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Short communication

EFFECT OF MALNUTRITION/SERUM DEPRIVATION ON RELEASE OF PROGESTERONE AND ESTRADIOL BY CULTURED PORCINE OVARIAN FOLLICLES AND THEIR RESPONSE TO METABOLIC HORMONES

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ABSTRACT

The aim of present studies is to understand hormonal mechanisms of the effect of malnutrition on ovarian functions. For this purpose we examined the effect of malnutrition/serum deprivation, addition of metabolic hormones IGF-I and leptin and their combination on the release of progesterone (P_4) and estradiol (E_2) by cultured whole ovarian follicles isolated from porcine ovaries. It was observed that in the presence of serum the addition of IGF-I promoted release of P_4 but not of E_2 by ovarian follicles. Exogenous leptin reduced output of E_2 but not of P_4. Serum deprivation did not affect a release of P_4 but reduced output of E_2. An addition of both IGF-I and leptin prevented effect of malnutrition on E_2 release by cultured ovarian follicles. Present observations (1) confirm the involvement of hormones IGF-I and leptin in control of secretory activity of ovarian cells, (2) demonstrate, that isolated ovarian follicles cultured in the absence of serum could be an adequate in-vitro model for the study of the effect of malnutrition on ovarian secretory functions, (3) suggest, that malnutrition could affect ovarian functions through the changes in ovarian estradiol release, and (4) suggest that metabolic hormones could be useful for a prevention of negative effects of malnutrition on ovarian functions.

Key words: stress; malnutrition; IGF-I; leptin; FSH; progesterone; estradiol; ovarian follicles; pig

INTRODUCTION

Calory intake provides control and synchronizes reproductive cycle via optimal nutritional conditions. Malnutrition can block or prolong reproductive cycles (Crowe, 2008; Scaramuzzi and Martin, 2008). Therefore, nutritional control of reproductive processes represents not only scientific, but also medical and economical problem, which could be solved by understanding and affecting mediators of the effect of nutrition on reproduction. Mechanisms of the nutrition effect on reproduction are, however, studied insufficiently. Malnutrition can suppress GnRH/LH surge, progesterone alters IGF-I release and prolongs or blocks ovulation of ovarian follicles, whilst food intake restores and promotes these processes (Crowe, 2008; Lucy, 2008; Scaramuzzi and Martin, 2008). It indicates that malnutrition inhibits reproductive processes by a suppression of the GnRH-

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gonadotropin-IGF-I-ovarian steroid hormone axis. This axis could be affected by metabolic hormones. Food restriction reduces a release of metabolic hormone - leptin, a product of adipose and some other tissues including ovarian cells, which can affect reproduction both through the hypothalamo-hypophysial system and by a direct action on gonads. Leptin is able to promote a release of gonadotropins FSH and LH, the well known stimulators of ovarian cell proliferation and follicular growth, regulators of apoptosis and stimulators of a release of ovarian steroid and peptide hormones (Hillier, 1991; Erickson and Danforth, 1995; Berisha and Schams, 2005; Sirotkin et al., 2005). In addition, leptin can affect gonads directly: regulates growth of ovarian follicles, *corpus luteum* development, suppress ovarian cell apoptosis, activates ovarian cell proliferation and affects a release of steroid hormones, oxytocin, prostaglandin, IGF-I and IGFBP-3 by the ovarian cells (Spicer, 2001; Smith et al.,2002; Sirotkin et al., 2005; Zieba et al., 2005). Both leptin (Spicer, 2001; Sirotkin et al., 2005; Zieba et al., 2005) and gonadotropins (Erickson and Danforth, 1995; Berisha and Schams, 2005) can control ovarian functions through a stimulation of a local production of insulin-like growth factor I (IGF-I), whose anti-apoptotic effect and stimulatory action on ovarian cell proliferation, folliculogenesis and hormone release are similar to the action of leptin and gonadotropins (Sirotkin et al., 1998; 2001; 2005; Makarevich et al., 2000; Berisha and Schams, 2005).

