Antioxidant effect of Epigallocatechin gallate on bovine IVM and IVF

Lomen W Singh, Pritviraj M Barua, Joyshikh Sonowal, Keshab C Nath, Probodh Borah, Rumi S Borah, Arunima Das, Dwijyoti Mahanta, Rubal Das and Waseem A Malla

Abstract

The present study examined the effect of epigallocatechin gallate (EGCG) during In-vitro maturation (IVM) and In-vitro fertilization (IVF) of bovine oocytes. Cumulus-oocyte complexes (COCs) were aspirated from the ovaries derived from slaughter house and cultured in Tissue culture medium-199 supplemented with 5, 10 or 15 µM of epigallocatechin gallate for 24 h. Oocytes of a control group were matured in a maturation medium without EGCG. After IVM, COCs were coincubated with frozen-thawed spermatozoa for 15–18 h. In comparison with the absence of EGCG, treatment with EGCG at 10 and 15 µM showed a significant increase in the proportion of cumulus cell expansion, 1st polar body and 2nd polar body. However, compared to control, the presence of 10 and 15 µM EGCG during IVM significantly (P < 0.01) increased the proportion of In-vitro maturation and fertilization rate. However, a further decrease to 5 µM EGCG reduced the In-vitro maturation and fertilization rate. The results suggest that at certain concentrations of EGCG (10 and 15 µM) in IVM medium has beneficial effects on In-vitro maturation and subsequent In-vitro fertilization of bovine oocytes.

Keywords: Oocyte, IVM, IVF, antioxidant, epigallocatechin gallate, green tea

1. Introduction

In-vitro embryo production (IVEP) technology has been successfully applied in a number of animal species with transferred embryos resulting in live offsprings. Incorporation of the various types of additives in tissue-culture media has been found to be useful to increase the rate of In-vitro maturation of domesticated livestock as well as wildlife oocytes [3]. In-vitro cultures of oocytes and embryos are maintained at higher concentrations of oxygen than the in-vivo environment, leading to an increased level of reactive oxygen species (ROS). In-vivo, the damaging effects of oxygen radicals are usually prevented or limited by endogenous antioxidants (or scavengers of free radicals). However, the level of antioxidants was lower than in-vivo during In-vitro culture of oocyte and embryo. Consequently, the addition of an antioxidant may be important. A new antioxidant, Green tea polyphenols have been found as an alternative for the In-vitro culture of oocyte and embryo. Green tea polyphenols are mainly epigallocatechin gallate (EGCG), epicatechingallate (ECG), epicatechin (EC) and epigallocatechin (EGC) etc. All these catechins have strong antioxidant activity [10, 11] and are effective in enhancing in-vitro maturation and fertilization rate [5, 9]. So, the present study was conducted with an aim to standardize the culture condition of bovine oocytes using epigallocatechin gallate as an antioxidant for in-vitro maturation and subsequent in-vitro fertilization of bovine oocytes.

2. Materials and Methods

The media and chemicals used to conduct the present study were procured from Sigma-Aldrich, USA.

2.1 Collection of ovary and oocytes: The cattle ovaries were collected from a local slaughter house in warmed (37 °C) normal saline solution (0.9%) containing Gentamicin (50 µg/ml) in a thermos flask and brought to the laboratory within 2 h after the animals was slaughtered. The extraneous tissues were removed from the ovaries with the help of scissors. The ovaries were then washed 3–4 times in physiological saline solution containing Gentamicin (50 µg/ml) prior to processing. The oocytes were retrieved using aspiration technique and slicing technique in

