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Research Article

Comparing the Effect of Bovine Albumin and Autologous Sera in Culture Medium (EmbryoCul-MHRM) in the Presence of Zn Supplementation on the Number of Embryos Produced by IVF in Mice

Mahdi Hadi[1-3]*, Fatemeh Ouroujzadeh[4], Mahnaz Azarnia[4] and Ghadireh Mirabolghasemi[4]

[1]Department of Reproductive Biology, Pishgam Paya zist Research and Knowledge Based Company, Tehran, Iran

[2]Department of Advanced Biological Technologies, Tamin Pharmaceutical Investment Company, Tofigh Darou Research and Engineering Company, Tehran, Iran

[3]Department of Embryology, Royan Research Institute, Reproductive Medicine Research Center, Tehran, Iran

[4]Department of Animal Biology, Kharazmi University, Faculty of Biological Sciences, Tehran, Iran

***Corresponding Author:** Mahdi Hadi, Department of Reproductive Biology, Pishgam Paya zist Research and Knowledge Based Company, Tehran, Iran.

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Abstract

Background: IVF techniques are developing as an effective treatment for the treatment of infertile couples. Accordingly, the present study designed to examine the effect of autologous serum and bovine serum albumin in the presence of zinc supplementation on the number of embryos produced.

Methods: 3 male and 1 female NMRI were used for the extraction of oocyte and sperm. Capacitate sperms were added to drops containing oocytes. The resulting compound was incubated for 4 - 6 hours in order to fertilization. Group design was performed in six groups: 1-control group, 2-control group + Zn (Zinc), 3-control group + AS (Autologous Serum), 4- AS + Zn, 5- AS (BSA free) and 6- free serum group with three replications in each group. After 24 and 48 hours, the 2 and 4 cells embryos were counted. Data were analyzed by ANOVA1. The difference between results in diverse groups was considered at $P \leq 0.05$ level.

Results: The data from the experimental groups showed the percentage of embryos in the group containing AS was lower than the control group and the Zn group. The Zn group moreover had more embryos than the control group, but this increase was not significant. And according to the results, the presence of BSA is essential in the culture medium.

Conclusion: According to the results, it seems necessary to use of BSA in cultures, but its combination with AS is unrecommended. It is also advisable to use zinc supplements to increase the number of embryos in the culture medium.

Keywords: Autologous Serum; Bovine Serum Albumin; Zinc Supplement; IVF

Introduction

According to the World Health Organization, about eighty million couples worldwide are now infertile [1]. Infertility is defined as the failure to reach a clinical pregnancy after regular intercourse for 12 months [2]. In the past few decades, assisted reproductive technology (ART) has been used to compensate for infertility of couples and commercially valuable animals. There have been improvements in many ART techniques, resulting in a significant increase in the proportion of viable oocytes and transferable embryos [3]. Assisted reproductive technology (ART) consists of procedures that involve the *in vitro* handling of both human oocytes and sperm, or of embryos, to establish a pregnancy [4]. A variety of assisted reproductive techniques include *in vitro* fertilization or IVF. With the beginning of this technology in 1978, it became possible to treat infertility in some couples who, for whatever reason, had gametes but were deprived of children [5]. The advances in the world of IVF during the last decades have been rapid and impressive, and culture media play a major role in this success. Until the 1980s fertility centers made their media in house. Nowadays, numerous commercially available culture media contain various components including nutrients, vitamins and growth factors. Since the birth of Louise Brown, the first child born as a result of IVF, the advances in the world of assisted reproduction have been rapid and impressive and the future holds even more. The number of children conceived by assisted reproduction technologies (ART) has reached 5 million. Evidence suggests that culture medium conditions are important as a result of IVF and affect pre-and post-implantation development and possibly children health [6]. There are important reasons to put a serum in the culture medium. In addition to the basal culture medium, the protein material in the serums is required to facilitate the fetus' interactions with the culture medium, and the serums provide conditions similar to the mother's fallopian tubes for the fetus [7]. There are a variety of growth factors in the serum, including beta-modifying growth factor, epidermal growth factor, and insulin-like growth factor. Bovine serum albumin is also one of the most important serum proteins and promotes cell growth and survival. This protein acts as a carrier of salts and lipids [8]. The autologous serum is a sort of serum reserved from a person's blood sample [9]. It seems that the use of the individual's serum is most compatible with *in vivo* and cellular phenomena that can have favorable effects. It is expected that this type of serum will not have the side effects caused by the use of non-autological serum.

Zinc is a very critical element in the reproductive cycle of species. In humans, it is necessary for the formation and maturation of spermatozoa, for ovulation, and fertilization. Zinc supplementation has already proven beneficial in male sterility and in reducing complications during pregnancy [10].

Culture media conditions in mammals lead to a reduction in developmental potential, in other words, embryonic survival is affected by environmental conditions. Therefore, culture media should be in optimal conditions in terms of concentration of nutrients, minerals and vitamins required by the fetus, pH, temperature, etc. In the present study, we tried to improve the efficiency of the culture system used for *in vitro* development of mouse embryos by adding zinc supplement and autologous mouse serum to the culture medium.

