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RESEARCH ARTICLE

The Effects of Various Protein Supplementations on *In Vitro* Maturation of Cat Oocytes [1]

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Summary

This study has been conducted to evaluate the effects of certain media containing various protein sources on the in vitro maturation (IVM) of cat oocytes. In this study 1013 oocytes obtained from 40 cats underwent ovario-hysterectomy surgeries for the purposes of spaying were used. Ovaries were brought to the laboratory in Dulbecco's PBS at 25-30°C. The recovered oocytes were left to mature for 48 hours in Ham's F-10 media supplemented with different protein sources. In this study four experimental groups were designed: Group I: Fetal Calf Serum (FCS), Group II: Bovine Serum Albumin (BSA), Group III: Estrus Cat Serum (OCS), Group IV: Control (No protein supplementation). The incubator conditions were 38.5°C temperature, almost 100% humidity and a gaseous mixture (5% O_2, 5% CO_2, 90% N_2). At the end of the maturation period, the oocytes were fixed and stained. The maturation rates were determined under 400 x magnification on a phase-contrast microscope. Metaphase II (M II) rates were 6.2% (14/255), 16.5% (43/260), 13.1% (36/273) and 13.3% (30/225) in Groups I, II, III and IV, respectively. The maturation rate in the Group I where FCS was used as protein additive was significantly lower ($P<0.001$) than other groups. The ratio of oocytes reaching M I+M II stages was 28.6% (73/255), 40.3% (105/260), 31.1% (85/273) and 30.2% (68/225), respectively. The difference in the Group II (BSA added), was significantly superior to the other groups ($P<0.05$). In conclusion, although homologous protein source OCS was ineffective and FCS has negative effects, it was determined that using BSA as the protein additive for the medium will be beneficial for in vitro maturation of cat oocytes.

Keywords: *Cat, Oocyte, In vitro maturation, Protein supplementation*

Kedi Oositlerinin *İn Vitro* Olgunlaştırılması Üzerine Değişik Protein Katkılarının Etkileri

Özet

Bu çalışma, medyuma katılan değişik protein kaynaklarının kedi oositlerinin in vitro olgunlaştırılması üzerine etkilerini araştırmak amacıyla yapıldı. Çalışmada, kısırlaştırma operasyonu uygulanmış 40 kediden kazanılan 1013 oosit kullanıldı. Ovaryumlar, 25-30°C sıcaklıktaki PBS solüsyonu içerisinde laboratuvara taşındı. Kazanılan oositler, farklı protein kaynaklarını içeren Ham's F-10 medyumu içerisinde ve 38.5°C sıcaklık, %100'e yakın nem ve gaz karışımının (%5 O_2, %5 CO_2, %90 N_2) sağlandığı inkübatör ortamında 48 saat süreyle olgunlaşmaya bırakıldı. Sunulan çalışmada dört uygulama grubu oluşturuldu. Grup I: Fetal Buzağı Serumu (FCS), Grup II: Sığır Serum Albumini (BSA), Grup III: Östrustaki Kedi Serumu (OCS), Grup IV: Kontrol (protein katkısı yok). Olgunlaşma sürecinin sonunda, oositler fikse edilerek boyandı. Olgunlaşma oranları, faz - kontrast mikroskopta (x400) yapılan incelemelerle belirlendi. Metafaz II (M II) dönemine ulaşma oranları Grup I, II, III ve IV için sırasıyla %6.2 (14/255), %16.5 (43/260), %13.1 (36/273) ve %13.3 (30/225) olarak bulundu. Protein kaynağı olarak FCS'nin kullanıldığı Grup I deki olgunlaşma oranları, diğerlerinden oldukça düşük olarak bulundu ($P<0.001$). M I+M II' ye olgunlaşan oositlerin oranları ise sırasıyla %28.6 (73/255), %40.3 (105/260), %31.1 (85/273) ve %30.2 (68/225) olarak gerçekleşti. BSA içeren Grup II' deki olgunlaşma oranının diğer gruptakilerden önemli derecede yüksek olduğu saptandı ($P<0.05$). Sonuç olarak, homolog protein kaynağı olan OCS'un etkisiz olduğu ve FCS'un zararlı etkisinin bulunduğu gözlenmesine rağmen, medyuma BSA katılmasının kedi oositlerinin in vitro olgunlaştırma oranlarını artırabileceği kanısına varıldı.

