The effects of different protein solutions used as supplement to the two-cell mouse embryo culture system as a quality control system

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Objective of the study presented was to demonstrate the effects of protein solutions used as supplement to the mediums which are utilized for embryo culture in our laboratory. Two-cell embryo culture system was used as the quality control system. CD 7 *X B6C3F1 strains were used to test various 5% albumin solutions such as plasma protein fraction (PPF), bovine serum albumin (BSA), human serum albumin (HSA) and Ham's F-10 without protein supplement. I be percentage of extended blastocyst development was significantly superior with proteinate (92.1%) compared to control (63.8%, P< 0.05). Our results emphasized the role and importance of a quality control system utilization and monitoring in achieving a consistent and standard laboratory procedure. [Turk J Med Res 1993 11(6): 249-251]*

Key Words: Tissue culture, Albumins, Embryo transfer

Quality control (QC) is an integral part of assisted reproductive technologies (ART). Many ART laboratories rely on mouse embryo system for QC. It can be used effectively to control and monitor the quality and the standardization of the laboratory. These are important in assessing the consistency and safety of the procedures, media, and the equipment used in the laboratory which may adversely effect the gamete and embryo quality resulting in low pregnancy rates. Despite its widespread use of mouse embryo QC, there are pro and cons on its effectiveness in ART (1-4). Extrapolation the results of mouse embryo QC to clinical ART remains a potential concern because of various inherent differences between human and mouse embryos (4).

The objective of the reported study is to test the effects of different brand products of protein solution on 2-cell mouse embryo system as an integral part of the QC system in our laboratory.

MATERIALS AND METHODS

In this study we used B6C3F1 female and CD1 male mouse strains. Female mice were superovulated by injecting 10 IU of PMSG intraperitoneally between 3:00 and 6:00 PM. Fortyeight hours after the PMSG injection 10 IU human chorionic gonadotropin (hCG) was administered and the female mated with a male mouse. The females were examined the next morning for the presence of sperm plug to check if mating occurred. Approximately 36 hours after the hCG injection the females were sacrificed for 2-cell embryos. After entering the abdominal cavity, the oviducts were outlined and carefully removed. The zygotes were collected by use of a dissecting microscope and transfered to a single dish for randomization.

Organ culture dishes were prepared with 1 ml medium (Ham's F-10) inside the center well and 1-2 ml medium in moat. The center well was covered with 1.5 ml oil and gassed at 37°C (5% O_2, 95% N_2). Besides the control medium, bovine serum albumin (BSA), plasma protein fraction (PPF), and human serum albumin (HSA) were tested. Each test were performed in triplicate and 5-8 embryos were placed in each dish for each variable tested. The culture dishes were kept in an CO_2 incubator at 37°C for 72 hours. The progress of the embryos was checked each day and development was recorded. In our laboratory the threshold for the embryo development was accepted to be 75% hatched and expanded blastocysts at 72 hour.

The analysis of variance (ANOVA) and student-t test were used to assess the statistical significance.

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Table 1. The percentage of extended blastocyst development on two-cell mouse embryo culture system using different protein solutions (Mean±SE, 95% CI).

Protein Solutions	% of extended bastocyst development	95 % CI
Proteinate	92.1 ±2.3"	87.5-95.7
BSA	85.3±1.6*	82.1-88.5
HSA	71.8+2.1*	67.4+75.5
Control	71.8+3.0	65.8-77.8

#p<0.05 (compared to control)

RESULTS

Ham's F-10 is used as the standard media in the mouse 2-cell embryo culture system. The percentage and 95% confidence interval (CI) of the results are depicted in Table 1. The percentage of hatched and expanded blastocyst formation differed among the study solutions (Figure 1, Table 2). Compared to control media, the protein solutions resulted in higher rates of blastocyst formation (73.8% vs 92.1%, 85.3%, 93.8% respectively, p<0.05). There was a significant reduction in the developing hatched and the expanded blastocyst formation in groups which are supplemented with BSA and Ham's F-10 only (Figure 1). The highest percentage of development was obtained in PPF groups (92.1%) and these were statistically significant compared to the other groups (p<0.05).

