Secretion of immunoreactive β -endorphin and GnRH by the pig uterus: effects of reproductive status and breed

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Immunoreactive β -endorphin (ir-BEND) and GnRH (ir-GnRH) have been identified in the pig uterus. The study reported here examined (i) possible biochemical differences between ir-BEND and ir-GnRH present in pig uterine fluids and standard synthetic peptides, and (ii) the secretory profiles of uterine ir-BEND and ir-GnRH during the oestrous cycle and early pregnancy for two breeds of pig, Large White and the highly prolific Chinese Meishan. Reverse phase (RP)-HPLC analysis of concentrated uterine fluids indicated that the majority of ir-BEND eluted with a hydrophobicity similar to that of synthetic BEND 1–31 (BEND) and α -N-acetylated BEND 1–31 (Nac-BEND), with a ratio of BEND to Nac-BEND of approximately 1.8:1. The RP-HPLC profiles of ir-GnRH demonstrated a major peak co-eluting with synthetic GnRH, along with a minor peak eluting at the void volume. Total content of ir-BEND (pg per uterus) was affected by the interaction of breed with day ($P < 0.001$), but was independent of reproductive status. In Large White gilts, uterine fluid content of ir-BEND was higher ($P < 0.05$) on days 10 and 11 than on days 8, 12 and 14; however, in Meishan gilts, ir-BEND decreased from day 8 to days 10 and 11, and remained low on days 12 and 14. Compared to Meishan gilts, Large White gilts had higher ir-BEND concentrations on day 10 ($P < 0.01$), day 11 ($P < 0.0001$), day 12 ($P < 0.01$) and day 14 ($P < 0.02$). Similar patterns were detected when ir-BEND in uterine fluid was expressed as pg mg^{-1} protein. Differences in total ir-GnRH content of uterine fluid were influenced by the interaction of breed, status and day ($P < 0.02$). For Large White gilts, total ir-GnRH content was similar on days 8, 10, 11 and 12 between cyclic and pregnant gilts, but increased markedly ($P < 0.0001$) on day 14 of pregnancy. In pregnant Meishan gilts, total ir-GnRH content was increased on days 8, 12 and 14, compared with cyclic Meishan gilts on the same days. However, concentrations of ir-GnRH in uterine fluid (pg mg^{-1} protein) depended only upon the interaction of status and day ($P < 0.03$). These results suggest that ir-BEND and ir-GnRH in uterine fluids possess biochemical similarities to their respective synthetic peptides. In addition, the uterine secretory profile of ir-BEND is different in Large White and Meishan pigs but the significance of this difference is unclear. The marked increase in ir-GnRH in uterine fluids between days 12 and 14 of early pregnancy suggests that uterine ir-GnRH may play a role in early pregnancy.

Introduction

The uterus synthesizes and secretes a number of peptide hormones. For example, oxytocin and arginine vasopressin have been identified in the human uterus during both proliferative and secretory phases (Wiquist *et al.*, 1984; Lundin *et al.*, 1989), relaxin has been reported in the uterus of women (Bryant-Greenwood, 1991) and guinea pigs (Bryant-Greenwood *et al.*,

1991), and endogenous opioid peptides (EOP), including immunoreactive β -endorphin (ir-BEND) and immunoreactive methionine-enkephalin (ir-MENK), have been detected in the endometrium and uterine fluid of women (Wahlstrom *et al.*, 1985), cows (Petraglia *et al.*, 1986), rabbits (Li *et al.*, 1989, 1991b), and pigs (Li *et al.*, 1987, 1991a). In addition, we have recently demonstrated the presence of immunoreactive GnRH (ir-GnRH) in the uterus of rabbits (Li *et al.*, 1990) and pigs (Li *et al.*, 1993).