Anahtar sözcükler: *Kedi, Oosit, In vitro olgunlaştırma, Protein katkısı*

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INTRODUCTION

The fact that wild cats comprise a significant part of the biological ecosystem and that the natural habitat of these species is rapidly deteriorating makes it mandatory to run biotechnological research on these species [1]. In recent years, increased interest in the domestic cats and dogs, both as companion animals and as model for rare carnivore species, has led to augmented research focusing on reproductive processes in this species [2-6]. Because of the enormous progress made with domestic cat artificial reproduction, the developed assisted reproductive technologies such as IVM, IVF and nuclear transfer will undoubtedly serve as important tools for strategic management of endangered species populations [7]. The aim of IVM effort which is the first step of IVF is to enable primary oocytes to complete the first meiotic division under favorable laboratory conditions to become secondary oocytes. This will only be possible by replacing the hormones, enzymes, minerals, energy and protein sources, as well as gaseous atmosphere, pH level and osmotic pressure found in the female genital canals; in other words, by achieving a lifelike *in vitro* environment [8].

Varying results ranging from 2% to 82% have been obtained from *in vitro* cat oocyte maturation studies [1,4,6-8]. Among the primary actors in this degree of variability are the nutritional condition of donors [9], the cyclic stage they are in [10-12], the quality of recovered oocytes [13], the transport and storage temperature of the ovaries [4,10,14], the proteins added to the medium [15] and types and quantities of hormones [16,17], as well as the type of medium used [18-20]. Accordingly, researchers have utilized many different media in their studies and have obtained various results [16-22]. The fact that IVM and in vitro fertilization efforts are a current issue and that the desired performance levels have not yet been reached, it requires specific attention to be paid to the studies in this field.

The present study has researched the effects of different protein additives such as FCS (Fetal Calf Serum), BSA (Bovine Serum Albumin) and OCS (Estrus Cat Serum) on the *in vitro* maturation of cat oocytes.

MATERIAL and METHODS

All chemicals and reagents were purchased from Sigma Chemical Company (St. Louis, MO, USA), unless otherwise indicated.

In order to obtain OCS to be used in the study, four street cats between the ages of 1 to 6 were taken to care in a home environment for 10 days. To improve their overall condition, the cats were fed ad libitum with dry food (Hill's Science Diet® Kitten Formula) developed for kittens and pregnant females and they were allowed continuous access to water. The environmental temperature was kept between 18 to 20°C, and the lighting cycle was adjusted to be 14 h of light followed by 10 h of darkness. Cats were taken to cages during dark hours, and left free in a 25 square meter area during light hours. Litters were cleaned and disinfected daily. Superovulation was stimulated by im application of PMSG (Synchroject, Vetimex). Application dosage was 100 IU on Day 0, 50 IU on Day 2, and 50 IU on Day 4. Within 2 to 4 days of the last application, clinical estrus symptoms were observed in three out of four cats. Following the sedation of these subjects with 0.3 mg/kg of acepromazine 1% (Vetranquil®, Sanofi), 6 to 7 ml blood samples were taken from their *V. jugularis*, centrifuged at 4000 to 5000 rpm, and the resulting serum was frozen at -20°C and stored until use.

Ovaries were obtained from 40 cats that underwent ovario-histerectomy for spaying purposes. The cats' ages ranged from 1 to 6, and weights were between 2.5 to 4.5 kilograms. Among the subjects, 12 were being cared for in houses, and 28 were street cats. Ovaries obtained from these cats were brought to the laboratory in a PBS solution at 25 to 30°C, their surfaces were sliced with a scalpel, washed with M2 medium, and the fluid was collected on a warm watch glasses. The criteria for the oocytes to be selected for *in vitro* maturation were an intact zona pellucida, a compact cumulus oophorus /corona radiata structure, and a homogeneous vitellus filling the zona pellucida.

