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In Vitro Bovine Embryo Production: The Role of FCS and Cysteamine on Cleavage Rate

İn Vitro Sığır Embriyo Üretimi: FCS ve Sisteaminin Bölünme Hızındaki Rolü

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Öz

Giriş ve Amaç: Bu çalışmanın amacı Sisteamin ve Fetal Buzağı Serumunun sığır embriyosu üretiminde bölünme oranları üzerindeki etkilerini araştırmaktır.
Gereç ve Yöntemler: Oositler, mezbahadan getirilen yumurtalıklardan doğrama yöntemi ile elde edilmiştir. Seçilen oositler iki gruba ayrılmış ve 24 saat olgunlaştırılmıştır. Olgunlaşan oositler 18-22 saat boyunca fertilizasyon medyumuna aktarılmıştır. Bu sürenin sonunda her gruba ait embriyolar iki farklı kültür medyumuna transfer edilmiştir. Bu amaçla 8 deney grubu oluşturulmuştur. Kültür döneminin başlangıcından itibaren 48. saat sonra embriyoların yarıklanma kontrolleri yapılmış ve kaydedilmiştir.
Bulgular: Çalışmanın sonuçları istatistiksel olarak değerlendirildiğinde, tüm gruplar arasında fark bulunmamıştır ($p<0.05$).
Sonuç: Fetal Buzağı Serumu ve Sisteamin kullanımının sığır embriyosu yarıklanma oranları üzerine etkisinin olmadığı görülmüştür.
Anahtar Kelimeler: Sığır, yarıklanma, sisteamin, embriyo, fetal buzağı serumu

Abstract

Objective: The aim of this study was to investigate the effects of Cysteamine and Fetal Calf Serum on cleavage rates in bovine embryo production.
Materials and Methods: The oocytes were obtained from the slaughtered ovaries by slicing method. They were selected and divided into two groups and maturated for 24 hours. The matured oocytes were transferred to fertilization media for 18-22 hours. At the end of this period, the embryos of each group transferred to two different culture media. For this reason 8 experimental groups were formed. After 48th hour from the beginning of the culture period, cleavage controls of the embryos were done and recorded
Results: When the results of the study were evaluated statistically, no difference was found ($p<0.05$) between all groups.
Conclusion: There was no effect of using Fetal Calf Serum and Cysteamine on bovine embryo cleavage rates.
Keywords: Bovine, cleavage, cysteamine, embryo, fetal calf serum

1. Introduction

Atmospheric oxygen pressure is routinely used in in vitro embryo production of mammalian species, but this high pressure causes reactive oxygen species (ROS) during in vitro embryo culture [1]. The known harmful effects of ROS include DNA damage, lipid peroxidation and oxidative modifications of proteins, and fusion inhibition of oocytes with spermatozoon [2]. In addition to these known adverse effects, ROS can be described as physiological in some conditions, such as an important factor of programmed cell death (apoptosis) [3]. Reactive oxygen species can originate directly from male and female gametes or embryonic developmental embryos, as well as completely environmental conditions. The most important endogenous source of ROS is oxidative phosphorylation. Inhibition of oxidative phosphorylation

reduces ROS production and has a positive effect on in vitro embryo development [4]. The most important exogenous factor that increases ROS production is oxygen pressure. The oxygen pressure in the oviduct is only ¼ of the atmospheric oxygen pressure. It is reported that in in vitro produced cattle embryos under low oxygen pressure (5-7%) synthesis of non-protein sulfhydryl compound of glutathione (GSH) and the resistance against freezing can be increased [5]. Antioxidants such as β-mercaptoethanol, cysteamine, cystine, cysteine, N-acetyl-L-cysteine (NAC) and superoxide dismutase (SOD), have been used in in vitro bovine embryo production to protect embryos against oxidative stress [6].

Proteins, especially essential amino acids that cannot be synthesized by the body, have an important role in embryo development [7]. Protein sources such as L-glutamine, fetal calf serum, fetal bovine serum and bovine serum albumin, which are used in in vitro embryo production, have been reported to be useful in the implantation stage of embryo [8]. Some researchers defend that proteins do not have a positive effect in the stage of reaching to blastocyst [9]. In particular, ammonia released as a result of the metabolism of glutamine has a negative effect on the embryo [10]. The most important effect of ammonia is that it is one of the main factors that constitute the formation of "large offspring syndrome" [11]. Therefore, some researchers reported that the regeneration of culture media using glutamine by changing every two days during the culture period optimizes the rate of embryo development [11]. However, it is stated that media refreshing eliminates metabolic residues as well as altering the ambient pH and gas pressure, thus eliminating factors that support development of the embryo [12].

Considering this information; the aim of this study was to investigate the effect of Cysteamine and Fetal Calf Serum in the production of bovine embryos in vitro up to the cleavage stage.

2. Material and Method

2.1 Oocyte recovery and selection

Ovaries were transferred to the laboratory within 2-3 hours from Tuzla slaughterhouse in Dulbecco's phosphate buffered salt solution (D-PBS / FTTS) added to antibiotics at 30-35 °C. Oocytes were obtained from 2-8 mm follicles on ovaries by slicing method. Oocytes washed in petri dishes and searched under a microscope with the properties of smooth cell wall, homogenous cytoplasm, smooth and undamaged zona pellucidas, and covered in a compact manner with at least 3-4 rows of cumulus ooforus cells were matured according to these criteria. The study was repeated 17 times in total.

