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.

PALABRAS CLAVE ADICIONALES

Cultivos de células de granulosa. Hormonas esteroides.

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% CO_2 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.

RESUMEN

Se estudiaron los efectos sinérgicos del factor de crecimiento II similar a la insulina (ICF-II) solo o asociado a hormona folículo estimulante (FSH), sobre la producción de progesterona y estradiol por las células de granulosa bovinas (GC) cultivadas *in vitro*. Las GC, obtenidas de ovarios de vacas sacrificadas, fueron cultivadas durante cuatro días en incubador óxido de carbono (IV) a 37°C y 5% de CO_2 en aire atmosférico y 100% de humedad relativa, usando el medio de cultivo de tejidos 199 (TCM 199). El medio, fue cambiado al segundo día y se añadió testosterona como sustrato para la producción de estradiol. El IGF-II solo o IGF-II (10 ng/mL) + FSH, se añadieron a las proporciones de 0; 0,1; 1,0; 10; 50 y 100 (ng/mL). Las concentraciones de progesterona y estradiol producidos se midieron mediante radioinmunoensayo. Los datos obtenidos se sometireron a análisis de varianza. Los resultados obtenidos demuestran que el IGF-II, solo o IGF-II + FSH tuvieron efectos significativos ($p<0,05$) sobre la progesterona y el estradiol producidos por las GC cultivadas. La asociación IGF-II + FSH mostró efecto sinérgico sobre la produccion, de progesterona y estradiol por la granulosa bovina.

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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al., 2002). 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 $NaHCO_3$ 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-

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).

IGF II (ng/mL)	Progesterone (ng/mL)	Estradiol 17β (pg/mL)
0	0.78^{d}±0.06	1.13^{d}±0.12
0.1	1.03^{c}±0.06	1.49^{c}±0.12
1.0	1.41^{b}±0.06	3.63^{a}±0.12
10	1.87^{a}±0.06	3.67^{a}±0.12
50	1.84^{a}±0.06	3.57^{a}±0.12
100	1.51^{b}±0.06	3.08^{b}±0.12

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

tration of progesterone was recorded at 10 ng/mL level of IGFII. Meanwhile, the results of IGF-II effects on estradiol production showed no significant differences among 1 ng/mL, 10 ng/mL and 50 ng/mL levels of inclusion.

SYNERGISTIC EFFECTS OF IGF-II WITH FSH ON BOVINE GRANULOSA CELLS CULTURED *IN VITRO* FOR STEROIDS 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 (Echtern-kamp *et al.*, 1990; Spicer and Enrighgt, 1991). Some studies revealed that IGF acts on the GC to augment hormonal actions of gonado-tropins (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, gonado-tropin and IGFII significantly influenced the steroidogenic activity of bovine GC.

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).

IGF II[1]	FSH[2]	P4[3]	E2[4]
10	0	1.40^{d} ±0.14	2.98^{d}± 0.14
10	0.1	1.89^{c}±0.14	4.44^{c}± 0.14
10	1.0	3.76^{b}± 0.14	11.40^{b}±0.14
10	10	5.04^{a}±0.14	15.12^{a}± 0.14
10	50	5.11^{a}±0.14	15.15^{a} ±0.14
10	100	4.67^{a} ±0.14	14.67^{a}± 0.14

^{abcd}means in the column with different superscript are significant ($p<0.05$).
[1]IGF II: IGF II inclusion level (ng/mL); [2]FSH: FSH inclusion level (ng/mL); [3]P4: progesterone (pg/mL); [4]E2: 17β-estradiol (pg/mL).

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