İstanbul Üniv. Vet. Fak. Derg.
34 (2), 1-5, 2008

J. Fac. Vet. Med. Istanbul Univ.
34 (2), 1-5, 2008

**Araştırma Makalesi**

# PARTHENOGENETİC ACTIVATION OF SHEEP OOCYTES

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Geliş Tarihi : 17.01.2008
Kabul Tarihi : 13.02.2008

## Koyun Oositlerinin Partenogenetik Aktivasyonu

**Özet:** Koyunlarda somatik ve embriyonik hücre nüklear transfer yöntemlerini laboratuvarımızda başlatabilmek için, iki farklı partenogenetik aktivasyon protokolü denendi. Oositler mezbahada kesilen koyunların ovaryumlarından elde edildi ve piruvat, hormonlar (FSH ve LH) ve %10 FCS katkılı medyum 199 içerisinde 24 saat olgunlaştırıldı. Olgunlaştırılan oositlere 80 µsec süreyle 1.25 kV/cm elektrik akımı verildi ve oositlerin tamamı önce 5 dk ionomisin içerisinde, daha sonra ise 30 dk sitokalasin B içerisinde bırakıldı. İki gruba rastgele ayrılan oositler, 1. grupta 6-DMAP'lı medyumda 2 saat, 2. grupta ise sikloheksimid'li medyumda 3 saat inkübe edildikten sonra kültür medyumuna aktarıldı. Grup I ve II'de elde edilen embriyolarda yarıklanma oranları sırasıyla %82.7 ve %81.3 olurken blastosist oranları %2.3 ve %0 olarak bulundu (P>0.05). Sunulan çalışma koyunların klonlanması konusunda önemli aşamalardan birini oluşturan partenogenetik aktivasyon konusunda ülkemizde gerçekleştirilmiş ilk çalışmadır.

**Anahtar Kelimeler:** Koyun, Oosit, Partenogenetik aktivasyon

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**Summary:** In order to establish somatic- and embryonic-cell nuclear transfer procedures in sheep in our laboratory, two activation protocols for parthenogenetic activation of sheep oocytes were tested. Oocytes obtained from ovaries of slaughtered ewes were matured for 24 h in medium 199 supplemented with pyruvate, hormones (FSH and LH) and 10% FCS. After maturation, all oocytes were exposed to single electrical stimulation of 1.25 kV/cm for 80 µsec, then placed into ionomycine and cytochalasin B for 5 min and 30 min respectively, and then divided into 2 groups randomly. While oocytes in Group I were incubated in 6-dimethylaminopurine (6-DMAP) for 2 h, oocytes in Group II were kept in cycloheximide (CHX) for 3 h before transferring into culture medium. Cleavage and blastocyst rates in groups I and II were 82.7% and 81.3%, and 2.3% and 0% respectively (P>0.05). These are our preliminary results for the parthenogenetic development of sheep oocytes in Turkey and represent promising results in the direction to achieve sheep cloning.

**Key Words:** Parthenogenetic Activation, Sheep, Oocyte

## Introduction

Since the birth of the first mammalian cloned by somatic-cell transfer, Dolly, **(15)** there have been very intensive studies in this area. In several species including cow **(7, 14)**, sheep **(2, 10, 11)**, pig **(12)**, horse **(8)**, cat **(6)**, dog **(9)**, rabbit **(5)** and mouse **(13)**, cloned offspring have been obtained by somatic cell nuclear transfer.

Activation of oocytes is an extremely important step of cloning studies. Different techniques are used for activation purposes and since the success rate developed to offspring is still lower than other *in vitro* studies further experiments are required.

In sheep, the most often used activators are electrical stimuli (ES) and different chemical reagents, including calcium ionophore (CI), cycloheximide (CHX) and 6-dimethylaminopurine (6-DMAP). Although blastocyst rate of parthenotes is slightly greater than cloned embryos, no offspring from parthenogenetic embryos is obtained in livestock because of the chromosomal abnormalities.

In this study, we investigated the effects of two activation procedures (using CHX and 6-DMAP) on the development of parthenogenetically activated sheep oocytes.

## Materials and Methods

All chemicals were purchased from Sigma-Aldrich unless otherwise indicated.