2.2 Maturation of the oocytes

The selected oocytes were washed 3 times in Hepes TCM-199 medium with or without 100 µM cysteamine and then washed in pre-gassed maturation media and transferred into four well petri dishes containing 700 µL IVM medium to each well of 20-30 oocytes were allowed to be matured in an incubator containing 5% CO2 at 38.8° C for 24 hours.

2.3 Fertilization of oocytes

After maturation, the oocytes that have expanded cumulus ooforus cells were accepted as matured and transferred to the pre-gassed in vitro fertilization medium (IVF-TALP) to allow them to get used to the new media until the time of fertilization. For fertilization, frozen 0,25 µL semen straws which were belonging to the two Holstein bulls (culture breed) were melted at 37 °C for 30 sec; in the post-melting motility examination at least 45-60% motile sperm were determined and the concentration of them was calculated by the thoma slide. The contents of the straws were transferred into 14 mL conical tubes containing 4 mL sperm-TALP medium to be washed. After centrifugation at 1500 g for 5 min the supernatant was discarded and the same amount of medium was added to complete over 4 mL of the remaining semen and centrifuged again for 5 minutes at 1500 g. After the last centrifugation, the supernatant was discarded without stirring the bottom residue and was completed with the same medium to the 0,5 mL. 400-600x103 washed spermatozoa were added with the help of automatic pipette to each well of four well dishes containing pre-gassed IVF-TALP medium and matured oocytes in which in vitro fertilization will take place. The mobility of oocytes and spermatozoon was then checked under a microscope and allowed to fertilize for 18-22 hours in an incubator containing 5% CO2 at 38.8 °C.

2.4 Transferring the fertilized oocytes to culture medium

The oocytes thought to be fertilized were vortexed separately for each experimental group. The reason of vortexing in this stage is to remove the spermatozoa and the cumulus cells around the oocytes. TCM-199 medium with Hepes was used for mixing. After mixing, the contents were poured into a 35 mm empty petri dish and embryo selection was performed under a stereo microscope. The selected embryos were washed three times in Hepes TCM-199 medium and once in SOF and CR1 media. The embryos that were thought to be fertilized were cultured for 48 hours in an incubator containing 5% CO2, 5% N2 and 90% humidity at 38.8 °C in embryo culture medium that was gassed in the incubator of 20-30 embryos per drop. Eight groups were formed as Group A1: (SOF C + F +), Group A2: (SOF C + F-), Group A3: (SOF C-F +), Group A4: (SOF C-F-), Group B1: (CR1 C + F +), Group B2: (CR1 C + F-), Group B3: (CR1 C-F +), Group B4: (CR1 C-F-). 5% FCS was used in the serum added groups.

2.5 Cleavage control of the embryos

At the end of the 48th hour from the beginning of the culture period, cleavage controls of the embryos were done and recorded.

2.6. Statistical analysis

Chi-square test in SPSS 13.0 package software was used for statistical analysis. p-value<0.05 was accepted statistically significant.

3. Results and Discussion

At the end of the study when we evaluated all of the groups' cleavage rates the best result was achieved with 69,9% in Group B2 (CR1 C+F-) and this result is followed by Group A2 (SOF C+F- 67,8%). The lowest group was SOF C-F+ with 57,8%. Similarly, CR1 C-F+ group shows low cleavage rate with 58,2% (Table 1).

Table 1. Cleavage rates of the embryos.

Groups	Cultured Embryos	Cleavage of Embryos	Cleavage Rates (%)
Group A1 (SOFC+F+)	144	90	62.5
Group A2 SOF C+F-)	115	78	67,8
Group A3 (SOF C-F+)	116	67	57,8
Group A4 (SOF C-F-)	120	78	65
Group B1 (CR1C+F+)	152	93	61,2
Group B2 (CR1 C+F-)	123	86	69,9
Group B3 (CR1 C-F+)	134	78	58,2
Group B4 (CR1 C-F-)	115	67	58,3

When the other experimental groups were examined, although there was a percentage difference between the cleavage rates, no statistically significant difference was found. Sovernigo et al. [13] evaluated the effects of different antioxidants including Cysteamine, Quercetin, Vitamin C, Resveratrol and Carnitine on embryo development in a study and found that they did not affect the rate of cleavage and reaching to blastocyst stage. These results are parallel with the study's results of Sandal and Özdaş [14] and Enginler et al. [15] reported that the addition of 100 µM cysteamine did not cause a statistical difference in cleavage rates. Although many studies have shown that FCS has a positive effect on embryo development, it is also known that large offspring syndrome cases are frequently encountered in calves born as a result of the transfer of embryos developed in high-protein media [16]. Therefore, it is preferred to use serum-free media in recent study [17]. Some researchers defend that the use of FCS in the in vitro culture stage is responsible for high lipid accumulation in blastocysts which leads poor quality embryos [18]. Similarly, as a result of metabolism of proteins, ammonia which is a toxic residue; causes increase Reactive Oxygen Species in the medium. The lowest cleavage rates we obtained in our study, regardless of the culture medium used in the serum and cysteamine-free groups (Group A3 and Group B3) support this view [11]. Some of them; as the growth supplement in mammalian in vitro cell culture, alternative protein sources replace the risk of possible pathogens such as Bovine Viral Diarrhea, Bovine Spongioform Encephalopathy, which can be transmitted from sera by replacing FCS. However, there is also evidence that the addition of low concentration of FCS to the culture medium increases cryotolerance [19].

4. Conclusion

The results obtained from experimental studies will support each other if Cysteamine, an antioxidant substance used in vitro production of bovine embryos, and fetal calf serum, which is a protein source, are used in combination.

In conclusion, this study suggests that Cysteamine and FCS used in different culture media such as SOF and CR1 have no effect on the cleavage rate of the embryos.

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