EFFECTS OF SERUM AND HORMONE ADDITIONS TO MATURATION MEDIUM ON IN VITRO MATURATION OF SHEEP OOCYTES

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Koyun Oositlerinin In Vitro Olgunlaştırılmasında Medyuma İlave Edilen Serum Ve Hormonların Etkileri

Özet: Olgunlaşma medyumuna ilave edilen serum ve hormonların koyun oositlerinin in vitro olgunlaştırılması üzerine etkilerini araştırmak amacıyla gerçekleştirilen bu çalışmada, mez-baha materyalinden elde edilen primer oositler (n=205) 5 gruba ayrıldı.

Grup 1: %20 oranında koyun serumu (SES), 10μg/ml FSH, 10μg/ml LH ve 1μg/ml estradiol 17β ilaveli TCM 199,

Grup 2: % 20 oranında fotal buzağı serumu (FCS), 10μg/ml FSH, 10μg/ml LH ve 1μg/ml estradiol 17β ilaveli TCM 199,

Grup 3: %20 oranında SES ilaveli TCM 199,

Grup 4: 10μg/ml FSH, 10μg/ml LH ve 1μg/ml estradiol 17β ilaveli TCM 199, ve

Grup 5: hormon ve serum ilavesi olmayan TCM 199 medyumları kullanıldı.

26 saat in vitro olgunlaştırımı takiben gruplara göre sırasıyla 27 (%67.5), 23 (%57.5), 26 (%63.4), 18 (%50.0) ve 21 (%43.8) oosit metafaz II (M II) dönemine ulaştı. Gruplar arasındaki farklar istatistiksel açıdan anlamli bulunmadı (p>0.05).

Anahtar Kelimeler: Koyun, in vitro olgunlaştırma, serum ve hormon ilaveleri.

* This work was supported by the Research Fund of The University of Istanbul. Project number: 753/280795.
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Summary: Primer oocytes collected from the ovaries of slaughtered ewes divided into 5 groups and matured in vitro in different supplementations of sera and hormones.

Group 1: TCM 199 medium supplemented with 20% sheep estrous serum (SES), 10μg/ml FSH, 10μg/ml LH and 1μg/ml estradiol 17β,

Group 2: TCM 199 medium supplemented with 20% foetal calf serum (FCS), 10μg/ml FSH, 10μg/ml LH and 1μg/ml estradiol 17β,

Group 3: TCM 199 medium supplemented with 20% SES,

Group 4: TCM 199 medium supplemented with 10μg/ml FSH, 10μg/ml LH and 1μg/ml estradiol 17β, and

Group 5: TCM 199 medium only.

After 26 hours incubation, oocytes reached to M II stage were 27 (67.5%), 23 (57.5%), 26 (63.4%), 18 (50.0%) and 21 (43.8%), respectively according to maturation groups. Differences between maturation rates among the groups were not important statistically (p>0.05).

Key Words: Sheep, in vitro maturation, serum and hormone additives.

Introduction

Turkey is one of the leading countries in sheep population, however the low rate of pure breeds and their hybrids and the poor yields of native breeds avoid satisfactory animal production. Currently, acceleration of genetic improvement and increase in animal production are possible by biotechnological methods. In in vitro fertilization which is one of these methods, collection of primary oocytes from slaughtered animals, maturation, fertilization and culture of these oocytes in vitro and transfer of in vitro fertilized embryos can be achieved. Therefore, thanks to the in vitro occurrence of these procedures, we can manipulate the oocytes and embryos at every stage for genetic improvement.

In most mammals, meiotic maturation is initiated near the time of birth but is not completed. The oocytes arrest at the prophase stage of meiosis I. If mammalian oocytes are removed from antral follicles and cultured in vitro, they will undergo meiotic maturation spontaneously in a hormone-independent manner (2).

In in vitro maturation of sheep oocytes tissue culture medium (TCM-199) is commonly used (3,4,6,7,8,9). Foetal Calf Serum (FCS) (1,5,6,7,9) or Sheep Estrous Serum (SES) (3,4,8,9) are usually added to the maturation medium.

Primer oocytes collected from slaughtered ewes were incubated in vitro under humidified 5% CO₂ in air at 38.5-39°C for 22-26 hours (1,3,4,5,6,7,8). After incubation, oocytes which extruded their first polar body (M II) were regarded as matured.

In this study, effects of adding hormones and sera to maturation media on in vitro maturation of sheep oocytes were investigated.
Material and Methods

Ovaries from slaughtered ewes were used. These ovaries transported in physiological saline (0.9% NaCl) at 30-35°C in a vacuum-flask. Time between the collection of the first ovary and culture did not exceed 4-5 hours.

