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Research Article

Comparison of the Effect of Autologous Mouse Sera, Human Albumin Serum and Bovine Albumin Serum in IVF Medium, In the Presence of Zinc Supplementation on the Quality and Number of Fetuses in the 2PN Stage of Laboratory Mice

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Abstract

Introduction: Infertility is one of the major health problems in the world. Infertility is defined as the inability to get pregnant after one year of regular intercourse, without the use of any fertility prevention methods. Infertility includes 40% men, 40% women, 10% both sexes and 10% unknown. There are various ways to treat infertility, one of them is IVF.

Materials and Methods: This study was done by using BSA and HSA serum and autologous serum from female parent mice in IVF medium. The effect of serum in the medium was performed in three groups with Zn and without Zn and three replications in each group. Data were analyzed by one-way ANOVA and then by Tukey's test using SPSS 20 software.

Results: The percentage of embryos in HSA-containing and autologous cultures was lower than the control group containing BSA ($P < 0.05$). The lowest percentage of embryos formed belonged to the autologous serum test group ($P < 0.05$). But in the medium containing zinc, no changes were found in any of the HSA, BSA and autologous serum due to the addition of zinc.

Conclusion: BSA did not show any change compared to BSA + Zn at 2Pn, BSA showed superiority over autologous and HSA.BSA + Zn showed superiority over autologous and HSA.

Keywords: IVF; Autologous Serum; Bovine Albumin Serum; Human Albumin Serum; 2PN Embryo; Zinc (Zn)

Introduction

Infertility is a chronic problem that always and everywhere causes distress and stress for infertile people. Because it impedes or at least poses a serious obstacle to the process of family transformation to later stages by impeding one of the main goals of marriage, namely, reproduction and happy parenthood. There are various ways to treat infertility, including medications, surgery and assisted reproductive techniques. Since 1970, the method of assisted reproduction (ART) has given a new approach to infertility [1]. In recent decades; new methods have been used to treat infertility. Laboratory methods for storing and freezing sperm, ovule and embryos have created a new field [2].

Fertility assist technology increases the probability of pregnancy by two mechanisms: 1. facilitates the interaction between sperm and oocytes; 2. Eliminates sperm abnormalities such as decreased

number and movement or increased morphological defects. Sperm morphology has an important role in determining fertility. It is associated with fertilization and pregnancy rates at normal fertilization stages as well as intrauterine fertilization and IVF treatment. Controlled ovarian stimulation is one of the key components of IVF treatment. Access to multiple oocytes for fertilization increases the chance of pregnancy [3].

In the IVF stimulation phase, the luteal phase is physiologically dysfunctional. The most plausible reason for this is the multi-follicle development during the follicular phase which leads to many Corpus luteum. Generation of general steroids of Corpus luteum will result in concentrations of progesterone and estradiol which directly inhibits LH secretion by the pituitary through the hypothalamic-pituitary-gonadal negative feedback section [4]. If this not completed the corpus luteum destroyed and pregnancy fails. So to ensure the result of reproduction, one method of assisting fertility is the correction of the vital luteal phase. This can be accomplished either by elevation and activity of primary luteal LH or by steroid hormone supplements [4]. As long as the value of chorionic gonadotropin (HCG) produced by implantation of the fetus is high enough to ensure luteum action. HCG is a standard for ovulation induction. Its role is as an alternative to the mid-phase of LH. HCG binds to the receptor like LH and activates it. Thus, by injecting a very small amount of HCG, it may trigger final oocyte maturation and ovulation [4]. Therefore the purpose of this paper is to compare the position of oocytes in autologous mouse sera, Human HSA, bovine albumin serum in In IVF medium containing zinc (ZN) on the quality and number of 2PN embryos of NMRI mice.

Materials and Methods

Test animals

In this experiment, adult NMRI mice were used. Some male and female rats weighing 28-30 g were purchased from Razi Serum Laboratory Animal Production and transferred to the animal storage room of Medical Sciences Tehran University. 50 female mice and 15 NMRI mice aged 8–6 weeks weighing approximately 35 g for male mice and 28 g for female mice at 12 h light and 12 h dark kept in the laboratory with average temperature of 22-19 ° C and 45% humidity with sufficient water and food. Male rats were kept in individual cages and female rats in 5 cages that each containing 10 rats. One week after storage in order to adapt to the new environment; 3 groups of female rats in groups of 5 for injection of ovarian stimulating hormones for ovulation and 3 medium including IVF medium containing BSA serum group IVF medium containing HSA serum and group IVF medium with autologous serum were categorized to compare the effect of these three serum on IVF medium on quality and number of embryos. A specific dose of 10% was administered for all.