Therefore, it might be proposed, that malnutrition can affect basic ovarian functions through the changes in a hormone release, which, in turn, can affect secretory and other functions of ovarian cells. If malnutrition affects ovarian cells through some reproductive hormones, (1) deficit of nutrients should change a release of hormones, and (2) treatment with these hormones should prevent or promote effects of malnutrition. In this case, manipulation with hormones can neutralize negative effect of malnutrition on ovarian functions. This hypothesis can be promising for understanding and eliminating effect of non-optimal nutrition on reproductive functions. This hypothesis requires a support with experimental data. Nevertheless, direct effect of malnutrition on the ovarian cells, as well as an influence of hormones on a malnutrition effect have not been examined yet. Lack of such evidence can be caused by the absence of an adequate experimental model enabling examination of a direct effect of malnutrition on ovarian cells. Such model could be isolated ovarian cells cultured under conditions of a reduced nutrition (deprivation of a blood serum, an obvious cells culture nutrient), addition of hormones (whose importance in control of the ovarian cell function and in mediating the effect of malnutrition is documented) and the combination of these factors.

The aim of present studies is to understand the hormonal mechanisms of effect of malnutrition on the ovarian functions. For this purpose, we examined effect of malnutrition/serum deprivation, addition of metabolic hormones IGF-I and leptin and the combination of serum deprivation and addition of IGF-I or leptin on a release of progesterone (P_4) and estradiol (E_2) by cultured whole ovarian follicles.

MATERIALS AND METHODS

Isolation, culture, and processing of ovarian follicles

Non-cycling Slovakian white gilts, 180 days of age and without visible reproductive abnormalities, were killed at a local slaughterhouse. Ovarian follicles (2.5-3.5 mm diam.) were collected, processed and cultured for 2 days in Falcon 24-well plates (Becton Dickinson, mesto, stat), 1 follicle per well in 2 ml of culture medium DME/F-12 (1:1 mixture) supplemented with 1% antibiotic-antimycotic solution, with or without 10% heat-inactivated fetal calf serum (all from Sigma), with and without hormones at doses listed above, as described previously (Sirotkin et al., 1998). Immediately after culture follicles were weighted and culture medium was collected and stored at –18°C to await RIA.

Immunoassays

Concentrations of hormones were determined by RIA in 25 µl samples of incubation medium. P_4 and E_2 were assayed using RIA/IRMA kits from DSL, Webster, USA) according to the instruction of a manufacturer. The characteristics of the assays were described previously (Makarevich et al., 2000; Sirotkin et al., 1998; 2001; 2005; 2008). The RIAs were validated for use in samples of culture medium by dilution tests.

Statistics

Each experiment was performed on ovaries obtained from 15-20 animals. Each experimental group was represented by 6 wells with ovarian follicles (1 follicle per well). The data shown are means of values obtained in 3 separate experiments performed in different days using separate pools of ovaries. Significant differences between the groups were determined using one-way ANOVA followed by Student's t-test by SigmaPlot 9.0 statistical software (Systat Software, GmbH, Erkrath, Germany). Differences from control at $P < 0.05$ were considered as significant.

RESULTS AND DISCUSSION

It was observed that in the presence of serum the addition of IGF-I promoted release of P_4 but not of E_2 by ovarian follicles. Exogenous leptin reduced output of E_2,

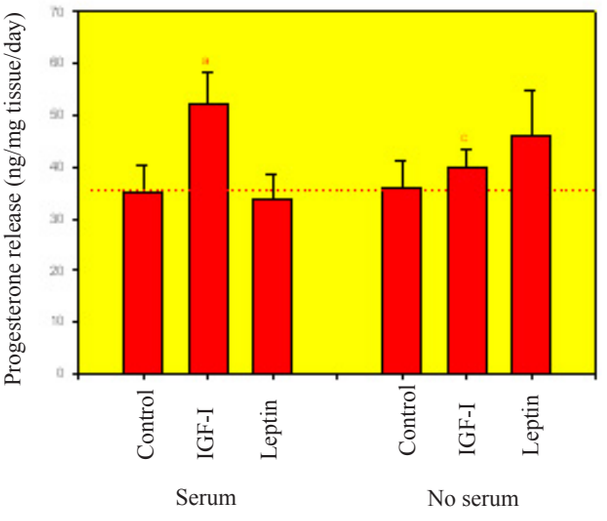

Fig.1: Release of progesterone by porcine ovarian follicles cultured with and without serum with and without hormones IGFgf-I and leptin. Vvalues are means+Ss.E.Mm. obtained in three separate experiments. Indications of significant differences between the groups: a) effect of hormones, shows a significant ($p<0.05$) difference between corresponding groups of cells cultured with and without hormones c) effect of serum deprivation, shows significant ($p<0.05$) differences between corresponding group of cells cultured with and without serum

