SHORT NOTE

SYNERGISTIC EFFECTS OF INSULIN-LIKE GROWTH FACTOR (IGF-II) AND FSH ON BOVINE GRANULOSA CELLS

EFECTOS SINÉRGICOS DEL FACTOR DE CRECIMIENTO SIMILAR A LA INSULINA (IGF-II) Y LA FSH SOBRE CÉLULAS DE LA GRANULOSA BOVINA

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ADDITIONAL KEYWORDS
Granulosa cells culture. Steroid hormones.

SUMMARY

The synergistic effects of insulin-like growth factor II (IGF-II) and follicle stimulating hormone (FSH) and the effect of IGF-II alone on progesterone and estradiol production by bovine granulosa cells (GC) cultured in vitro were determined. Granulosa cells obtained from ovaries of slaughtered cows were cultured for four days in carbon (IV) oxide incubator at 37°C, 5% CO2 in atmospheric air and 100% relative humidity, using Tissue Culture Medium 199 (TCM 199) as culture medium. The medium was changed on the second day and testosterone added to serve as substrate for estradiol production. IGF-II alone, or IGF-II (10 ng/mL)+ FSH was added to culture medium at 0, 0.1, 1.0, 10, 50, and 100 (ng/mL) levels of inclusion. Concentrations of progesterone and estradiol produced were measured by radioimmunoassay. Data collected was statistically analysed using analysis of variance method. The results obtained showed that IGF-II alone and IGF-II + FSH had significant effects (p<0.05) on progesterone and estradiol produced by cultured GC. From the result obtained, it can be concluded that IGF-II + FSH gave better synergistic effects on bovine granulosa progesterone and estradiol production.

INTRODUCTION

The insulin-like growth factors (IGFs) are members of a family of low molecular weight of single-chain polypeptide named for their structural and functional similarity to insulin (Strauss and Barbieri, 2009). The stimulatory effects of insulin, IGF-I and -II on estradiol production by mammalian granulosa cells are documented (Guidice, 1992) and are likely due in part to their ability to enhance the action of gonadotropins on ovarian follicular steroidogenesis (Spicer et
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The IGFs enhance FSH actions in vitro, including stimulation of estradiol production (Spicer and Echternkamp, 1995) by granulosa cells of multiple species including cattle (Minegishi et al., 2000). In cattle, evidence indicates that IGFs play an important regulatory role once follicles reach the antral stage and become gonadotropin dependent (Monget and Bondy, 2000). Consequently, this study aimed to evaluate the effects of preincubated granulosa cells of ovaries obtained from local abattoir with IGF-II and FSH on steroid hormones production.

MATERIALS AND METHODS

The study was conducted at the Department of Animal Physiology, College of Animal Science and Livestock Production, University of Agriculture, Abeokuta, Nigeria. Ten pairs of ovaries were collected from the local abattoirs and were kept in 0.9% normal saline containing 100 mg/mL streptomycin sulfate and 100 IU/mL Penicillin-G Sodium. They were transported to the laboratory within one hour after slaughter.

PREPARATION OF CULTURE MEDIA

The medium used for the cell culture was TCM-199 (Sigma Aldrich Chemical Co., USA). It was diluted in 1.0 litre of de-ionized water and 3.36 mM of NaHCO₃ was added to each litre of TCM-199 solution. Also, 1% Bovine Serum Albumin (BSA) was then added to a half of the TCM-199 while 5% Fetal Calf Serum (FCS) was added to 47.5 mL from the remaining part of TCM-199 while 0.8% antibiotics was also added to each half of the medium, after which 0.2 µm Supor® membrane filter was used to filter both media for the purpose of sterility.

METHODOLOGY

Immediately returning to the laboratory, ovaries were washed in fresh sterile saline. All visible follicles were aspirated into the culture medium. Granulosa cells collected were separated from follicular fluid by centrifuging at 3000 rpm for 10 min. Cell pellet were then mixed thoroughly with the culture medium and aliquoted at 50 µL/mL per well into 24-well micro plates containing 450 µL of medium per well. Cultures were incubated at 37°C in a 95% air and 100% relative humidity. Media was changed after 2 days of incubation and cells were maintained in the presence of 10% fetal calf serum for the first 48 hours of culture. Testosterone (10 ng/mL each) was added as substrate for estradiol production at the third day of the culture. On the 4th day, the experiment was completed.

DATA COLLECTION

At the termination of the experiment, cultured medium samples was collected for determination of estradiol and progesterone concentration by Radioimmunoassay method. Samples were packaged and sent to the Laboratory for Physiology and Immunology, Department of Animal Science, Katholeic University of Leuven, Belgium. Hormone was assayed in maturation media according to Abraham et al. (1972).

