EFFECT OF CYSTEAMINE ON THE RATE OF *IN VITRO* MATURATION OF OOCYTES IN TWO MEDIA

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Abstract- Rate of *in vitro* maturation of oocytes is one of the challenges of assisted reproductive techniques. In this study we investigated the effects of supplementation of cysteamine on the rate of in *vitro* maturation of oocytes in two different media. Germinal vesicle oocytes were collected from mouse ovary and cultured in two media (TCM199 and MEME) with 0, 50, 100, 200, 500 µM/ml cysteamine. Number of germinal vesicle breakdowns and metaphase II oocytes were recorded. The results showed that the rate of *in vitro* maturation in 100 µM/ml cysteamine was significantly higher compared to control ($P < 0.05$). Evaluation of two media in this study showed that TCM199 improved the rate of *in vitro* maturation and oocyte maturation better than MEME; however, this difference was not statistically significant. These findings indicate that TCM199 as compared to MEME was better in rate of in vitro maturation of oocytes.

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INTRODUCTION

In vitro maturation (IVM) of oocytes from small antral follicles could potentially provide increased numbers of oocytes for *in vitro* fertilization (IVF) reduce the need for exogenous gonadotrophin treatment and offer an alternative to superovulation.

In the early follicular phase, several small antral follicles exist but the majority of them regress by atresia in the few days before ovulation. Following IVM and fertilization of oocytes retrieved from these follicles, pregnancies and live births have been achieved but the rates of clinical pregnancy are still lower compared to the rates achieved using standard IVF (1, 2).

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To undergo a successful fertilization and embryonic development, complete cytoplasmic and nuclear maturation is necessary (3). Studies done on cows and mice have shown that commercially available culture media used in IVF have widely different effects on spontaneous nuclear maturation and subsequent preimplantation development (4, 5). Some of the culture media can result in a spectrum of abnormalities collectively referred to as large offspring syndrome (6, 7). Moreover, in all *in vitro* culture systems, a major culture-induced stress is enhanced oxidative damage, with increased reactive oxygen species (ROS) (8-15).

Adding thiols compounds like cysteamine to culture mediums could have different effects depending on the concentration used, the species and types of oocytes and medium in study (16). Low molecular weight thiols such as cysteamine when present during IVM of oocytes, stimulate GSH synthesis and decrease hydrogen peroxidase levels (15-21). Furthermore, Aerobic organisms possess

protective mechanism like the glutathione (GSH) peroxidase/reductase system (10-15).

In this study we investigated the effects of different doses of cysteamine in two culture mediums and compared the rate of oocyte maturation in two media.

MATERIALS AND METHODS

All the materials were purchased from Sigma except of TCM 199 and FBS which were from Gibco. Oocytes were obtained from immature female Balb/c mice with age 6 weeks which were kept under controlled light and temperature conditions and free access to water and food. They had successively 12 hour light and 12 hour dark condition. The animals received an intra-peritoneal injection of 10 IU pregnant mare's serum gonadotrophins (PMSG, IRAN).

Forty-five to 50 hours later the animals were sacrificed by cervical dislocation. The ovaries were dissected out and placed in TCM199 or MEME (Sigma). Medium for collection of oocytes was composed of 3 mg/ml BSA, 0.23 mM sodium pyruvate, 100 IU penicillin/streptomycin. Follicles were dissected from ovary by using insulin syring. Oocytes were denuded by frequent pipetting by bore-ended pipettes. Culture medium was composed of 0.23 mM sodium pyruvate, 3 mg/ml BSA, 100 IU penicillin/streptomycin, 75 mIu/ml FSH, 10% FBS and 0, 50, 100, 200, 500 µM cysteamine. Oocytes were cultured in 50 µl culture medium which was covered by mineral oil (embryo tested) in 5% CO_2 and 37º C. After 4 and 24 hours, oocytes with germinal vesicle (GV) (Fig1), germinal vesicle breakdown (GVBD) and metaphase II (MII) were counted using an inverted microscope. Fragmented oocytes were omitted from results. Differences among treatments in each experiment were analyzed by ANOVA and $P < 0.05$ was considered as significant.