The selected oocytes were passed through the washing medium three times, and then were taken to their relative maturation media after passed three more times. The media volume was 500 µl, and incubation conditions were 38°C temperature and a gaseous mixture of 5% CO_2, 5% O_2, 90% N_2. The oocytes were left to mature for 48 hours. All media were adjusted to have a pH of 7.3 and an osmolarity of 286 mOsm. Ten to 20 oocytes were placed in each well containing 500 µl medium.

Group I: Ham's F-10 + FSH (1 µl/mg, Sigma) + LH (1 µl/mg, Sigma) + FCS (5%, Biological Industries),

Group II: Ham's F-10 + FSH (1 µl/mg) + LH (1 µl/mg) + BSA (3 mg/ml),

Group III: Ham's F-10 + FSH (1 µl/mg) + LH (1 µl/mg) + OCS (5%),

Group IV (Control): Ham's F-10 + FSH (1 µl/mg) + LH (1 µl/mg).

Following the maturation process, the cumulus cells of oocytes were removed with 0.1% Hyaluronidase. Oocytes were kept in a 0.7% KCl solution for 2 to 3 min, taken between microscope slides, and fixed in a 1 : 3 acetic acid : ethanol solution. Following at least 24-30 h of fixation, oocytes were stained with 2% aceto-orcein and evaluated under phase-contrast microscope (x400).

Chi-square test was applied in SPSS 13.0 program to compare the developmental stages of oocytes *in vitro*.

RESULTS

Maturation results are summarized in *Table 1*. A total of 1013 oocytes (Group I= 255, Group II= 260, Group III= 273 and Group IV= 225 oocytes) were used in the study. The meiotic resumption rates (D+MI+MII) of the oocytes were 76.1% (194/255), 75.8% (197/260), 63.7% (174/273) and 70.7% (159/225), respectively in groups I, II, III and IV.

The rate of oocytes matured (MI+MII) in BSA group was significantly higher than the oocytes in the other all groups (P<0.001). However, there were no differences among oocytes matured in FCS, OCS and control groups (P>0.05).

Table 1. *The in vitro developmental competence of the cat oocytes matured with different protein sources*
Tablo 1. *Medyuma farklı protein kaynakları katılarak olgunlaştırılan kedi oositlerinin in vitro gelişim düzeyleri*

Group	Oocyte Number	UDNM (%)	GV (%)	D (%)	M I (%)	M II (%)	M I+M II (%)
FCS	255	53 (20.8)	8 (3.1)	121 (47.4)	59 (23.1)	14 (5.4)[b]	73 (28.6)[d]
BSA	260	46 (17.7)	17 (6.5)	92 (35.3)	62 (23.8)	43 (16.5)[a]	105 (40.3)[c]
OCS	273	83 (30.4)	16 (5.8)	89 (32.6)	49 (17.9)	36 (13.1)[a]	85 (31.1)[d]
Control	225	51 (22.7)	15 (6.6)	91 (40.4)	38 (16.8)	30 (13.3)[a]	68 (30.2)[d]

The differences between numbers having different letter in a same column are significant. a,b: P<0.001; c,d: P<0.05
UDNM: Undetermined nuclear material, GV: Germinal vesicle, D: Diakinesis, M I: Metaphase I, M II: Metaphase II

DISCUSSION

Three important observations were made in this study. At first, protein supplementation was not necessary for cat oocyte maturation *in vitro*. Second, meiotic nuclear maturation was clearly inhibited when oocytes were cultured in the presence of FCS, and third, the addition of 3 mg/ml BSA into the maturation medium can be beneficial in reaching to the M II stage.