Material and Methods

Animals: This study was performed using 3 female mice and 1 male NMRI mice in each experimental group. The studied mice aged were 7 - 10 weeks that kept under controlled conditions (12 hours light/dark) and free access to water and food. All rules and ethical principles regarding the use of laboratory animals were observed. This study has been approved by the ethics committee of Kharazmi University.

Oocyte and sperm extraction

Intraperitoneal injection of PMSG (Foligon, Netherlands) at 12 noon and 48 hours after that, HCG (Pergnil, Germany) was performed, respectively. 20 hours after HCG injection (8 am the next day), the mice were killed for oocytes collection. The isolated oviducts were transferred to a petri dish containing EmbryoCul-MHRM medium (Tawfiq Daroo, Iran) and by two insulin syringes, the oocytes with the Cumulus Oocyte Complex were gently removed from the fallopian tube (Figure 1). The oocytes and their surrounding cell mass were then transferred to EmbryoCul-MHRM drip culture medium containing 15 mg/ml BSA and coated with mineral oil (Sigma, USA) and incubated for 12 hours.

Adult and motile sperm isolated from the epididymal tail were incubated for one hour in EmbryoCul-MHRM culture medium containing 15 mg/ml BSA (Sigma, USA) for one hour. Then sperms were added to the mature oocytes in the droplets for fertilization and incubated for 6 hours.

Preparation of autologous mouse serum

To prepare the autologous serum after collecting blood from the animal's heart inside the Falcon tube, we allowed the blood to clot at laboratory temperature for half an hour. The tube was then centrifuged to separate blood cells and serum. The yellow liquid at the top of the tube was the same autologous mouse serum used for the eggs of the same mouse. The serum was placed in Ben Mari for deactivation for 10 minutes at 56°C.

Experimental groups

In this investigation, six experimental groups contain different embryonic culture media, were studied. Also, 3 replicates in each group with 20 - 25 embryos in each replicate were considered. These groups were: a control group with EmbryoCul-MHRM culture medium containing 4 mg/ml bovine serum albumin (BSA), and five treatment groups including: 1 - base culture medium or control+ autologous serum (control + AS), 2 - Basic culture medium or control + zinc supplement (control + Zn), 3 - Basic culture medium + AS + Zn (control+ AS+ Zn), 4 - Culture medium containing autologous serum and without BSA, 5 - Culture medium without any type of serum and zinc supplement. For this purpose, the zinc supplement (Sigma Albridge, USA) was added to the culture medium of Zn + control group in the amount of 1 mg/ml. Also, 10% of autologous serum was used for drip in groups containing AS.

Two cells embryos were counted 24 hours and four cells embryos 48 hours after fertilization by inverted microscopy. Degenerated embryos were also studied.

Statistical analysis

Data were analyzed by one-way ANOVA and also Tukey test using SPSS software (V.24). Data scatter was normal and was considered significant at the P-value level of less than 0.05.

Results

100 adult oocytes from female mice oviducts were randomly assigned to each of the experimental groups. According to the graphs presented below (Chart 1-3), the results of embryo culture up to the two-cell stage (Figure 2) showed that the percentage of development of two-cell embryos in the BSA + AS group compared to the control group had a significant decrease. On the other hand, although there was no significant difference between the Zn group compared to the control group, however, the Zn group embryos showed a higher percentage of development compared to the control group. The group containing AS + Zn showed a significant decrease in the number of embryos compared with the control group (Figure 2). Also, the data obtained from embryo culture in the 4-cell stage (Figure 3) had similar results compared to the results of embryo culture up to the 2-cell stage. Thus, the percentage of 4-cell embryo development in the AS group was significantly reduced compared to the control group. However, there was no significant difference in the percentage of four-cell embryo development between the Zn group compared to the control group.

The results show the presence of BSA is essential for embryonic development and if replaced with autologous serum in the culture medium, the number of embryos will be greatly reduced. However, the percentage of 4-cell embryo development in the Zn group was higher than the control group (Figure 3).

Also, the results show that the absence of BSA due to its nutritional role prevents the fertilization of oocytes, causes fragmentation (Figure 4) and loss of sperm stimulation and loss of sperm motility. Also, adding the zinc supplement to the culture medium containing bovine serum albumin improves the forward development of embryos.

Chart 1: Mean percentage of embryo formation in the 2-pn stage. Mean ± SE, P ≤ 0.05.

Significance relative to the control group is indicated by the * sign at the top of the columns. According to the graph, the group containing AS and serum-free group show a significant decrease compared to the control group. The group that had zinc supplement in their culture medium increased compared to the control group, but this increase was not significant. (P < 0.05 **, P < 0.01*, *** P < 0.001).

Chart 2: Mean percentage of embryo formation in the two-cell stage. Mean ± SE, P ≤ 0.05.

Significance relative to the control group is indicated by the * sign at the top of the columns. According to the graph, the group containing AS and serum-free group show a significant decrease compared to the control group. The group that had zinc supplement in their culture medium increased compared to the control group, but this increase was not significant. (P < 0.05 **, P < 0.01*, *** P < 0.001).