Process

On the first day and at 12 pm, PMSG was injected to 15 female rats by scar inhibitory method and were kept in separate labeled cages. No need to do anything on the second day. 48 hours after PMSG injection on day 3 at 12 pm IU5/7 hormones was injected into the same 15 mice. Since we need 24 hours before ovulation and incubation respectively IVF medium, sperm capacity medium and disc medium for the fourth day, on the same day 10% BSA, HSA or autologous mouse serum (each serum individually for each female group with 15 mice) was prepared for 90% of MHRM-Embryocul medium purchased from TODACO Company and suitable for all IVF stages. The medium used was mixed with 10 mg of zinc and then we used it in the relevant steps. In sterile conditions, beneath a Class II laminar hood, we drop 50 microliters into petri dishes. And, along with the drops, we pour the mineral fertilizer for mice purchased from Sigma until it is completely cover the drops. This is to prevent pH changes due to exposure of the medium to the air, to prevent evaporation of droplets in the incubator and to prevent the droplets from disrupting and contaminating the medium as a result of transferring petri dishes to the incubator. Then transferred the petri dishes to a CO_2 incubator with 37 ° C, 80% humidity and 5% CO_2. In the other two Petri dishes, place a 500 Lund drop with the same percentage of serum as IVF (10% BSA, HSA and mouse autologous) and cover with the above-mentioned mineral oil. This is the environment to find sperm capacity and then kept in the incubator on the stated conditions. The dicast medium also contains MHRM medium without serum and oil. To cut the ampulla and remove the ovum from the oviduct, we flush the culture medium in Petri dish and transfer to the incubator. To cut the ampulla and remove the ovum from the oviduct, put the culture medium in a flat Petri dish and transfer it to the incubator. On the fourth day at 8 am killed the male by the cervical dislocation method. And by a surgery remove the epididymal tail, which is full of mature and active sperm. Cut it by a forceps and sterile scissors and put each tail of the epididymis individually in a drop of 500 Landau in capacity environment which was the day before in a co2 incubator at 37 ° C and 80% humidity. And put it in the incubator for another 1 hour to

sperm capacity. Approximately 16 to 18 hours after HCG injection and 15 min after sperm capacity expired, female rats were killed by cervical dislocation and mature ovum in the culture medium under a stereo microscope equipped with a warm stage; it was removed from the oviduct ampoule area and made by hand with an oral pipette. All three ova were placed in an IVF drop. Then remove the sperm's capacity drop after 1 hour of incubation. With oral pipette remove sperm from the margin and dome of surface, Sperm were transferred to each drop of IVF containing some ovum (about 5 µl) and again for 5-4 hours to fertilize and form PN2 embryos transferred to co2 incubator. PN2 embryos are embryos undergoing fertilization and the two nuclei of the sperm and oocytes coexist in the oocyte cytoplasm and it is visible under the microscope.

Statistical analysis

In this study examined the effect of BSA, HSA and autologous serum medium supplemented with zinc and zinc-free media on mouse embryo development in 6 groups with three replications in each group and the data were analyzed by One-way ANOVA.

Results

Quantification of 2PN embryos in zinc free medium

In the first group, IVF medium contained BSA (heterologous serum). The first replication consisted of 90 ovum, after sperm culture and adjacent spermatozoa, 33 2PN embryos were obtained. The second replicate produced 130 ovum and 42 embryos and the third replicate 140 ovum and 47 embryos. In the second group that had IVF medium containing HSA serum; The first replicate was produced 70 ovum and 17PN 2 embryos, the second replicate 123 ovum and 34 embryos and the third replicate 114 ovum and 19 2PN embryos. In the third group, the culture medium contained autologous serum (homologous from female mice); The first replicate consisted of 89 ovum and 20 2PN embryos, the second replication of 128 ovum and 30 embryos, the third replication of 111 ovum and 25 2N fetuses.

