Dose dependent effect of pregnant mare serum gonadotropin and human chorionic gonadotropin on in vitro maturation of goat oocytes

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ABSTRACT

This study was aimed to evaluate the dose dependent effect of pregnant mare serum gonadotropin and human chorionic gonadotropin on in vitro maturation goat oocytes. The study was planned in 2 experiments. In the first experiment effect of pregnant mare serum gonadotropin supplementation on nuclear maturation of goat oocytes using 5 different concentrations of PMSG, was studied. The treatment was divided into group 1 (control medium containing TCM-199, sodium pyruvate (0.25mM), gentamycin (50µg/ml), L-glutamine (100µg/ml), estradiol 17-β (1µg/ml), BSA (3mg/ml), supplemented with 10% FBS); group 2 (control medium + 10 IU/ml PMSG); group 3 (control medium + 20 IU/ml PMSG); group 4 (control medium + 30 IU/ml PMSG); group 5 (control medium + 40 IU/ml PMSG); and group 6 (control medium + 50 IU/ml PMSG). The oocytes of these treatment groups were matured in maturation media for 27 h in humidified atmosphere of 5% CO₂ at 38.5°C in a CO₂ incubator. The maturation rates achieved in groups 1–6 were 20.0, 48.51, 74.63, 74.15, 75.0 and 76.33%, respectively. In experiment 2, the effect of hCG supplementation on nuclear maturation of goat oocytes using 3 different concentrations of PMSG, was studied. Second experiment was divided into group 1 (control medium + 20 IU/ml PMSG +10IU/ml hCG), group 2 (control medium + 20 IU/ml PMSG +20IU/ml hCG) and group 3 (control medium + 20 IU/ml PMSG +30IU/ml hCG). The maturation rates achieved in groups 1–3 were 77.0, 85.19 and 84.22%, respectively. It can be concluded that supplementation of 20 IU/ml PMSG and 20IU hCG in maturation media significantly increased the maturation rate.

Key words: Goats, hCG, In vitro maturation, Oocytes, PMSG

Small ruminants are good models for developing technologies like in vitro embryo production, transgenesis and cloning (Freitas et al. 2007). Selection of protein supplements and hormones such as follicle stimulating hormone (FSH) and luteinizing hormone (LH) for IVM medium is an important event in the subsequent in vitro fertilization (IVF) and embryonic development (Pawshe et al. 1996). Estradiol may be involved in ooplasmic maturation by stimulating DNA polymerase β and enhancing the synthesis of presumed male nucleus growth factors (Rahman et al. 2008). The beneficial effect of gonadotropins in the IVM medium proved to be more pronounced for the oocytes of pre-pubertal females (Ledda et al. 1997). Researchers are using FSH ranged from 0.1 µg/ml (Cognie et al. 2003) to 10 µg/ml (Jimenez-Macedo et al. 2007), LH 3 µg/ml (Ongeri et al. 2001) to 100 µg/ml (Keskintepe et al. 1994) and estradiol up to 1µg/ml (Jimenez-Macedo et al. 2007, Kharche et al. 2008, Yadav et al. 2010) to determine the developmental potential of in vitro produced embryos as gonadotropins support oocytes maturation and also induce major alteration in the protein profile of oocytes (Schorer and Meinecke 1995). Caprine culture media supplemented with gonadotropins and estradiol-17B improve maturation rates (Keskintepe et al. 1994, Pawshe et al. 1996, Mogas et al. 1997a, b, Kharche et al. 2008, Yadav et al. 2010, Kharche et al. 2011). Moreover, FBS increased cleavage rate over goat serum in an LH, FSH and E2 hormonal milieu (54%; P<0.05, Seydou et al. 1999).

Commercial PMSG is less expensive than FSH and LH. However, to our knowledge, dose dependent effects of PMSG and PMSG+hCG supplementation on in vitro maturation of goats oocytes have not been previously investigated in detail. Thus, in the present study attempts were made to study the effect of different concentrations of PMSG and the effect of different concentrations of hCG in media supplemented with PMSG on in vitro maturation of goats oocytes.