Karja et al.[6] have used 0.4% BSA added TCM 199 medium in a similar study and determined that 59.1% of oocytes continued meiosis. Similarly, Evecen et al.[21] who used 0.4% BSA added Ham's F-10 have reported a meiosis resumption ratio of 60.89%. In the present study, the meiotic resumption ratios in all groups were higher than that obtained by the scientists. In another study [16] where 5% FCS added TCM 199 was used a meiotic oocyte ratio has reported of 31.55%, which is much lower than the ratio obtained in all groups of the present study. The important factor in this difference is thought to be the use of different media (TCM 199 and Ham's F 10).

In a study [5] DMEM (Dulbecco's Modified Eagle Medium) and 0.4% BSA protein additive were used, and the ratios of M II oocytes after 32 to 34 h of incubation were reported to be 65.5%. In another study [20] where SOF medium was used, the ratio of M II oocytes following 40 h of incubation were reported to be 82.8%. This is the highest ratio among all studies. Considering that most studies used the same percentage of BSA as the protein additive, the differences can be explained by the medium changes [16,21].

Katska et al.[23] have reported that the ratio of oocytes reached to the M II stage in 24 h was 40% in a study where 0.4% BSA added SOF medium was used and that this was higher than the obtained from the group using TCM 199 medium (29.7%) in the same study. The reported M II ratio, although being higher than the TCM 199 medium group, remains inferior to the results of other studies where SOF medium were used. Possible reasons could include the reproductive stage of the donor [10-12], nutritional conditions, seasonal and climactic differences [2,9,12,21].

Goodrowe et al.[22] found that BSA had more beneficial effects on IVM of cat oocytes compared to OCS. Our results were parallel with these findings and BSA added group was superior to all the other groups. Moreover, it was found that BSA had more beneficial effects than FCS on maturation of cat's [15] and dogs' oocytes [24] *in vitro.* According to our findings, it was interesting that control group oocytes were matured more successfully than the FCS group oocytes. Our findings

supported the claim that although serum factors can promote egg maturation *in vitro*, there are species specificities, and in cats, it is probable that FCS contains inhibitory elements [15].

In conclusion, although it was found that protein supplementation was not necessarily for cat oocyte maturation *in vitro*, it can be suggested that the use of 3 mg/ml BSA to the medium for *in vitro* maturation of cat oocytes will be a supportive factor in more oocytes reaching to the M II stage. However, homologous protein source OCS as a protein additive has ineffective and FCS has negative effect on reaching to the M II stage

REFERENCES

1. Pope CE: Embryo technology in conservation efforts for endangered felids. *Theriogenology*, 53, 163-174, 2000.

2. Evecen M, Cirit Ü, Demir K, Özdaş ÖB, Taş M, Birler S, Pabuccuoğlu S: Effects of estrous cycle stage and transport temperature of ovaries on *in vitro* maturation of canine oocytes. *Anim Reprod Sci,* 117, 160-165, 2010.

3. Evecen M, Cirit Ü, Demir K, Karaman E, Bakırer G, Hamzaoğlu Aİ, Alkan S, Birler S, Pabuccuoğlu S: Comparison of two different media for *in vitro* production of dog embryos. *Kafkas Univ Vet Fak Derg,* 16 (6): 897-902, 2010.

4. Taş M, Evecen M, Özdaş ÖB, Cirit Ü,Demir K, Birler S, Pabuccuoğlu S: Effect of transport and storage temperature, of ovaries on *in vitro* maturation of bitch oocytes. *Anim Reprod Sci,* 96, 30-34, 2006.

5. Yindee K, Saikhun J, Pavasuthipaisit K: Somatic cell nuclear transfer in domestic cat oocytes treated with IGF-I for *in vitro* maturation. *Theriogenology*, 59, 1775-1786, 2003.

6. Karja NWK, Otoi T, Murakami M, Fahrudin M, Suzuki T: *In vitro* maturation, fertilization and development of domestic cat oocytes recovered from ovaries collected at three stages of the reproductive cycle. *Theriogenology*, 57, 2289-2298, 2002.