Chart 3: Mean percentage of embryo formation in the four-cell stage. Mean ± SE, P ≤ 0.05.

Significance relative to the control group is indicated by the * sign at the top of the columns. According to the graph, the group containing AS and serum-free group show a significant decrease compared to the control group. The group that had zinc supplement in their culture medium increased compared to the control group, but this increase was not significant. (P < 0.05 **, P < 0.01*, *** P < 0.001).

Figure 1: Swelling in the fallopian tube due to the accumulation of oocyte mass.

Figure 2: 2-cell embryos (×200 magnification).

Figure 3: 4-cell embryos (×200 magnification).

Figure 4: Fragmented embryo next to a four-cell embryo (×200 magnification).

Discussion

The researchers found the presence of 1 - 5% bovine serum albumin in the culture medium was necessary to prevent the hardening of zona plusida and consequently the failure of sperm to enter the oocyte [11]. In a study for the effect of bovine serum albumin on motility and survival of equine sperm in cold conditions. The results showed the mobility at 48h was significantly higher in 5 and 10 mg/ml BSA diluents compared to diluents without BSA and 15 mg/ml BSA. Also, 10 mg/ml BSA diluents resulted in higher progressive motility compared to non-BSA and 15 mg/ml BSA diluents. Membrane and viability activity in 15 mg/ml BSA diluent per hour 48 decreased significantly compared to other diluents [12]. Bovine serum albumin has been shown to improve embryonic development in rabbits [13]. Bovine serum albumin (BSA) acts as a source of protein in the culture medium. Studies show the favorable effects of BSA on embryonic development and the survival of cow blastocysts [14]. In 2017, a study on goat oocyte showed that due to the influence of various level of BSA on *in vitro* maturation (IVM), fertilization and subsequent embryonic development of goat oocytes. It can be advantageous as a supplement of maturation, fertilization and embryo culture media to increase the developmental rate of goat oocyte [15]. Another study was performed at the University of Bangladesh on maturation and *in vitro* fertilization rate of buffalo oocytes in the culture medium containing BSA. This study data show that the addition of 5% BSA in culture media enhances the chances of maturation and oocyte fertilization [16]. Study on the influences of zinc supplementation during the IVM of porcine oocytes showed a positive effect of this supplement on increasing the blastocyst formation rates. Intracellular glutathione (GSH) levels increase with increasing dose of zinc and the level of reactive oxygen species (ROS) decreases with increasing dose. This result can be explained by the antioxidant role of zinc [17]. Experiments have shown that adding 10 µg/ml zinc to the IVM medium inhibits fertilization in bovine oocytes [18]. Many substances influence ART results by affecting follicular development, oocyte maturation, fertilization and embryo development subsequently [20]. Fragmentation happens in both *in vitro* and *in vivo* embryo, where anucleate cells of varying size and number are found along with nucleate cells in the embryo. Studies have shown that fetuses with a lower percentage of fragmentation have a better chance of reaching the blastocyst stage [21]. In the present study, the addition of 1 mg/ml zinc improved the development of mouse embryos. Also, the control group containing BSA in the culture medium had a significant increase compared to the group containing AS without BSA, which is consistent with many previous studies. Also, in this study, autologous serum and bovine serum albumin were used simultaneously in the culture medium, which in this group was significantly reduced compared to the control group. A culture without BSA does not produce any embryos. This confirms previous studies that serums provide growth factors and proteins for embryonic development. The presence of zinc supplement, autologous serum and bovine serum albumin (BSA) simultaneously in the culture medium did not show any increase in embryonic development rate compared to the control group. It can be due to the interaction of substances and compounds in serums and thus reduce the effect of each of them through the negative feedback mechanism. According to the results, group AS + BSA had a significant decrease ($P \leq 0.001$) in the number of embryos compared to the control group. The results indicate zinc supplementation has a positive effect on IVF embryos, which leads to an increase in the number of embryos. The results also show that the rate of fragmentation in the culture medium contains the autologous serum was higher than the control group and the group containing zinc supplementation. Autologous serum may have been unable to meet all the nutritional needs of fetuses, including growth factors.

Conclusion

According to the results, the use of bovine serum albumin in research is necessary because of its nutritional role, in its absence, embryos will be unable to continue to develop even up to the 2-cell stage. If the positive effect of zinc is proven, this study can be a basis for further studies to use this supplement to increase the rate of embryonic development because reducing costs and saving time are the basic principles in research work. Since the maximum amount of blood that can be taken from the heart of a mouse is about 2 cc, which after centrifugation, filtration and Ben Marie is finally given the amount of 200 landa, and due to the possibility of trial and error during in case of total specimen loss, it is recommended to use rats or larger specimens. In this way, the accuracy of the work will be higher. On the other hand, like human laboratories, it is better to examine the animal for blood or infectious diseases before taking autologous serum sample to access to a healthier culture medium and, of course, greater success.

Conflict of Interest

Authors declare no conflict of interest.

Acknowledgements

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