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# The Effect of Oxamate on Fertilization Capacity of Mouse Sperm *in vitro*

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**Abstract:** This study was conducted *in vitro* to show the effect of oxamate on motility and fertility of the mouse. The spermatozoa were extracted from the caudal part of epididymis. The study animals divided into four groups: (1) control group in TYH medium+5 mg $mL^{-1}$ BSA ; (2) test groups in TYH medium in which contain 10 Mm oxamate; (3) test groups in TYH medium in which contain 20 Mm oxamate and (4) test groups in TYH medium in which contain 30 Mm oxamate. All four groups were incubated for 90 min to obtain capacitation. Further their motility was checked after incubation time. The mice were super ovulated with PMSG and HCG hormones to obtain oocytes. Total of 600 oocytes were collected and cultured in drops of KSOM medium+5 mg $mL^{-1}$ BSA, then for fertilization process received spermatozoa from different groups of mentioned above. After 24-26 h, the rate of fertilization was checked. The results of this research indicated that oxamate at the concentration of 20 and 30 Mm significantly reduce ($p<0.05$) the progress of motility and fertility. Statistical analysis showed that percentage of the sperm progress motility in both concentration of 20 and 30 Mm was significantly differ ($p<0.05$) in compare with control group and in concentration of 10 Mm, respectively. The same results were obtained in the case of fertility. These findings suggested that oxamate has an inhibitory role on motility and fertility of mouse sperm.

**Key words:** Oxamate, motility, fertility, mouse, sperm

## INTRODUCTION

The world outrageous of population leads to development of various methods to prevent pregnancy. Some of these methods are designed to be based on the enzymes involved in the process of fertilization (Suri, 2004; Bone *et al*., 2001). Lactate dehydrogenase $C_4$ (LDH-$C_4$) is an isozyme of lactate dehydrogenase (LDH: L-lactate-NAD oxidoreductase EC1.1.1.27) that is found in mature testes and spermatozoa of many species with internal fertilization (Rodriguez-Paez *et al*., 2002; Golberg, 1972). Recently, it found in mouse oocyte and in early embryo until blastocyst stage of development (Coonrod *et al*., 2006). The importance of glycolysis to motility was rediscovered recently by Mukai and Okuno (2004) that showed inhibition of oxidative phosphorylation had little effect on sperm ATP levels or flagellar activity. Some investigators disrupted LDH-C4 by gene targeting glyceraldehyde 3-phosphate dehydrogenase-S, a mouse gene expressed only during spermatogenesis and a key glycolytic enzyme (Miki *et al*., 2004).

Freshly ejaculated spermatozoa are not fertile and are sequentially activated, despite their lack of cytoplasmic protein synthesis, during their transit in the female genital tract. They first undergo a complex and timely series of changes, termed capacitation, before they can bind to the zona pellucida surrounding the oocyte; this contact triggers the acrosome reaction, an exocytotic process by which proteolytic enzymes are released to help a spermatozoon to penetrate the zona pellucida, reach and fertilize the oocyte (De Lamirande *et al*., 1997; De Jonge, 2005). Duan and Goldberg (2003) showed that oxamate may inhibit sperm LDH activity that lead to block capacitation.

In this research we studied oxamate as a possible inhibitor of sperm motility and fertility in mice, as an approach to the development of a male contraceptive.

## MATERIALS AND METHODS

This project was conducted in Ahwaz Jondishapour University of Medical Sciences form 16 May 2007 to 21 Oct 2008.

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**Oocyte collection:** Female NMRI mice that were 6 to 8 week-old were administered intraperitoneally with 10 IU Pregnant Mare Serum Gonadotropine (PMG) for superovulation; this was followed 46-48 h later by the intraperitoneal administration of 10 IU Human Chorionic Gonadotropine (HCG). Mice were scarified 12-14 h after HCG injection by cervical dislocation method. After disinfection with 70% alcohol and opening the abdomen wall, the Y shaped uterus, ovaries and oviducts were identified. The oviducts were excised as follows: clamping cornuas, dissecting the peritoneum and fat between ovary and tube and then cutting the fallopian tube from the proximal end and cumulus-oocyte complexes were collected in KSOM medium. The granulosa cells of oocytes were removed by pipetting in KSOM medium containing 80 IU $mL^{-1}$ hyaluronidase and mature oocytes obtained and randomly divided into four parts (O'Flaherty *et al*., 2005).

**Sperm preparation:** Males of proven fertility were scarified and the cauda epididymis removed and immediately inserts into a 150 μL drop of TYH medium+5 mg $mL^{-1}$ Bovine Serum Albumin (BSA) under mineral oil (Sig., embryo-tested, cat. No. M8410). The epididymis contents were squeezed out. The spermatozoa were extracted from the caudal part of epididymis randomly divided into four groups: (1) control group in TYH medium+5 mg $mL^{-1}$ BSA ; (2) test groups in TYH medium in which contain 10 Mm oxamate; (3) test groups in TYH medium in which contain 20 Mm oxamate and (4) test groups in TYH medium in which contain 30 Mm oxamate. All four groups were incubated for 90 min at 37°C in 5% $CO_2$ in humidified air for the purpose of capacitation (O'Flaherty *et al*., 2005).