7. Goodrowe KL: Cat reproduction and artificial breeding technologies. *Anim Reprod Sci,* 28, 389-397, 1992.

8. Farstad W: Current state in biotechnology in canine and cat reproduction. *Anim Reprod Sci*, 60-61, 375-387, 2000.

9. Robinson JJ: Nutrition and reproduction. *Anim Reprod Sci,* 42, 25-34, 1996.

10. Johnston LA, O'Brien SJ, Wildt DE: *In vitro* maturation and fertilization of domestic cat follicular oocytes. *Gam Res*, 24, 343-356, 1989.

11. Freistedt P, Stojkovic M, Wolf E: Efficient in vitro production of cat embryos in modified synthetic oviduct fluid medium: Effects of season and ovarian status. *Biol Reprod,* 65, 9-13, 2001.

12. Evecen M, Özdaş ÖB, Pabuccuoğlu S, Birler S, Tek Ç, İleri İK: The effects reproductive status of queens on in vitro maturation of cat oocytes. *Indian Vet J,* 81, 1005-1009**,** 2004.

13. Pope CE, McRae MA, Plair BL, Keller GL, Dresser BL: In vitro and *in vivo* development of embryos produced by *in vitro* maturation and *in vitro* fertilization of cat oocytes. *J Reprod Fert,* 51, 69-82, 1997.

14. Evecen M, Cirit Ü, Demir K, Karaman E, Hamzaoğlu Aİ, Bakırer G: Developmental competence of domestic cat oocytes from ovaries stored at various durations at 4°C temperature. *Anim Reprod Sci,* 116**,** 169-172, 2009.

15. Wood TC, Byers AP, Jannette BD, Wildt DE: Influence of protein and hormone supplementation on in vitro maturation and fertilization of domestic cat eggs. *J Reprod Fert,* 104, 315-323, 1995.

16. Evecen, M, Baran A, Tek Ç: The effects of gonadotrophins on *in vitro* maturation of cat oocytes. *Tr J Vet Anim Sci*, 26, 1315-1320, 2002.

17. Schramm RD, Bavister BD: Effects of gonadotrophins, growth hormone and prolactin in developmental competence of domestic cat oocytes matured *in vitro*. *Reprod Fertil Dev*, 7, 1061-1066, 1995.

18. Evecen M, Baran A, Alkan S, Özdaş ÖB, Tek Ç: The effects of BSA and FCS on *in vitro* maturation of bitch oocytes. *Tr J Vet Anim Sci,* 26, 1453-1458, 2002.

19. Evecen M, Şahin BE, Baran A, Ak K, İleri İK: *In vitro* fertilization of cat oocytes. *J Fac Vet Univ Istanbul,* 29, 253-258, 2003.

20. Bogliolo L, Leoni G, Ledda S, Naitana S, Zedda M, Carluccio A, Pau S: Intracytoplasmic sperm injection of *in vitro* matured oocytes of domestic cats with frozen-thawed epididymal spermatozoa. *Theriogenology,* 56, 955-967, 2001.

21. Evecen M, Atalla H, Birler S, Baran A, Ak K: Studies on the *in vitro* maturation of cat oocytes. *Tr J Vet Anim Sci*, 27, 1409-1412, 2003.

22. Goodrowe KL, Hay M, King WA. Nuclear maturation of domestic cat ovarian oocytes *in vitro. Biol Reprod,* 45, 466-470, 1991.

23. Katska L, Rynska B, Kania G, Smorag S, Gajda B, Pienkowski M: Timing nuclear maturation of nonstored and stored domestic cat oocytes. *Theriogenology*, 59, 1567-1574, 2003.

24. Evecen M, Baran A, Alkan S, Özdaş ÖB, Tek Ç: The effects of BSA and FCS on *in vitro* maturation of bitch oocytes. *Tr J Vet Anim Sci,* 26, 1453-1458, 2002.