Effects of Progesterone on in Vitro Developmental Competence of Bovine Embryos

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ABSTRACT

Progesterone plays a key role in the establishment and maintenance of pregnancy in mammalian. Increasing levels of circulating progesterone in the post-conception period are associated with conceptus elongation and high pregnancy rates in cattle. Contradictory results are available on the direct role of progesterone in early embryo development. The objective of this study was to evaluate direct effects of progesterone on in vitro development of cattle embryos. Immature oocytes collected from slaughtered animals and cultured in the presence of different concentrations of progesterone (25, 50, 100 ng/mL) following in vitro fertilization. Cleavage rates in 25 and 50 ng/mL concentrations of progesterone were significantly higher than those in controls and 100 ng/mL. Rate of embryos that reached to the morula stage was similar in all groups. Supplementation of 25 and 50 ng/mL progesterone to the culture media significantly increased blastocyst yield while 100 ng/mL progesterone resulted in a decrease. As a conclusion, we can suggest that progesterone supplementation in in vitro culture may support embryo development at low levels.

KEYWORDS:
Bovine
Embryo culture
Progesterone
In vitro fertilization
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Introduction

Most of the embryonic losses in cows occur during the early embryonic development period (Carter et al., 2008). The embryonic environment is crucial in shaping the embryonic development rate in the post-fertilization period (Carter et al., 2008). The elevated concentration of circulating progesterone immediately after pregnancy is closely related to the establishment of pregnancy in cattle (Carter et al., 2008; Clemente et al., 2009). In vivo and in vitro studies on both the direct (Ferguson et al., 2005; Merlo et al., 2006; Larson et al., 2011; Ferguson et al., 2012) and indirect (Bazer et al., 2010, Lonergan et al., 2016) effects of progesterone on embryo is available.

Existing contradictory results may be due to the differences in culture systems or the period in which the embryo is exposed to progesterone (Clemente et al., 2009). As a matter of fact, progesterone concentration in cows increases from day 3 following estrus. Progesterone treatments prior to the fertilization lead to a decrease in cleavage rates (Ferguson et al., 2012). Data on negative effects of progesterone during in vitro maturation (Fukui et al., 1982) and in vitro fertilization (Fukushima and Fukui, 1985) stages are also available. However, elevated concentrations of progesterone are accepted as an indicator of infertility (Ferguson et al., 2012). Timing of exposure to progesterone is crucial for embryo development in mammals, in this regard. Therefore, the main objective of this study was to determine whether supplementation of progesterone during in vitro culture directly alerts embryo development or not.

Materials and Methods

Ethical Statement

No approval from the research ethics committee was requested since embryos are not included in the list of organisms that require a specific authorization according to EC Directive 86/609/EEC for animal experiments.

Chemicals

Cell culture media for in vitro production (IVP) of bovine embryos were purchased from Caisson Labs (East Smithfield, UT, USA) unless otherwise indicated. Sperm preparation (SP)–Tyrode’s Lactate (TL), IVF-TL, Heps-TL and potassium simplex optimized medium including
Table 1. Cleavage rates of embryos

<table>
<thead>
<tr>
<th>Developmental stages</th>
<th>Control (n=193)</th>
<th>25P (n=210)</th>
<th>50P (n=212)</th>
<th>100P (n=201)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-cell</td>
<td>78.8±1.23ab</td>
<td>88.6±0.35b</td>
<td>82.1±0.45b</td>
<td>53.7±2.68b</td>
<td>0.027</td>
</tr>
<tr>
<td>Egg</td>
<td>21.2±1.23ab</td>
<td>11.4±0.35a</td>
<td>17.9±0.45a</td>
<td>46.3±2.68b</td>
<td></td>
</tr>
</tbody>
</table>

Values are %±SEM, different letters of superscript in a row represent different groups according to Duncan
Supplementation of the culture media with progesterone in varying concentrations significantly enhanced blastocyst yield at physiological levels (25 and 50 ng/mL), but resulted in a decrease at a supra-optimal concentration (100 ng/mL), in the present study (Table 2). These findings suggest that progesterone may act directly as a survival factor on embryo development. Further research has to be conducted in order to determine the direct effects of progesterone on Blastomere count, quality grade and diameter of embryos.

Acknowledgement

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References


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