EXPERIMENTAL DESIGN

The experimental design included: (1) Supplementation of additives; i.e. (a) addition of IGF-II and FSH; (b) IGF-II alone, and varied levels of inclusion: (a) 0 ng/mL, (b) 0.1 ng/mL, (c) 1.0 ng/mL, (d) 10 ng/mL, (e) 50 ng/mL and (f) 100 ng/mL. Each treatment was replicated 3 times and data collected was statistically analysed by analysis of variance. Significant means were separated using Duncan Multiple Range Test (Duncan, 1955).

RESULTS

IGF-II EFFECTS ON BOVINE GRANULOSA CELLS CULTURED IN VITRO FOR STEROIDS PRODUCTION

Table I showed the effects of IGF-II on bovine granulosa cells cultured in vitro for estradiol production. The highest concen-
EFFECTS OF IGF-II AND FSH ON STEROID HORMONE PRODUCTION

The combination of IGF-II (at 10 ng/mL) with FSH inclusion in culture medium for bovine granulosa cells culture has significant effect on the progesterone production as shown in Table II (p<0.05). FSH inclusion level at 50 ng/mL recorded the highest value of progesterone (5.11 ng/mL). It could be deduced from the same table that there was significant difference in the value of estradiol recorded by varying levels of IGF-II and FSH combination.

DISCUSSION

The results obtained in this study showed a significant effect on progesterone and estradiol production - a pointer to the proliferation of GC. This is validated by the report of Lowe (1991) that IGF-II stimulates uptake of both amino acids and glucose, and acts as progression factor in the cell cycle. It was also reported by Savion et al. (1981) that in addition to the known metabolic effects of insulin and IGFs, they have been shown to stimulate both progesterone production and mitosis of bovine ovarian granulosa cells cultured in vitro. In vivo data also compliment the result of this study by revealing a positive correlation between follicular fluid IGF-II and progesterone concentration in post-partum anestrous and cyclic cows (Echternkamp et al., 1990; Spicer and Enrighgt, 1991). Some studies revealed that IGF acts on the GC to augment hormonal actions of gonadotropins (Veldhuis and Rodgers, 1987; LaVoie et al., 1999). Veldhuis and Rodgers (1987) suggested that the synergism between FSH and IGF augmented rates of progesterone synthesis by activating dual mechanisms: enhancement of effective cellular uptake and utilization of low-density lipoprotein (LDL)-borne sterol substrate and stimulation of functional cholesterol side chain cleavage activity. It can be concluded that, gonadotropin and IGFII significantly influenced the steroidogenic activity of bovine GC.

Table I. Effects of IGF-II alone on bovine granulosa cells cultured in vitro for steroids production. (Efectos del IGF-II solo sobre las células de granulosa bovinas cultivadas in vitro para producción de progesterona y estradiol).

<table>
<thead>
<tr>
<th>IGFII (ng/mL)</th>
<th>Progesterone (ng/mL)</th>
<th>Estradiol 17β (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.78±0.06</td>
<td>1.13±0.12</td>
</tr>
<tr>
<td>0.1</td>
<td>1.03±0.06</td>
<td>1.49±0.12</td>
</tr>
<tr>
<td>1.0</td>
<td>1.41±0.06</td>
<td>3.63±0.12</td>
</tr>
<tr>
<td>10</td>
<td>1.87±0.06</td>
<td>3.67±0.12</td>
</tr>
<tr>
<td>50</td>
<td>1.84±0.06</td>
<td>3.57±0.12</td>
</tr>
<tr>
<td>100</td>
<td>1.51±0.06</td>
<td>3.08±0.12</td>
</tr>
</tbody>
</table>

abcd means in the column with different superscript are significant (p<0.05).

1IGFII: IGF-II inclusion level (ng/mL); 2FSH: FSH inclusion level (ng/mL); 3P4: progesterone (pg/mL); 4E2: 17β-estradiol (pg/mL).

Table II. Synergistic effects of IGF-II and FSH on bovine granulosa cells cultured in vitro for steroids production. (Efectos sinérgicos de IGF-II y FSH sobre las células de granulosa bovinas cultivadas in vitro para producción de progesterona).

<table>
<thead>
<tr>
<th>IGFII (10 ng/mL)</th>
<th>FSH (50 ng/mL)</th>
<th>P4 (pg/mL)</th>
<th>E2 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0</td>
<td>1.40± 0.14</td>
<td>2.98±0.14</td>
</tr>
<tr>
<td>10</td>
<td>0.1</td>
<td>1.89±0.14</td>
<td>4.44±0.14</td>
</tr>
<tr>
<td>10</td>
<td>1.0</td>
<td>3.76±0.14</td>
<td>11.40±0.14</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>5.04±0.14</td>
<td>15.12±0.14</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>5.11±0.14</td>
<td>15.16±0.14</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
<td>4.67±0.14</td>
<td>14.67±0.14</td>
</tr>
</tbody>
</table>

abc means in the column with different superscript are significant (p<0.05).
ACKNOWLEDGEMENT

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REFERENCES


