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Evaluation of the Effects of Sera Originating from Goats Supplemented with Vitamin E and Fish Oil on *In Vitro* Maturation of Murine Oocytes

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Abstract

In Vitro Maturation (IVM) of oocyte is an inextricable step of *in vitro* fertilization process. The positive effect of vitamin E and fish oil, as a source of n-3 fatty acids, on the quality and maturation of oocyte has been reported previously; however, the impact of sera of animal supplemented with such elements on maturation of oocytes has not been investigated. Therefore, the present study was conducted to examine the effect of sera of goats supplemented with vitamin E, fish oil or their combination on *in vitro* maturation of oocytes in mice. Sera were harvested from estrous goats that had received basic diet (control), basic diet plus vitamin E, basic diet plus fish oil and basic diet plus combination of vitamin E and fish oil for 10 weeks. Subsequently, the respective sera were added to IVM media at concentration of 10% and the proportion of oocytes at various stages of development was analyzed following IVM. Sera enriched in vitamin E enhanced the development of oocytes towards metaphase II stage ($P< 0.05$). However, sera enriched in n-3 fatty acids did not influence maturation of oocytes ($P>0.05$). In conclusion, the present study indicated the positive effects of sera of goat's supplemented vitamin E on efficiency of IVM in mice.

Keywords: IVM of murine oocyte; Fortified caprine serum; Vitamin E; Fish oil

Introduction

Conventional *In Vitro* Fertilization (IVF) of oocyte, in which matured oocytes are provided through ovarian stimulation by gonadotropins, could not be an option in some patients due to FSH resistance, risk of ovarian hyper stimulation in polycystic ovary syndrome and urgency of fertility preservation in cancer patients prior to oncotherapy [1]. Therefore, *In Vitro* Maturation (IVM) of oocytes has been developed as an alternative reproductive technique prior to IVF so as to overcome the disadvantages of conventional IVF [1,2]. Yet more studies are required to optimize the IVM protocols [1].

In this regard, there is evidence indicating that vitamin E contributes to maturation of oocyte and the quality of resultant embryo [3]. Moreover, addition of vitamin E to culture media improved not only maturation of oocytes toward metaphase II stage under *in vitro* condition but also development of fertilized oocytes up to blastocyst [4]. It has also been demonstrated that n-3 fatty acids, which account for the prominent proportion of fish oil fatty acids [5,6], could modulate molecular behavior of oocyte and its surrounding cumulus cells [7], thereby enhancing the development and quality of oocytes [8]. However, to our knowledge, the effect of vitamin E and fish oil combination on *in vitro* maturation of oocyte has not been investigated.

Although vitamin E can serve as an antioxidant both under *in vitro* and *in vivo* condition, the *in vivo* effect is definitely differ from *in vitro* impact since in the former vitamin E would pass through the complex hepatic metabolism prior to reaching the target organ [9]. Likewise, liver plays an indispensable role in metabolism of n-3 fatty acid, which could contribute to discrepancy between *in vitro* and *in vivo* supplementation with fish oil [10]. Accordingly, the present study was conducted to evaluate the influence of sera belonging to goats supplemented with vitamin E, fish oil or their