Quantification of 2PN embryos with zinc

In the first group, the IVF medium contained BSA serum with Zn. The first replicate contained 45 ova, after sperm culture and adjacent spermatozoa, 20 2PN embryos were obtained. The second replicate produced 150 ovum and 62 embryos and the third replicate 115 ovum and 45 embryos. In the second group that had IVF medium containing HSA serum and zinc; The first replicate was produced 65 ovum and 25 PN 2 embryos, the second replicate 91 ovum and 40 embryos and the third replicate 114 ovum and 19 2PN embryos. In the third group, the culture medium contained autologous serum (homologous from female mice) with zinc; The first replicate consisted of 80 ovum and 25 2PN embryos, the second replication of 130 ovum and 40 embryos, the third replication of 110 ovum and 35 2N fetuses.

The results of the impact of culture medium serum BSA, HSA and autologous mouse in vitro fertilization in mice

The results of the study, the effect of BSA, HSA and autologous sera *in vitro* fertilization of mice are shown in the following tables. Due to the individual differences between the mice, the number of ovule in the replicates was not equal; therefore the results were expressed as percentages. Also, data analysis was performed on the basis of mean percentages. In each iteration, the embryo to ovule ratio was calculated and multiplied to 100; this will determine the percentage of each iteration in each group. Then the mean of the duplicates of each group and their standard deviation were calculated.

Group	Iteration	Number of ovule	Number of fetuses	Percentage
BSA	Iteration 1	90	30	35/86
	Iteration 2	130	42	34/86
	Iteration 3	140	47	34/33
HSA	Iteration 1	70	17	25/784
	Iteration 2	123	34	27/84
	Iteration 3	114	19	21/85
Autologous	Iteration 1	89	20	22/85
	Iteration 2	128	30	24/114
	Iteration 3	111	25	23/414

Table 1: Percentage of fetuses in experimental group 1 (HSA serum) and experimental group 2 (autologous serum) compared to control group (BSA) with 10% concentration of serum medium.

As can be seen in Tables 1 and 2, the percentage of embryos in cultures containing HSA and autologous serum was lower than in the control group containing BSA. The difference was significant, with the lowest percentage of embryos forming the autologous experimental group.

Group	Iteration	Number of ovule	Number of fetuses	Percentage
BSA + Zn	Iteration 1	45	20	43/78
	Iteration 2	150	63	40/372
	Iteration 3	115	45	39/182
HSA + Zn	Iteration 1	65	25	28/89
	Iteration 2	91	40	35/8
	Iteration 3	145	45	31/014
Zn + Autol-ogous	Iteration 1	80	25	31/68
	Iteration 2	130	40	30/92
	Iteration 3	110	32	32/558

Table 2: Percentage of fetuses in experimental group 1 (HSA serum) and experimental group 2 (autologous serum) compared to control group (BSA) with 10% concentration of serum medium plus 10 µg zinc.

Groups	Autologous serum	HSA serum	BSA control
Percentage of fetus	23/46 ± 4/49	24/892 ± 4/31	34/99 ± 2/40

Table 3: Percentages of embryos in experimental group 1 (HSA serum) and experimental group 2 (autologous serum) compared to control group (BSA) with 10% concentration of serum medium ($P < 0.05$).

Groups	Autologous Serum + Zn	HSA + Zn serum	Control + Zn (BSA)
Percentage of fetus	31/54 ± 2/49	31/91 ± 3/55	40/97 ± 4/43

Table 4: Percentages of embryos in experimental group 1 (HSA serum) and experimental group 2 (autologous serum) compared to control group (BSA) with 10% concentration of serum medium plus 10 µg zinc ($P < 0.05$).

As the results of Tables 3 and 4 show, the lowest percentage of formed embryos belonged to the autologous experimental group. The percentage of embryos in cultures containing HSA and autologous serum was lower than in the control group containing BSA and the difference is significant.

As the results of Table 5 show, there is a significant difference between the 6 groups. Also, for a more detailed examination of the differences between groups, pairwise comparisons were made using the Ben Ferry test.

Variable	P	F	Mean squares	df	Sum of squares
Number of fetuses	0/00	5/92	33/69	5	168/45

Table 5: Results of ANOVA test to examine the difference of sample groups in the number of embryos.