Although the biological significance of EOP and ir-GnRH in the pig uterus has yet to be determined, understanding the hormonal regulation of their synthesis and secretion may help in elucidating their physiological function(s). Experimental results have indicated the involvement of ovarian steroids in the

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uterine secretion or synthesis of EOP and ir-GnRH. In ovariectomized gilts, progesterone treatment stimulated the secretion of ir-BEND, ir-MENK and ir-GnRH, whereas oestradiol treatment produced no detectable effect. However, compared with treatment with progesterone alone, the combined treatment with progesterone and oestradiol resulted in divergent actions: it decreased uterine secretion of ir-BEND, produced no effect on ir-MENK secretion, and enhanced uterine secretion of ir-GnRH (Li *et al.*, 1987, 1991a, 1993). Concentrations of ir-BEND in the pig endometrium and ir-MENK content in uterine fluids increased between day 8 and day 14 of the oestrous cycle and early pregnancy (Li *et al.*, 1991a, 1992a). These results suggest that EOP and ir-GnRH may play a role in the establishment of a successful pregnancy.

The current study determined the (i) biochemical identity of ir-BEND and ir-GnRH present in uterine secretagogues; (ii) secretory profiles of uterine ir-BEND and ir-GnRH during the oestrous cycle and early pregnancy; and (iii) secretory profiles of uterine ir-BEND and ir-GnRH in Large White and highly prolific Chinese Meishan gilts.

Materials and Methods

Collection of uterine fluids

For biochemical characterization of ir-BEND and ir-GnRH present in pig uterine fluids, reproductive tracts of cyclic gilts were collected from an abattoir. Uterine fluids were harvested using a modification of methods described by Bazer *et al.* (1978). Twenty milliliters of 0.9% saline solution were introduced into each uterine horn via a catheter. The saline solution was forced toward the oviduct and out through the catheter. Uterine fluids from these gilts were pooled and centrifuged at 3000 *g* for 10 min at 4°C to remove cellular debris. The supernatant was stored at -20°C until lyophilized.

Reverse phase (RP)-HPLC

The pooled uterine fluids were lyophilized (Labconco Corp., Kansas City, MO), reconstituted in deionized water, centrifuged at 10 000 *g*, and filtered through 0.80 µm and 0.22 µm filters (Millipore, Bedford, MA). In RP-HPLC analysis of ir-GnRH, the filtered uterine fluid was applied directly to the RP-HPLC system. For ir-BEND, to avoid possible interference of substances in the sample, the filtered uterine fluid (1.5 ml, equivalent to 35 ml of original uterine fluid) was first subjected to Sephadex G-50 gel filtration chromatography (1.5 cm × 110 cm column), using 0.01 mol phosphate buffer l⁻¹ containing 0.1% NaN₃ at 4°C. The G-50 column was precalibrated with blue dextran (void volume, *V*₀), BSA, soybean trypsin inhibitor, cytochrome C, aprotinin (Sigma, St Louis, MO), human β-lipotropin (BLPH) (a gift from NIAMDD, National Institutes of Health, Bethesda), and synthetic porcine BEND 1-31 (BEND) (Peninsula, Belmont, CA). The recovery rate of BEND following gel filtration was 76.1 ± 5.7%. Fractions corresponding to the peak of BEND were pooled, lyophilized, reconstituted in deionized water, centrifuged at 10 000 *g*, and filtered through 0.80 µm and 0.22 µm filters before RP-HPLC.

The HPLC system included two M510 pumps, a U6K sample injector with a 200 µl loop, a M481 absorbance detector, an automated gradient controller, M740 data modules (Waters Associate, Milford, MA), and a FRAC-100 fraction collector (Pharmacia LKB, Inc., Piscataway, NJ). Reconstituted uterine fluid samples (50 µl) were applied to a Waters µBondapak C18 column (0.39 cm × 30 cm) and eluted with HPLC grade H₂O containing 0.1% trifluoroacetic acid for 5 min. A linear gradient was initiated at a rate of 2% acetonitrile min⁻¹ for 30 min, followed by a linear gradient of 4% acetonitrile min⁻¹ for 5 min. The flow rate was 2 ml min⁻¹. Fractions (1 ml) were collected, lyophilized, reconstituted in radioimmunoassay buffer, and assayed for BEND or GnRH by radioimmunoassay. The column was precalibrated with synthetic BEND, α-N-acetylated BEND 1-31 (Nac-BEND) and GnRH (Peninsula). The recovery rates for BEND and GnRH from RP-HPLC were 79.6 ± 10.1 and 81.3 ± 7.8%, respectively.