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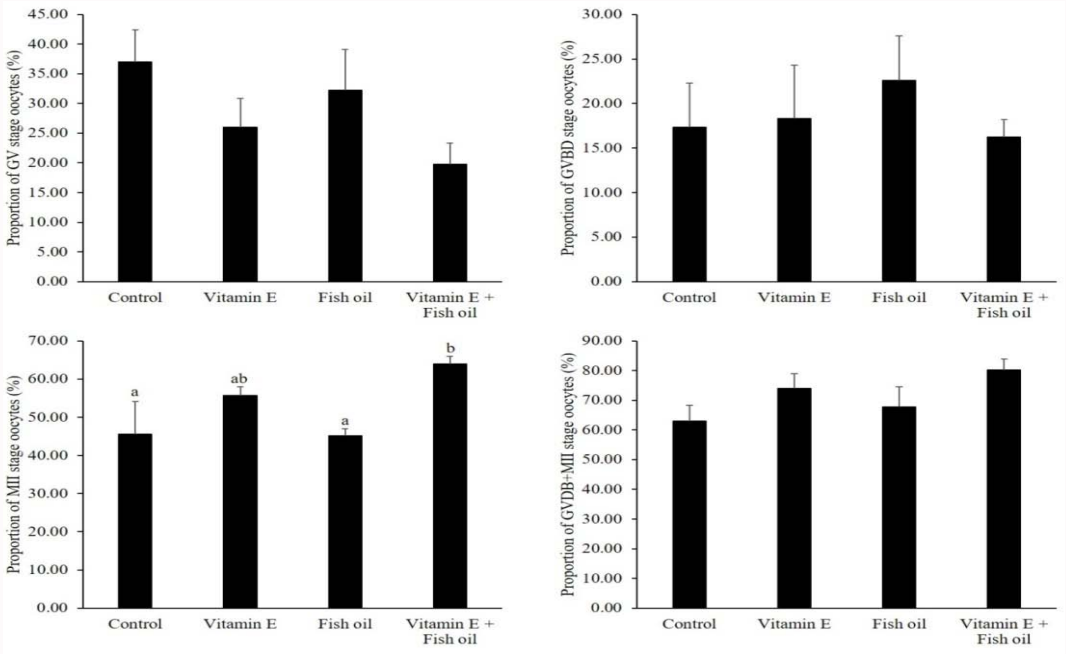

Figure 1: Proportion of various stages of oocytes following IVM in the control, vitamin E, fish oil and combination of vitamin E and fish oil groups. Data are presented as mean ± SEM. Dissimilar letters (a,b) indicate significant difference (P <0.05).

combination on *in vitro* maturation of murine oocytes.

Materials and Methods

Animals

Crossbred female goats (three to four years old) were used for preparation of fortified sera. Besides, ovaries of NMRI mice (six to eight weeks old), which were kept under controlled conditions (Temperature: 20-25°C; Humidity: 40-60%) with 12-h light cycle, were used for IVM in the present study All procedures of present study were performed under approval of Royan Ethics Committee (IR.ACECR.ROYAN.REC.1395.135).

Nutritional supplementation of goats

Goats (n=41), which were maintained at a commercial farm located in Qom province of Iran, were randomly assigned to four groups to receive different experimental diets including diet with no supplementation (control), diet supplemented with vitamin E, diet supplemented with fish oil and diet supplemented with combination of vitamin E and fish oil. The basic diet was composed of alfalfa hay, wheat straw, barley grains and molasses [11]. Vitamin E (5500 IU Vitamin E Premix, Vetaque Co., Tehran, Iran) and fish oil (Sahar Kilka Powder Co., Rasht, Iran) were added to basic diet at levels of 80 IU/day/goat and 20 g/day/goat, respectively. Goats received the respective diets for 10 weeks, including a 2-week adaptation period. The experimental diets were fed as total mixed rations twice daily in the morning and afternoon. All animals had ad libitum access to fresh water.

Blood sampling and serum preparation

Goats were estrus-synchronized using intravaginal sponges (Esponjavet, Hipra, Spain) for 11 days and blood samples (50 ml from each animal) were collected from goats using syringe at estrus. Following collection, blood samples were centrifuged for 10 min at 1000×g and sera were stored at -70°C in 500μl microtubes.