Lomen W Singh
College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

Pritviraj M Barua
College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

Joyshikh Sonowal
ICAR-IVRI, Iżnagar, Uttar Pradesh, India

Keshab C Nath
College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

Probodh Borah
College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

Rumi S Borah
College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

Arunima Das
College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

Dwijyoti Mahanta
College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

Rubal Das
ICAR-IVRI, Guwahati, Assam, India

Waseem A Malla
ICAR-IVRI, Iżnagar, Uttar Pradesh, India

Correspondence
Lomen W Singh
College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India
2.2 In-vitro maturation: COCs were washed three times each in washing medium (TCM-199 supplemented with Fetal bovine serum (10%), sodium pyruvate (0.8 mM), L-glutamine (0.7 mM) and gentamicin sulphate (50µg/ml)) and in maturation medium [1] with the different concentration of EGCG @ 5, 10 or 15 µM and control without EGCG. COCs were then incubated in 50 µl droplets (8–10 oocytes per drop) of maturation medium. The droplets were covered with mineral oil and then incubated at 38.5 °C in 5% CO2 with 90-95% humidity for 24 h.

2.3 In-vitro fertilization: The IVF was carried out as described by Wang et al. 2007 [9]. Briefly, matured COCs were washed thrice in washing TALP (Tyrode’s Albumin Lactate Pyruvate Solution) and twice in fertilization TALP and then placed in 50µl droplets (10–12 COCs per droplet) of fertilization medium. Frozen bull semen was semen was prepared and a swim-up procedure. Sperm cells were added to the fertilization drops at a concentration of 2 million per ml. Incubation was carried out at 38.5 °C in 5% CO2 and 90-95 per cent humidity for 15–18 h.

2.4 Evaluation of oocytes after IVM and IVF: IVM of oocytes was carried out with TCM-199 based maturation medium in the presence of 5, 10 or 15 µM EGCG and without treatment. The rates of maturation were counted after IVM of 24 h of culture and then matured oocytes were subjected to fertilization in standard fertilization medium. Fertilization rate is assessed after 18 h of co-culture. After 24 h of incubation in a CO2 incubator maintaining the temperature at 38.5°C with 5% CO2 in the humidified air, oocytes were washed thrice in washing medium (TCM-199) and twice in fertilization medium (TCM-199). The degree of expansion of cumulus cell expansion was observed as (i) Full cumulus cell expansion: Expansion of the cumulus cell mass to at least 3X of its original diameter away from the zona pellucida (ZP), (ii) Moderate cumulus expansion: Expansion of the cumulus cell to at least twice of its original diameter away from the ZP, (iii) Slight or no expansion of the cumulus cell mass: Cumulus cells tightly adhered to the ZP. Fertilization status was assessed based on extrusion of the 2nd polar body in the perivitelline space after 18 h of co-culture in a CO2 incubator at 38.5°C with 5% CO2 in the humidified air, under the Phase contrast inverted Microscope at 40 × 10X zoom.