MATERIALS AND METHODS

Collection of ovaries: Goat ovaries (355) were obtained within 4 h of slaughter from a local abattoir and were transported to the laboratory in a thermos flask containing sterile warm (35–37°C) physiological normal saline solution.
(NSS) supplemented with antibiotics (100 IU/ml penicillin G and 100µg/ml streptomycin sulphate). All ovaries were cleared off the attached tissue and mesoovarium (trimming). The trimmed ovaries were subject to washings (5–6 times) with warm saline fortified with antibiotics and transferred into laminar flow. All subsequent experimental procedures were conducted in laminar flow.

**Recovery of oocytes:** Oocytes (1,216) were recovered from ovaries by slicing (Yadav et al. 2007) with a sterile surgical blade separately in a sterile disposable Petri-dish containing oocyte collection medium (Dulbecco’s phosphate-buffered saline (D-5773) with 3 mg/ml BSA). The oocytes were searched in a Petri-dish under stereozoom microscope. Oocytes were searched, picked up with the help of a fine bore pipette and subsequently placed in another Petri-dish containing oocyte holding medium. The collected oocytes were finally graded as excellent (A), good (B), fair (C) and poor (D) quality under the inverted phase contrast (Kharche et al. 2008). Only grade A and B quality oocytes were chosen. The before said graded cumulus oocyte complexes (COCs) were selected, picked up and placed in a sterile disposable vile containing oocyte holding medium (TCM-199 containing L-glutamine (100 µg/ml), sodium pyruvate (0.25 mmol) and gentamycin (50 µg/ml)). The selected oocytes were picked up from vile and subjected to serial drop washing. Each oocyte was washed 14 times (100 ml each) through oocyte holding medium (OHM).

**Experiment 1:** Dose dependant effect of pregnant mare serum gonadotropin (PMSG) on in vitro matured goat oocytes

After washing with oocyte holding medium, selected cumulus oocyte complexes (n=856) were divided into 6 groups. Oocytes were then washed 3–4 times with different maturation media depending on the group selected.

- **Group 1** (control medium): TCM-199 medium containing sodium pyruvate (0.25mM), L-glutamine (100µg/ml), estradiol 17-B (1µg/ml), BSA (3mg/ml), supplemented with 10% fetal bovine serum (FBS).
- **Group 2:** Control medium + 10 IU/ml PMSG.
- **Group 3:** Control medium + 20 IU/ml PMSG.
- **Group 4:** Control medium + 30 IU/ml PMSG.
- **Group 5:** Control medium + 40 IU/ml PMSG.
- **Group 6:** Control medium + 50 IU/ml PMSG.

Oocytes of each group were matured in 100 µl droplets of maturation media (MM) covered with sterile mineral oil in a polystyrene culture dish (35 mm x 10mm), previously incubated for 2h in humidified atmosphere of 5% CO₂ at 38.5°C in a CO₂ incubator for 27 h. Maturation media contained oocyte complexes (n=360) were transferred to a 100 µl droplets of maturation media (MM) covered with sterile mineral oil in a polystyrene culture dish (35 mm x 10mm), previously incubated for 2h in humidified atmosphere of 5% CO₂ at 38.5°C in a CO₂ incubator for 27 h. Maturation media consisted of:

- **Group 1:** control medium + 20 IU/ml PMSG +10IU/ml hCG
- **Group 2:** control medium + 20 IU/ml PMSG +20IU/ml hCG
- **Group 3:** control medium + 20 IU/ml PMSG +30IU/ml hCG

After 27 h of *in-vitro* maturation, cumulus cells were removed by treatment with 0.1% hyaluronidase enzyme (H-4272) followed by gentle pipetting for 1 min. Denuded oocytes after exposing in hypotonic solution of KCl for 5 min. were fixed on a rectangular cover glass slide (22x30 mm) with 2.5% glutaraldehyde for 5 min at room temperature followed by staining with 0.1µg/ml 4,6-diamidino-2-phenylindole (DAPI).

**Statistical analysis:** The maturation stage of oocytes was calculated as a percentage. Maturation rates between the different treatment groups were compared using the Chi-square test. The level of significance was recorded at the 5% level of confidence (Snedecor and Cochran 1989).