**Assessment of sperm motility:** After incubation in medium with different concentration of oxamate, the sperm motility was assessed and classified as (1) progressive; (2) non-progressive and (3) immotile spermatozoa. Initial sperm motility was manually assessed by a single individual in duplicate for each sample by evaluating 100 sperms (Giraud *et al*., 2000).

**Fertilization *in vitro*:** Fertilization *in vitro* was carried out in drops of KSOM medium+5 mg $mL^{-1}$ BSA without oxamate under mineral oil. A preincubated capacitated sperm suspension into different study groups as mentioned above was gently added to the freshly ovulated ova which divided in four parts of oocytes to give a final motile sperm concentration on 100000 $mL^{-1}$. The mixture of sperm-oocyte suspension was incubated for 4-6 h. The ova were then washed through several changes of medium and finally incubated in drops of KSOM medium+5 mg $mL^{-1}$ BSA without oxamate under mineral oil. Fertilization was assessed by recording the number of bi-cells embryos 24-26 h after completion of *in vitro* fertilization (Yu *et al*., 2000).

**Statistical analysis:** Statistical analysis were done by SPSS 13.0. Chi-squire test was used to compare 4 experimental groups. A $p<0.05$ was considered as significant.

## RESULTS AND DISCUSSION

The percentage of spermatozoa showing progressive motility, non-progressive, immotile expressed as Mean±SD as well as the fertilization capacity are expressed as percentage in Table 1.

Present results indicated that oxamate at the concentrating of 20 and 30 Mm reduce significantly ($p<0.05$) the progress of motility and fertility. Statistical analysis showed that percentage of motility in both concentration of 20 and 30 Mm was significantly differ ($p<0.05$) to control group and in concentration of 10 Mm, respectively. The same results were obtained in the case of fertility.

The rapidly global population has turned the attention of family planning and associated reproductive health programs and providers towards providing safe and reliable method that can be used to limit family size. Due to overwhelm increasing in human population, it is necessary to develop new, safe, effective contraceptive methods as a possible strategy for controlling fertility.

Recently, Duan and Goldberg (2003) have confirmed the participation of LDH-C4 in mouse sperm capacitation. They presented a blocking effect on capacitation of mouse sperm *in vitro*, if an inhibition of LDHC4 by oxamate was performed. In the present study, we suggest that the participation of LDH-C4 in order to trigger the motility and fertility in capacitated mouse spermatozoa. This isoenzyme is important for obtaining metabolic energy for sperm motility and survival necessary to reach

Table 1: Percentage of motility and fertilization capacity of mice sperm after incubation

| | Parameters | | | |
|---|---|---|---|---|
| Experimental groups | Prog-motility (Mean±SD) | Non-prog-motility (Mean±SD) | Immotile (Mean±SD) | Fertilization rate (%) |
| 1 (n* = 5) | 48.25±3.97 | 26.12±2.48 | 25.62±3.50 | 55.3 |
| 2 (n* = 5) | 43.05±3.78 | 29.00±3.16 | 27.95±1.14 | 46.0 |
| 3 (n* = 5) | 28.65±2.19 | 40.52±2.77 | 30.88±0.89 | 25.3 |
| 4 (n* = 5) | 23.87±2.58 | 43.26±3.19 | 32.87±1.73 | 20.0 |

Prog-motility, Progressive motility, Non-prog-motility, Non-progressive motility, SD: Standard deviation, Group 1: Control, Group 2: 10 Mm of oxamate, Group 3: 20 Mm of oxamate, Group 4: 30 Mm of oxamate, *No. of replications

oocytes in the female reproductive tract and attain a successful fertilization (Miki *et al*., 2004).

The results of present study indicated that oxamate inhibits the progress motility of the mouse sperm in a dose- and time-dependent manner, although in relatively high concentration (20-30 mM). Earlier study showed that the other substances also inhibit sperm motility and energy metabolism only at high concentration. For example, 20 Mm 2-deoxyglucose and 20 mM α-chlorohydrins have employed to inhibit motility of ram (Breitbar and Nass-Addeny, 1995) and guinea pig spermatozoa (Mújica *et al*., 1991), 10 mM KCN blocks mitochondrial respiration in carp spermatozoa (Perchec *et al*., 1995) and 30 mM theophyline inhibits the cAMP phosphodiesterase in goat spermatozoa (Centola *et al*., 1998). Present data is consistent with Duan and Goldbrg (2003) that established a functional role for LDH-C4 as a key enzyme necessary for capacitation and motility. Present study also demonstrated that the oxamate with concentrating of 20 and 30 Mm significantly decreased the rate of fertilization capacity of mouse sperm witch was other earlier study by Rodriguez-Paez *et al*. (2002).

In conclusion, it appears that high concentrations of oxamate in the mM range are beneficial for inhibition of sperm motility and fertility capacitation. However, this effect could be applied in male contraceptive process.

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