%)	Media without LIF	Media+3 ng mL ⁻¹	Media+5 ng mL ⁻¹ LIF	Media+10 ng mL ⁻¹ of LIF	Media+50 ng mL ⁻¹ of LIF
Progress motility	33.00±8.07	30.87±7.41	29.20±7.47	35.73±6.61	32.20±8.62
Survival rate	55.23±7.67	35.87±8.47	55.53±7.63	70.33±7.13	54.40±6.01

Values are expressed as Mean±SD

Table 3: Sperm motility and survival rate of asthenospermic men, 48 h after cultured in different concentration of leukemia inhibitor factor

Variable (%)	Media without LIF	Media+3 ng mL ⁻¹	Media+5 ng mL ⁻¹ LIF	Media+10 ng mL ⁻¹ of LIF	Media+50 ng mL ⁻¹ of LIF
Progress motility	7.00±5.37	16±6.13	16.33±4.01	15±5.68	22.20±6.89
Survival rate	80.47±4.678	22.73±3.23	25.80±6.50	26.47±74.83	41.33±6.35

Values are expressed as Mean±SD

and 52.40±10.13, respectively. Statistical analysis shows that the forward motility of sperm cultured in media with and without of LIF is not significant ($p>0.05$).

The survival rate of sperm in control and medium with 3, 5, 10, 50 ng mL⁻¹ of LIF is 88.53±5.86, 87.25±4.58, 86.40±6.04, 86.60±5.87 and 87.20±5.34, respectively. Statistical analysis shows that at difference between different group of study are not significant ($p>0.05$).

Evaluated of sperm motility and survival rate of asthenospermic men 24 h after cultured in medium containing 0 to 50 ng mL⁻¹ of leukemia inhibitor factor.

As shown in Table 2 the Mean±standard deviation of progress motility of sperm 24 h cultured in control medium and medium with 3, 5, 10, 50 ng mL⁻¹ of leukemia inhibitory factor was 33±8.07, 30.87±7.41, 29.20±7.47, 35.73±6.61 and 32.20±8.62, respectively. Statistical analysis shows that the forward motility of sperm cultured in media with 10 and 50 ng mL⁻¹ of LIF significantly increased ($p<0.05$). The difference between two last groups was not significant ($p>0.05$).

The survival rate of sperm is 55.23±7.67, 35.87±8.47, 55.53±7.63, 70.33±7.13 and 54.40±6.01 in control and medium with 3, 5, 10, 50 ng mL⁻¹ of LIF, respectively. The survival rate of sperm cultured in media with 50 ng mL⁻¹ of LIF significantly increased compared to another groups ($p<0.05$).

Assessment of sperm motility and survival rate of asthenospermic men 48 h after cultured in medium containing 0 to 50 ng mL⁻¹ of leukemia inhibitor factor.

As shown in Table 3 the Mean±standard deviation of progress motility of sperm 48 h cultured in control medium and medium with 3, 5, 10, 50 ng mL⁻¹ of leukemia inhibitory factor was 7±5.37, 16±6.13, 16.33±4.01, 15±5.68 and 22.20±6.89, respectively. Statistical analysis shows that the forward motility of sperm cultured in media with 50 ng mL⁻¹ of LIF compared to another media significantly increased ($p<0.05$).

The survival rate of sperm is 80.47±4.678, 22.73±3.23, 25.80±6.50, 26.47±74.83 and 41.33±6.35 in control and medium with 3, 5, 10, 50 ng mL⁻¹ of LIF, respectively. The survival rate of sperm cultured in media with 50 ng mL⁻¹ of LIF significantly increased compared to another groups ($p<0.05$).

DISCUSSION

This study determined that no significant change in the progress sperm motility and as well as survival rate of sperm of asthenospermic men cultured in medium containing different concentrations of 3 to 50 ng mL⁻¹ of leukemia inhibitor factor for 6 h. The results of this study in agreement with results of previous study (Attar *et al.*, 2003). Earlier studies have shown the co-culture of fallopian tube epithelial cells and sperm that increases the survival rate and sperm motility. But this effect is not effective in time less than 5 h (Yao *et al.*, 2000). This result is similar to the results of present study. The fact that very limited information about the effects of tubal secretions of human on sperm function. The mechanisms of action of tubal fluid on sperm are two theories. First it is stated that the nutritional effect for sperm that provides all the basic necessities for survival of sperm and some other believe that the discharge tube adjusts the operation of gamete through interaction. It also can affect the growth and differentiation of the embryo (Quintero *et al.*, 2005). Yao *et al.* (2000) in their studies were found that tubal epithelial cells secreted the leukemia inhibitor factor *in vitro*. This factor maintenance the sperm motility. These findings indicate that the sperm has receptor for this factor. Earlier study has shown that a medium with Mullerian Inhibitory Factor (MIF) can improve movement and survival rate of sperm *in vitro* (Siow *et al.*, 1998).

Leukemia inhibitor factor is a cytokines and regulate the cell function through specific receptors. Effect of leukemia inhibitor factor on sperm is the same. Therefore, this factor must be interacting with sperm surface specific receptors (Jenab and Morris, 1998). Attar *et al.* (2003) in their study demonstrated that if normal human sperm culture in medium containing different concentrations of leukemia inhibitory factor for 24 h the concentration of 3 to 10 ng mL⁻¹ of LIF will improve the movement of sperm and highest percentage of sperm movement can be seen in medium with 5 ng mL⁻¹. This study shows that sperm motility is improved in medium containing 10 or 50 ng mL⁻¹ of LIF. Difference between two studies is probably due to differences in sperm motility evaluation.

Because attar used the Sperm Quality Analyzer device while the in the present study visual device used. Attar *et al.* (2003) studied about the normal sperm, but in our study the low motile sperm was evaluated. We observed that cultured of astheno-spermic men's sperm for 48 h in medium containing 50 ng mL⁻¹ of leukemia inhibitor factor can improved the motility and survival rate significantly. The final conclusion from this study is that certain concentrations of leukemia inhibitor factor can increase the motility and survival rate of sperm. It seems that more research and further studies may be able to design the medium can improved the motility and also survival rate of infertile patients who suffering from low motility of sperm.

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