2.5 Statistical analysis: The statistical analysis was done by using SAS enterprise guide 4.3.

3. Results and Discussion
In the present study, 10 µM and 15 µM of Epigallocatechin Gallate was found best in both IVM and IVF than other groups (Table 1). Similar finding was also reported by Wang et al. [9] with Green tea polyphenols (GTP) that treatment with 10 and 15 µM GTP significantly enhanced in-vitro maturation rate and subsequent development to the blastocyst stage in bovine oocytes. This improvement is due to increase of intracellular glutathione (GSH) concentration after IVM of oocytes. However, a further increase in GTP concentration from 20 to 25 µM did not improve the fertilization competence or the proportion of oocytes reaching the blastocyst stage. Wang et al. [6] reported that 15 µM GTP (green tea polyphenols) during IVM and IVC improved pregnancy rates after Embryo Transfer, this improvement is due to the increase of relative transcript abundance (RA) of antioxidant enzyme genes, SOD1, CAT, and GPX, and the decrease in apoptosis index (AI) in bovine blastocysts. Further increase in concentration failed to improved rates. Roychoudhury et al. [1] demonstrated that at highest dose (200 µg/ ml) of green tea extract apoptosis is markedly increased than lower dose (0.1, 1, 10 and 100 µg/ml) which is due to the increase accumulation of caspase-3 and p53 apoptotic markers in granulosa cells of Porcine. Barakat et al. [3] found that GTE (green tea extract) at concentrations of 0.3 mg/ml in IVM medium enhanced the in vitro maturation and embryo development of sheep oocytes to blastocyst stage. Addition of GTE at 0.6 mg/ml and more to IVM medium had little benefit in increasing the maturation rate and blastocyst formation. Another constituent of Green tea polyphenols i.e Epigallocatechin-3-gallate was found to have similar effect as reported by Spinaci et al. [8] that at certain concentration it enhanced in vitro maturation and fertilization in pig. However, it also exerts a diphasic effect on fertilization rate that is improved at medium (0-25 µg/ml) dosages while it is inhibited at high (more than 25 µg/ml) concentrations [8]. Similar to Yavari et al. [3] that Epigallocatechin-3-gallate (EGCG) at 10 and 50 µM was apparently harmful for in vitro development of porcine parthenotes [7]. On fertilization, addition of Epigallocatechin-3-gallate at 25 µM and 50 µM in thawing extender exhibited a significantly (P<0.01) increase in vitro penetration rate and total fertilization efficiency in boar [2]. Above discussion is well supported by Sakagami et al. [12] report that GTP has two different actions: an antioxidant action at lower, and a pro-oxidant action at higher concentrations [12]. In the present study, oocytes treated with 10 µM and 15 µM EGCG had higher in vitro maturation and fertilization rates than control (Table 1). This improvement might have been partly because of the increase relative transcript abundance (RA) of antioxidant enzyme genes, SOD1, CAT, and GPX and due to increase of intracellular glutathione (GSH) concentration. However, the precise reasons for this improvement are unclear, and need to be clarified in future investigations. In conclusion, treatment with Epigallocatechin Gallate at 10 µM and 15 µM on in-vitro maturation medium significantly improved the rate of in-vitro maturation and subsequent fertilization. So, Epigallocatechin Gallate alone might have the similar effect of GTP and GTE at lower concentration on in-vitro culture of oocytes.
Table 1: *In-vitro* Maturation and fertilization rate of Bovine Oocyte based on Cumulus cell expansion, 1st Polar body extrusion and 2nd Polar body extrusion in TCM-199 Based medium containing different concentration of Epigallocatechin Gallate

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>No. of oocytes used for IVM</th>
<th>Cumulus Cell Expansion</th>
<th>1st Polar body extrusion</th>
<th>2nd Polar body extrusion</th>
<th>IVF Rate Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of oocytes matured</td>
<td>IVM rate (Mean ± SE)</td>
<td>No. of oocytes matured</td>
<td>IVM rate (Mean ± SE)</td>
<td>No. of Oocytes use for IVF</td>
</tr>
<tr>
<td>EGCG (5 µM)</td>
<td>304 (15)</td>
<td>163 53.97±2.06</td>
<td>131 43.24±1.03</td>
<td>131 52</td>
<td>39.79±1.57</td>
</tr>
<tr>
<td>EGCG (10 µM)</td>
<td>299 (15)</td>
<td>221 74.10±1.96</td>
<td>171 57.27±1.31</td>
<td>171 94</td>
<td>55.34±2.24</td>
</tr>
<tr>
<td>EGCG (15 µM)</td>
<td>313 (15)</td>
<td>228 73.22±1.98</td>
<td>177 56.68±1.40</td>
<td>177 94</td>
<td>53.54±2.06</td>
</tr>
<tr>
<td>Control</td>
<td>298 (15)</td>
<td>184 56.39±1.76</td>
<td>139 45.26±1.73</td>
<td>139 55</td>
<td>41.82±2.20</td>
</tr>
</tbody>
</table>

Means with the different superscripts in a column differ significantly (*P* < 0.01)

Figure in the parenthesis indicate no. of trails

4. Acknowledgement

The authors are thankful to the department of ARGO, CVSc, AAU, Guwahati, India-781022 for technical supports and the laboratory facilities.

5. References