**In Vitro Maturation (IVM) of Oocytes:** IVM of sheep oocytes was performed according to the procedures reported previously **(3, 4)**. Briefly, sheep ovaries were obtained from a local abattoir and brought to the laboratory in phosphate bufferred saline (PBS) at 30$^\circ$C in a vacuum flask. After slicing and washing follicles with oocyte

washing medium, cumulus-oocyte complexes (COCs) were chosen regarding their appearance. Only oocytes with homogeneous vitellus and at least 4 layers of cumulus cells were chosen for maturation and matured for 24 h in medium 199 supplemented with 0.1 mg/ml pyruvate, hormones (1 µg/ml FSH and 1 µg/ml LH) and 10% FCS (Biological Industries), in a humidified atmosphere at 38.5 °C, and 5% $CO_2$ in air.

**Parthenogenetic Activation:** After denudations of oocytes with hyaluronidase by pipetting, oocytes were transferred into mannitol based fusion medium in hepes buffered synthetic oviduct fluid (HSOF, 1:1) for 30 min and immediately after into fusion chamber filled with fusion medium. Oocytes were exposed to single electrical stimulation of 1.25 kV/cm for 80 µsec using electroporator (BTX, ECM 830, Harvard Aparatus, USA). After washing in HSOF medium, oocytes were placed into 5 µM ionomycine in HSOF for 5 min and cytochalasin B for 30 min, respectively, and randomly divided into 2 groups. While oocytes in Group I were incubated in 6-dimethylaminopurine (6-DMAP) for 2 h, oocytes in Group II were kept in cycloheximide (CHX) for 3 h before transferring into culture medium. The oocytes were cultured in SOF medium in the humidified atmosphere of 5% $O_2$, 5% $CO_2$ and 90% $N_2$ at 38.5°C. After 72 h of culture, cleavage rates were recorded and cleaved embryos were transferred into SOF medium supplemented with 2.4 mM glucose and cultured for 4 days.

**Statistical analyses:** Data were analysed by chi-square test using the SPSS program v. 10.0 for windows (SPSS Inc, Chicago, IL).

## Results

In this experiment 200 oocytes were obtained from the ovaries and 127 of them having first polar body were chosen for parthenogenesis. Cleavage rates of activated oocytes in Groups I and II were 82.7% (43/52) and 81.3% (61/75), respectively (P>0.05). After 7 days of culture, the rates of embryos developed to blastocyst stage in Groups I and II were 2.3% and 0%, respectively. Results are shown in Table 1.

**Table 1:** Development of parthenogenetic sheep embryos
**Tablo 1:** Partenogenetik koyun embriyolarının gelişimi

| | Oocyte Number | Cleavage (%) | Blastocyst (%)* |
|---|---|---|---|
| Group I (6-DMAP) | 52 | 43 (82.7) | 1 (2.3) |
| Group II (CHX) | 75 | 61 (81.3) | 0 (0.0) |
| P value | - | >0.05 | >0.05 |



* From cleaved embryos

## Discussion

In this study, the first embryos developed up to the blastocyst stage were obtained after parthenogenetic activation of sheep oocytes in Turkey. After parthenogenetic activation of sheep oocytes, different cleavage (80-83%) and blastocyst (15-21% *in vitro* and 19-58% *in vivo*) development results have been declared in several studies **(2)**. In this study, high rates of cleavage were obtained after both activation treatments and the difference between groups were insignificant.

Embryo development rates after parthenogenetic activation and nuclear transfer (NT) procedures are influenced from different conditions like as source of oocytes (*in vitro* or *in vivo* matured), source of karyoplasts (for NT only), activation type (chemical or electrical) and activation protocol. For activation treatment of oocytes, some kinase inhibitors (like as 6-DMAP) and protein synthesis inhibitors (like as CHX) are usually used after Ca ionophore **(1, 2)**. Although these treatments are accepted equally successful, Alexander et al **(2)** reported 21% and 14.9% blastocyst rates after using 6-DMAP and CHX treatments respectively for parthenogenetic activation of sheep oocytes. In this study, only one oocyte was reached to the blastocyst stage in 6-DMAP group. This lower blastocyst yield in the present study could be related to the *in vitro* culture conditions in our laboratory.

For the first time, *in vitro* matured sheep oocytes reached to the blastocyst stage after parthenogenetic activation in Turkey. Although blastocyst rate was very low when compared with cleavage rates, this study demonstrated that activation protocols with 6-DMAP and CHX are useful for activation of sheep oocytes. Further studies are needed to improve blastocyst rates.

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