Ovaries were washed with 0.9% NaCl at 30-35°C and left in this solution until used. Ovaries were then sliced and washed with 0.9% NaCl supplemented with 1% FCS in a watch glass. This fluid was examined under stereomicroscope and oocytes which had at least 4 layer of compact cumulus and homogen vitellus were selected for in vitro maturation. Selected oocytes were washed 3 times in 0.9% NaCl supplemented with 10% FCS and divided into 5 groups randomly.

Group 1: TCM 199 medium supplemented with 20% sheep estrous serum, 10μg/ml FSH, 10μg/ml LH and 1μg/ml estradiol 17β,

Group 2: TCM 199 medium supplemented with 20% foetal calf serum, 10μg/ml FSH, 10μg/ml LH and 1μg/ml estradiol 17β,

Group 3: TCM 199 medium supplemented with 20% sheep estrous serum,

Group 4: TCM 199 medium supplemented with 10μg/ml FSH, 10μg/ml LH and 1μg/ml estradiol 17β, and

Group 5: TCM 199 medium only.

Primary oocytes were incubated under humidified 5% CO₂ in air at 39°C for 26 hours. After maturation all of the oocytes were fixed in ethanol-acetic acid (at a ratio 3:1) for 24 hours, stained with 2% aceto-orcein and evaluated under phase-contrast microscope (x400). For evaluation, germinal vesicle break down (GVBD), M I and M II (after extrusion of the first polar body) were considered.

χ² analysis were used for statistical evaluations.

Results

In this study, total 205 oocytes were used. 40, 40, 41, 36 and 48 oocytes were contained in the experiment groups, respectively. Oocytes reached to metaphase stages (M I + M II) were 39 (97.5%), 39 (97.5%), 39 (95.1%), 34 (94.4%) and 45 (93.8%), respectively. Oocytes reached to M II stage were 27 (67.5%), 23 (57.5%), 26 (63.4%), 18 (50.0%) and 21 (43.8%), respectively (Table 1).
Table 1. Developmental stages of oocytes matured in TCM-199 with different supplantations.

<table>
<thead>
<tr>
<th>Maturation groups</th>
<th>N. of oocytes</th>
<th>N. of Degenerated oocytes</th>
<th>Germinal Vesicle (GV)</th>
<th>Metaphase I (%)</th>
<th>Metaphase II (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>40</td>
<td>-</td>
<td>1</td>
<td>12 (30.0)</td>
<td>27 (67.5)</td>
</tr>
<tr>
<td>Group 2</td>
<td>40</td>
<td>-</td>
<td>1</td>
<td>16 (40.0)</td>
<td>23 (57.5)</td>
</tr>
<tr>
<td>Group 3</td>
<td>41</td>
<td>-</td>
<td>2</td>
<td>13 (31.7)</td>
<td>26 (63.4)</td>
</tr>
<tr>
<td>Group 4</td>
<td>36</td>
<td>1</td>
<td>1</td>
<td>16 (44.4)</td>
<td>18 (50.0)</td>
</tr>
<tr>
<td>Group 5</td>
<td>48</td>
<td>-</td>
<td>3</td>
<td>24 (50.0)</td>
<td>21 (43.8)</td>
</tr>
</tbody>
</table>

Group 1: TCM 199 medium supplemented with 20% sheep estrous serum, 10μg/ml FSH, 10μg/ml LH and 1μg/ml estradiol 17β.

Group 2: TCM 199 medium supplemented with 20% foetal calf serum, 10μg/ml FSH, 10μg/ml LH and 1μg/ml estradiol 17β.

Group 3: TCM 199 medium supplemented with 20% sheep estrous serum.

Group 4: TCM 199 medium supplemented with 10μg/ml FSH, 10μg/ml LH and 1μg/ml estradiol 17β, and

Group 5: TCM 199 medium only.

Discussion

Among the treatment groups, while the highest maturation rate (67.5%) was achieved in Group 1 in which SES and hormones were used, the lowest maturation rate (43.8%) was observed in Group 5 in which media was not supplemented with hormones and sera. Differences among the groups were not important statistically (p>0.05).

The maturation rates of this study were lower than the rates of O’Brien et al. (5) who reported 100% maturation. However, the rate in the first group (67.5%) was close to maturation rates of Byrd et al. (1) and higher than the rates (47.4%) of Yadav et al. (9).

In this study, although supplementation of maturation media with hormones and 20% SES gave the best maturation rate, we suggest that the serum addition is necessarily obligatory but hormone addition can be ignored for in vitro maturation of sheep oocytes.

This conclusion of ours is in agreement with Downs (2) who pointed out that meiotic maturation occurs spontaneously in a hormone-independent manner if mammalian oocytes are removed from antral follicles and cultured in vitro.

Literature