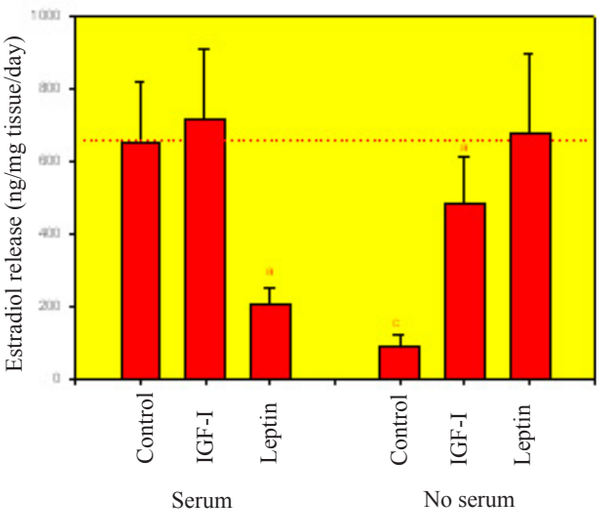

Fig.2: Release of estradiol by porcine ovarian follicles cultured with and without serum with and without hormones IGFgf-I and leptin. are means+E.obtained in three separate experiments. Indications of significant differences between the groups: a) effect of hormones, shows a significant ($p<0.05$) difference between corresponding groups of cells cultured with and without hormones c) effect of serum deprivation, shows significant ($p<0.05$) differences between corresponding group of cells cultured with and without serum

but not of P_4. Serum deprivation did not affect a release of P_4 but reduced an output of E_2. Addition of both IGF-I and leptin prevented effect of malnutrition on E_2 but not on P_4 release by cultured ovarian follicles (Fig.1, 2).

Release of P_4 and E_2 by porcine ovarian follicles into the culture medium observed in present experiments corresponds to our previous observations (Sirotkin et al., 1998, 2001, 2008).

Our observations of hormones-induced changes in secretory activity of whole follicles confirm previous reports on the involvement of IGF-I (Erickson and Danforth, 1995; Sirotkin et al., 1998; 2005; Makarevich et al., 2000; Berisha and Schams, 2005) and leptin (Spicer, 2001; Smith et al.,2002; Barb et al., 2005; Sirotkin et al., 2001; 2005; Zieba et al., 2005) in control of steroid hormone release by the ovary. Some effects of hormones could be primary, but other could be secondary. For example, leptin can affect ovarian functions through a stimulation of ovarian IGF-I release (Sirotkin et al., 2005).

In our experiments, ovarian follicles cultured without serum produced similar amounts of P_4 but less E_2, than the follicles cultured in a serum-supplemented medium. This suggests that malnutrition can reduce release of estrogen but not of progestagen by the ovarian cells. Different effects of serum deprivation on P_4 and E_2 output by ovarian follicles suggest on a different nutritional control of these hormones in ovarian follicles. Action of serum deprivation on ovarian steroid hormone provides first evidence, that negative effects of malnutrition on reproduction could be result of an abnormal release of estrogen and/or its regulators, whose important role in control of ovarian functions is well-documented (Hillier, 1991; Erickson and Danforth, 1995; Berisha and Schams, 2005). The second evidence could be an ability of IGF-I and leptin to prevent effect of malnutrition on E_2 release. It suggests that deficit of estrogen could be a hormonal signal of lack of the nutrients for reproductive system. It is known that food intake and development of adipose tissue activates production of leptin, an activator of gonadotropins and IGF-I release, which are known promoters of reproductive processes (Spicer, 2001; Smith et al., 2002; Sirotkin et al., 2005; Zieba et al., 2005). From practical viewpoint our observations suggest, that IGF-I,

leptin or their regulators could be potentially used not only for control of basic reproductive functions, but also for the prevention or neutralization of negative action of malnutrition on these processes in animal production and medicine.

Taken together the results of present studies (1) confirm the involvement of hormones IGF-I and leptin in control of secretory activity of ovarian cells, (2) demonstrate, that isolated ovarian follicles cultured in absence of serum nutrients could be an adequate in-vitro model for the study of the effect of malnutrition on ovarian secretory functions, (3) suggest, that malnutrition could affect ovarian functions through changes in ovarian estradiol release, and (4) that metabolic hormones could be useful for preventing negative effect of malnutrition on ovarian functions.

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