Mouse immature oocytes retrieval and IVM

Female mice were scarified by cervical dislocation to excise the ovaries. Next, the ovaries were placed in a drop of α-MEM culture medium (Gibco, Paisley, UK) containing 10% of fetal bovine serum (FBS; Sigma-Aldrich, Deutschland, Germany), which was covered with mineral oil and murine oocytes at Germinal Vesicle (GV) stage were retrieved by dissection of ovarian tissue. Following retrieval, oocytes were placed in 20-μl drops of α-MEM containing 100 mIU/ml FSH (Merck, Darmstadt, Germany), 7.5 IU/ml hCG (Choriomon®, IBSA, Switzerland) and 10% fortified serum harvested from goats supplemented various diets and were incubated for 18-24 hours at 37°C with 100% humidity and 5% CO_2. Fortified sera were melted at room temperature and were placed in water bath at 56°C to become inactivated prior to be used for IVM media. Following IVM, the proportion of oocytes at various stages including GV, Germinal Vesicle Breakdown (GVBD) and Metaphase II (M-II) were recorded. The oocytes in which the germinal vesicle had disappeared were considered as GVBD stage oocytes and the oocytes in which the first polar body had extruded were considered as M-II stage oocytes [12].

Statistical analysis

Initially, binary variables of percentage of oocytes at various stages were arcsine-transformed prior to analysis. Data were analyzed using GLM procedure. All analyses were conducted in SAS version 9.4 (SAS Institute Inc., Carry, NC, USA). Differences at $P \leq 0.05$ and $0.05 < P < 0.10$ were considered statistically significant and tended to be statistically significant, respectively.

Results

Fish oil and interaction of fish oil by vitamin E did not influence the proportion of GV stage oocytes ($P > 0.05$), but vitamin E decreased the proportion of GV stage oocytes ($P < 0.05$; Figure 1).

Fish oil, vitamin E and interaction of fish oil by vitamin E did not affect the proportion of GVBD stage oocytes ($P > 0.05$; Figure 1).

The proportion of M-II stage oocytes in vitamin E and fish oil combination group was higher than that in the control and fish oil group ($P<0.05$; Figure 1). It was not influenced by fish oil ($P>0.05$); however, it increased in response to vitamin E ($P<0.01$; Figure 1).

Fish oil and interaction of fish oil by vitamin E did not impact the proportion of GVBD and M-II stage oocytes ($P> 0.05$), but vitamin E tended to increase the proportion of GVBD and M-II stage oocytes ($0.05<P<0.10$; Figure 1).

Discussion

The findings of the present study revealed that incorporation of serum of goats supplemented with vitamin E in IVM media could enhance maturation of mouse oocytes. In agreement with the results of the current study, Farzollahi *et al.*, found greater development of oocytes towards M-II stage in response to addition of vitamin E to IVM media [4]. In addition, beneficial effects of the combination of vitamin E and selenium on maturation of porcine oocytes under *in vitro* condition have been reported [13].

The positive impact of sera fortified with vitamin E could be attributed to antioxidant activity of this vitamin. Although optimum level of Reactive Oxygen Species (ROS) contribute to normal development and maturation of oocyte [14], excessive amount of ROS could trigger apoptosis [15] and disrupt steroidogenic activity of somatic cells surrounding oocyte [16], which eventually would lead to atresia [14]. Hence, antioxidants play a crucial role in regulation of normal maturation of oocytes either under *in vivo* or *in vitro* condition [14,17]. In this context, vitamin E is recognized as a potent antioxidant [9], and in turn, could have potentially contributed to controlling of oxidative stress during IVM in the present study.

Unlike sera enriched in vitamin E, sera harvested from goats supplemented with fish oil, as a source of n-3 fatty acids, did not influence the maturation of oocytes in the present study. By contrast, consumption of diet containing high levels of n-3 fatty acids has been observed to enhance maturation and quality of oocytes [8]. Nevertheless, it seems that the impact of n-3 fatty acid on oocyte quality is dose-dependent [18-20], which could partly explain the insignificant effect of fish oil in the present study.

In conclusion, the present study revealed that sera of goats supplemented vitamin E improved efficiency of IVM in mice, whereas sera of goats supplemented fish oil failed to enhance IVM. This finding is of significance since it could be applied for fortification of sera used for preparation of culture media so as to augment IVM success.

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