**RESULTS AND DISCUSSION**

Collection, quality and quantity of oocytes harvested from goat slaughterhouse ovaries are important for *in vitro* embryo production. In this study, on an average 3.40±0.06 oocytes (A and B) recovered per ovary by using slicing technique. Several techniques were used for oocytes recovery in goats and sheep, and they reported better recovery and quality of oocytes per ovary than puncture and aspiration technique in sheep and goats (Martino et al. 1994, Wani et al. 2000, Wang et al. 2007, Yadav et al. 2007) and cattle (Yoo et al. 1993). Hormonal stimulation of oocytes during the course of *in vitro* maturation is of paramount importance in achieving nuclear and cytoplasmic maturation, which is essential for the preparation of oocytes for fertilization. Our results demonstrated that maturation rate increases with addition of 20IU/mL PMSG (Table 1; Fig. A, B, C, D, E and F). Furthermore, supplementation with higher concentrations of PMSG (from 30IU/mL to 50IU/mL) did not increase the progression to metaphase II. Feng et al. (2002) studied the effect of serum and
Table 1. Nuclear status of goat oocytes matured in vitro with different concentrations of PMSG

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Total COCs</th>
<th>GV(%)</th>
<th>GVBD (%)</th>
<th>MI(%)</th>
<th>MII(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Control)</td>
<td>100</td>
<td>21 (21)</td>
<td>27 (27)</td>
<td>32 (32)</td>
<td>20 (20.0)$</td>
<td></td>
</tr>
<tr>
<td>Group 2 (Control+10IU/ml PMSG)</td>
<td>101</td>
<td>1 (1)</td>
<td>12 (11.88)</td>
<td>39 (38.61)</td>
<td>49(48.51)$</td>
<td></td>
</tr>
<tr>
<td>Group 3 (Control+20IU/ml PMSG)</td>
<td>205</td>
<td>1 (0.49)</td>
<td>28 (13.66)</td>
<td>23 (11.22)</td>
<td>153 (74.63)$</td>
<td></td>
</tr>
<tr>
<td>Group 4 (Control+30IU/ml PMSG)</td>
<td>147</td>
<td>2 (1.36)</td>
<td>14 (9.52)</td>
<td>22 (14.97)</td>
<td>109 (74.15)c,d</td>
<td></td>
</tr>
<tr>
<td>Group 5 (Control+40IU/ml PMSG)</td>
<td>164</td>
<td>0 (0.00)</td>
<td>16 (9.76)</td>
<td>25 (15.24)</td>
<td>123(75.00)e</td>
<td></td>
</tr>
<tr>
<td>Group 6 (Control+50IU/ml PMSG)</td>
<td>131</td>
<td>0 (0.00)</td>
<td>11(8.4)</td>
<td>20(15.27)</td>
<td>100(76.33)c,d,e</td>
<td></td>
</tr>
</tbody>
</table>

Values in a column with different superscripts are significantly different (P < 0.05).

The second experiment was aimed to know the addition of human chorionic gonadotropin to maturation media with 20IU/mL PMSG can increase the maturation rate. Although a high maturation rate of 85.19% (Table 2) was obtained with 20IU/mL hCG, but it did not differ significantly with supplementation of 10IU/mL and 30IU/mL concentration of hCG. In Egyptian sheep, Farag et al. (2009) demonstrated that the supplementation of hormone combinations (PMSG+hCG+E2) plus FBS to culture media (TCM-199) improved maturation rate (41.25%) of sheep COCs as compared to the control media (3.5%). Serum provides nutrients to an oocyte and apart from this, tends to nurture the cells surrounding the oocyte, rather than the oocyte itself. It also decreases the possibility of the zona pellucida (ZP) hardening when an oocyte is liberated from its follicular environment. The addition of serum to the culture medium during in vitro maturation of oocytes is partly responsible for the induction of maturation. Combination of FBS and BSA are most commonly used protein source for in vitro culture system goat oocytes. Therefore the supplementation of hormone combination (20IU/mL PMSG+ 20IU/mL hCG+1µg/mL E2) with 10% FBS to culture media (TCM-199) could significantly improve the IVM of goat oocytes especially COCs. Inclusion of these gonadotropin for IVM enhances oocyte quality and developmental potential by possibly altering metabolic processes (Brackett and Zuelke 1993). The results indicated that supplementation of 20 IU/mL PMSG in maturation media significantly (P<0.05) increased maturation rate which was further enhanced by addition of 20IU hCG and may be used in in vitro maturation protocols.

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REFERENCES


Fig. 1. A. Mature oocytes; B. germinal vesicle; C. germinal vesicle breakdown; D. metaphase I; E. mataphase II with 2 chromatin spots; F. metaphase II with polar body.
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