RESULTS

Means ± SD of GVBD (Fig. 2) and MII oocytes (Fig. 3) development in TCM199 medium with different doses of cysteamine are presented in table 1. A significant improvement was observed in oocytes development with 100 µM addition of cysteamine to TCM199 ($P < 0.05$). Also a significant relationship was observed between 100 µM and 500 µm cysteamine in IVM of oocytes ($P < 0.05$). Compared to the control group (without cysteamine), the media with 500 µM cysteamine significantly decreased the development of IVM of oocytes ($P < 0/05$). Mean ± SD of GVBD and MII oocytes development in MEME with different doses of cysteamine are shown in table 1.

MII development improved significantly in MEME with 100 µM cysteamine ($P < 0.05$). GVBD development was also increased in 100 µM cysteamine but not significantly. Comparison between means ± SD of development of GVBD in two TCM199 and MEME medium, with different doses of cysteamine are presented in table 1.

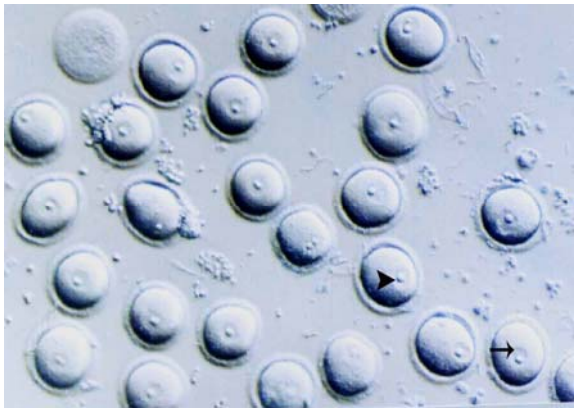

Fig. 1. Oocytes in germinal vesicle stage. Nucleus and nucleololus are shown with arrow and arrow tip respectively.

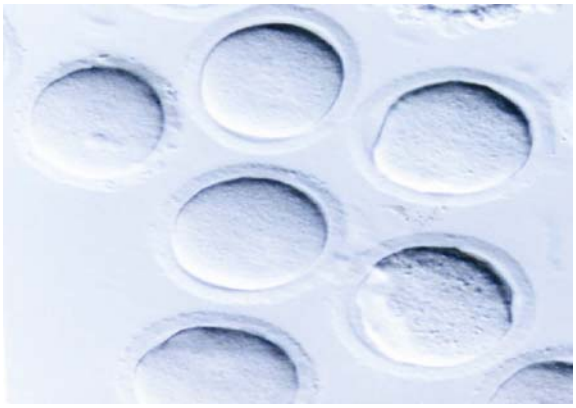

Fig. 2. Oocytes in GVBD. Germinal vesicle disappears in this stage.

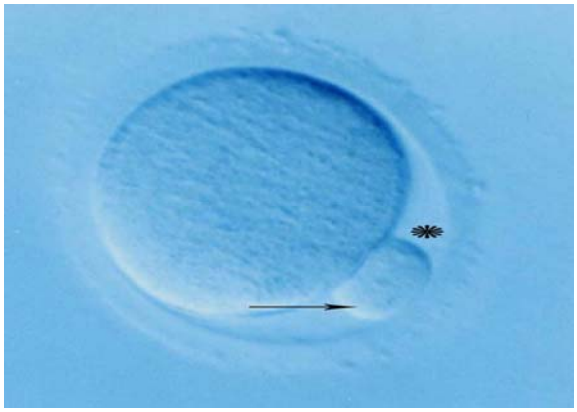

Fig. 3. Oocytes in MII stage. First polar body is shown with arrow in perivitelline (star) line space.