Variable name	Meaningful	Standard error	Average difference	Group	Group
Number of fetuses	0/24	0/87	1	HSA +Zn	BSA+Zn
	0/05	0/87	1/66	ATO+Zn	BSA+Zn
	0/93	0/87	-0/06	BSA	BSA+Zn
	0/00	0/87	3/20	HSA	BSA+Zn*
	0/00	0/87	3/33	ATO	BSA+Zn*
	0/44	0/87	0/66	ATO+ZN	HSA+Zn
	0/009	0/87	2/33	ATO	HSA +Zn
	0/22	0/87	-1/06	BSA	HSA +Zn
	0/01	0/87	2/20	HSA	HSA +Zn*
	0/05	0/87	-1/73	BSA	ATO+Zn
	0/08	0/87	1/53	HSA	ATO+Zn
	0/05	0/87	1/66	ATO	ATO+Zn
	0/05	0/87	3/26	HSA	BSA
	0/00	0/87	3/40	ATO	BSA*
	0/87	0/87	0/13	HSA	ATO

Table 6: Binary comparison of sample groups using the Ben Ferry test.

As Table 6 shows, the BSA + Zn group is significantly different from the HSA group and the ATO group. Also, BSA group was significantly different from ATO group, HSA + Zn group and HSA group ($P < 0.05$). There was no significant difference between the other groups.

Discussion

The family is an institution that its foundation is formed by marriage. Forming a family is the beginning of fertility and the source of birth. Fertility is the most important phenomenon in every couple's life. The general desire of human to have children is obvious. In addition to physiological aspects, human fertility also has social and psychological dimensions. Infertility is a chronic problem that always causes distress to infertile people. Therefore, the purpose of this paper was to compare the position of oocytes in autologous mouse serum, human HSA and bovine BSA in IVF medium containing Zn on the quality and number of 2PN embryos of NMRI mice.

Ashwood-Smith., *et al*. [5] and Staessen., *et al*. [6] compared the results of human serum albumin in embryo culture medium. The study showed that there was no difference in fertilization and implantation rates between the two groups, but in aluminous medium, embryo morphology was more appropriate and pregnancy rate was higher [5,7].

In a study performed at the University of Bangladesh on the maturation and *in vitro* fertilization of buffalo oocytes in culture medium containing BSA serum, the control group considered their medium without serum and the experimental group as 5% BSA medium. The differences were striking. The level of oocytes reaching metaphase 2 was 58.02 for the control group and 68.10 for the BSA-containing group. The normal fertilization rate was 19.63 for the control group and 29.52 for the BSA-containing group. Therefore, the data suggest that adding 5% BSA in both the media of oocyte maturation and fertilization increases the chances of oocyte maturation and fertilization of buffalo oocytes. In the present study, the addition of BSA in the culture medium increased the number of embryos obtained [8].

A comparative study by Léveillé., *et al*. [9] showed that mouse embryos do not continue to grow in the presence of human fetal cord serum and female serum and grow only in the presence of human serum albumin (HSA).

In another study by Dadhich., *et al*. [10], the reproductive organs of adult male mice were examined for 28 and 48 days after consumption of zinc-free food. Reports indicated that the seminal vesicles were emptied of germinal cell layers in the zinc deficient group compared to the control group. Some of these lumens contain only Sertoli cells with stem cells. In this study, severe zinc deficiency causes a severe decrease in semen ducts, which in turn causes loss of testicular cells. These declines eventually resulted in sexual weakness and infertility due to tissue changes in the reproductive organs [10].

In the present study, the relationship between zinc concentration in semen plasma and male infertility was investigated. In 9 studies, zinc concentrations in the semen plasma of infertile men were significantly lower than those of normal men [11-16].

Studies show that there is no significant difference between infertile and normal men. In this meta-analysis, zinc concentrations in semen from infertile men were significantly lower than those of normal men [17,18].

Conclusion

The percentage of embryos in HSA-containing and autologous cultures was lower than the control group containing BSA ($P < 0.05$). The lowest percentage of embryos formed belonged to the autologous serum test group ($P < 0.05$). According to statistical analysis conducted in this paper four following results were obtained: 1) BSA did not show any change compared to BSA + Zn at 2Pn stage. 2) BSA showed superiority over autologous and HSA. 3) BSA + Zn showed superiority over autologous and HSA. 4) BSA serum has better ability to fertilize mouse oocytes and give birth to fetuses than HSA and autologous sera, and with the increase in zinc (Zn) this effect gets better.

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