DISCUSSION

One of the most crucial factors in ART is the establishment of accuracy and quality in laboratory procedures to achieve successful results. To establish an ideal laboratory with increased implantation rates, all the materials and media used in the laboratory needs to be tested on a regular basis to observe their effect on embryo development. Each laboratory has their bioassay system for QC.

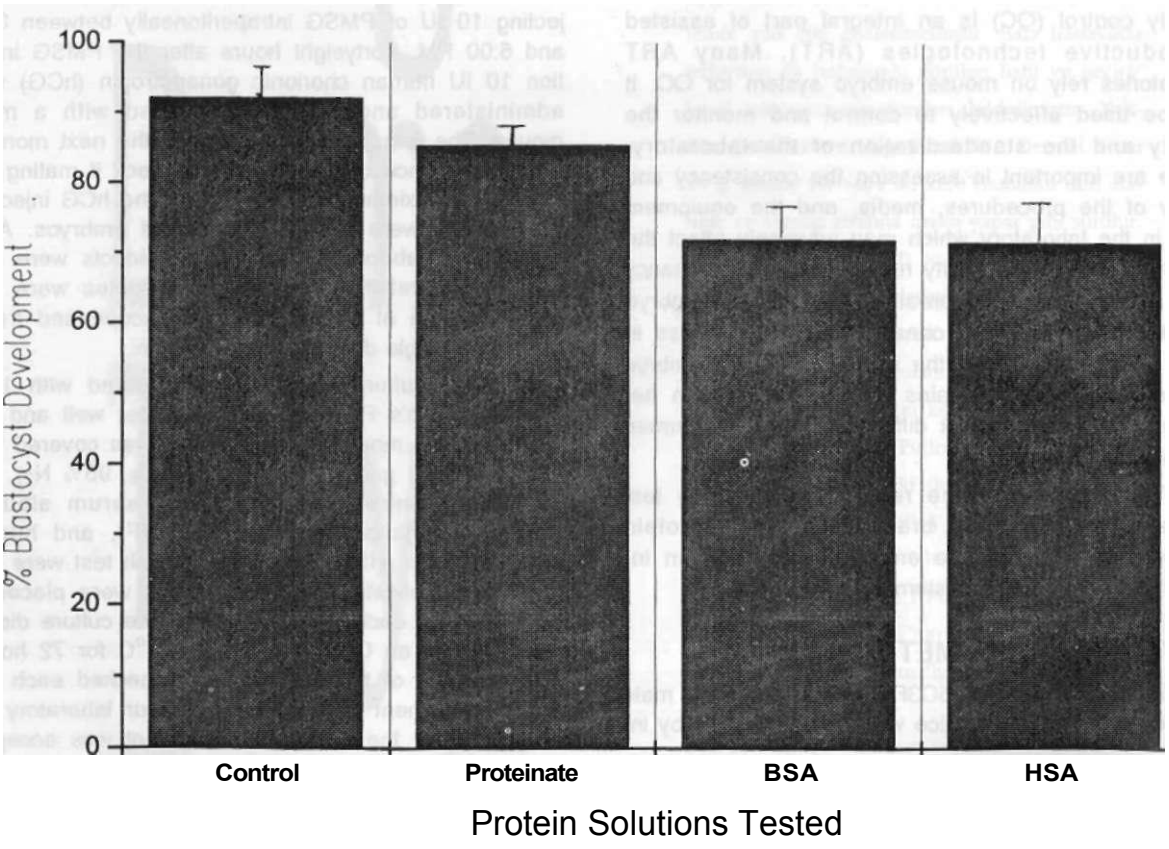

Figure 1. Mean percentage of extended blastocyst development with different protein solutions compared to controls (MeaniSE)