TCM199 compared to MEME improved development of GVBD but this difference was still not significant. Moreover, TCM199 improved MII development better than MEME but not significantly.

DISCUSSION

In the present study, the effect of cysteamine on IVM of oocytes was evaluated in two media. Adding this low molecular weight thiols during IVM resulted in an efficient improvement in the IVM of oocytes. The dose response trial showed that the optimal level of cysteamine among the four concentrations assessed was 100µM. In this concentration cysteamine significantly improved the efficiency of IVM of oocytes in the mice. Gasparini *et al.* in 2000 showed that 50µM/L cysteamine among the three concentrations (50, 100, 200) was the optimal dose in IVM and in vitro culture (IVC) of bovine. The different results between our study with Gasparini may have resulted from the type of species that had been used and can affect results of IVM (16-17). The oxidative damage of cell compounds via reactive oxygen species is one of the major damaging processes for proper cell function. Efficient antioxidant systems such as superoxide dismutase or catalase as well as thiol compounds that act as metabolic buffers may reduce oxidative stress by scavenging reactive oxygen species. It is well known that GSH protects mammalian cells from oxidative damage and its synthesis is essential for normal growth in the rat (17).

In general terms, MEME is a less complex medium than TCM199. Both contain a similar range of inorganic salts, but MEME has a higher glucose concentration, fewer non-essential amino acids and a relatively smaller number of vitamins. TCM199 has a lower glucose concentration, both essential and non-essential amino acids, a large range of vitamins and several other components such as cholesterol and ribose (21). Amino acids are known to be beneficial for embryo development, and oocyte maturation (19).

MEME has glucose and glutamine in high concentration compared to TCM199. It has been known for many years that glucose and glutamine are poor energy substrates for the cumulus cell – free rodent oocytes (19) and this is probably related to the low development of oocytes in MEME. In addition, glucose transit through the pentose phosphate pathway generates ribose–5 phosphate which is readily converted to phosphoribosyl pyrophosphate, the starting substrates for purine de novo synthesis (21, 19). Glutamine is utilized in two separate steps of the purine de novo pathway and contributes two nitrogen atoms to the purine back bone.

Table 1. Means ± SD of GVBD and MII oocytes development in TCM199 and MEME mediums with different doses of cysteamine

	Medium			
	TCM199		MEME	
Cysteamine dose	GVBD	MII	GVBD	MII
Control	18.166 ± 5.944	5.707 ± 2.330	22.166 ± 6.882	15.83 ± 4.535
50 µM	24.16 ±10.128	19.33± 8.017	18.166 ± 6.735	14.833 ±5.307
100 µM	33 ± 6.512	28.5 ± 6.65	28.33 ± 7.86	27.833 ± 3.971
200 µM	20.166 ± 8.329	17.155 ± 7.055	18.33 ± 6.522	16.5 ± 7.179
500 µM	8.33 ± 5.466	7.166 ± 2.787	10.166 ± 2.787	7.33 ± 1.033

Abbreviations: GVBD, germinal vesicle breakdown; MII, metaphase II.

These compounds may results in an increase of purine which has an inhibitory effect on IVM of oocytes. In 2000, Ruth *et al.* evaluated effects of two media on human oocyte maturation. They showed that MEME improved IVM of oocytes better than TCM 199. Absence of cysteamine in culture medium and type of oocytes can affect the results of study.

Contrary to the results of Ruth *et al.* who showed that MEME improved IVM of oocytes more efficiently than TCM 199, we found that TCM 199 was better. The most probable reasons could be the supplementation of media with cysteamine and different species. We also suggest that the beneficial effect on oocyte maturation following adding cysteamine might be mediated by an increase in GSH synthesis but this hypothesis was not investigated in this study and further research is needed for detection of the mechanism by which cysteamine improves oocytes development.

Conflict of interests

We have no conflict of interests.

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