Secretion of ir-BEND and ir-GnRH during the oestrous cycle and pregnancy

A total of 83 gilts, 39 Large White and 44 Meishan, was used for the study of uterine secretion of ir-BEND and ir-GnRH during the oestrous cycle and early pregnancy. The experiment was initiated after all gilts had experienced at least three oestrous cycles. Gilts assigned to be mated were artificially inseminated at 12 and 24 h (Large White) and 24 and 36 h (Meishan) after the onset of oestrus (Bazer *et al.*, 1988b). Gilts were assigned randomly to day 8 (four Large White and four Meishan gilts), day 10 (four Large White and three Meishan gilts), day 11 (three Large White and four Meishan gilts), day 12 (four Large White and four Meishan gilts), and day 14 (four Large White and five Meishan gilts) of the oestrous cycle, and day 8 (four Large White and four Meishan gilts), day 10 (four Large White and three Meishan gilts), day 11 (four Large White and five Meishan gilts), day 12 (four Large White and six Meishan gilts), and day 14 (four Large White and six Meishan gilts) of pregnancy, at which time uteri were obtained at slaughter. The presence of apparently normal conceptuses in uterine fluids confirmed that inseminated gilts were pregnant, and uterine fluids were collected (Bazer *et al.*, 1978). Inseminated gilts that were not pregnant were eliminated from the study. The total volume (per uterine horn) of recoverable uterine fluid was 19.1 ± 0.4 ml for Large White gilts (*n* = 39) and 19.5 ± 0.4 ml for Meishan gilts (*n* = 44).

Protein determination

The protein concentration in the uterine fluids was determined by the method of Lowry *et al.* (1951), using BSA as the standard.

BEND radioimmunoassay

The BEND radioimmunoassay was validated by Li and Chen (1987), and consists of an antiserum against porcine BEND (no. 83-1), BEND, and radioiodinated BEND. The antiserum cross-reacts with BEND and Nac-BEND of various species, and only slightly with porcine BLPH (5% on a mass basis). Na¹²⁵I was

purchased from the Amersham Corp. (Arlington Hts, IL). Phosphate buffer (0.05 mol l^{-1}) containing 0.2% BSA, 0.05% bacitracin, and 0.1% NaN_3 was used in the assay. The sensitivity for the measurement of porcine BEND was 12–15 pg per tube, and inter- and intra-assay coefficients of variation were 14.3% and 7.5%, respectively.

GnRH radioimmunoassay

The GnRH antiserum, CRR11, was a gift from V. D. Ramirez (University of Illinois). The GnRH radioimmunoassay (Hartter and Ramirez, 1980) has a sensitivity of 1 pg per tube at 90% binding. Inter- and intra-assay coefficients of variation were 10.4% and 5.8%, respectively.

Statistical analysis

Data were analysed by least squares analysis of variance using the General Linear Model procedure of the Statistical Analysis System (SAS Institute Inc., 1988) to detect main effects of breed (Large White versus Meishan), status (cyclic versus pregnant), day (oestrous cycle or pregnancy), their interactions on the total contents of ir-BEND and ir-GnRH, and concentrations of ir-BEND and ir-GnRH (pg mg^{-1} protein in uterine fluid). When significant interactions were detected, effects of day within each reproductive status and breed were determined.

Results

RP-HPLC characterization of ir-BEND

The RP-HPLC chromatographic profiles of BEND and Nac-BEND indicated a single peak for each at fractions 30–31 and 34–35, respectively (Fig. 1a). The majority (92.4%) of ir-BEND in pig uterine fluids eluted with a hydrophobicity similar to that of these two related peptides, with the major peak (59.4% of total ir-BEND applied) eluting at fraction 30–31 and a minor peak at fraction 34–35 (32.9%). Thus the ratio of BEND to Nac-BEND was approximately 1.8:1.

RP-HPLC characterization of ir-GnRH

The GnRH eluted as a single peak at fraction 44, whereas ir-GnRH of pig uterine fluids eluted as a major peak (83.6% of total ir-GnRH applied) at fraction 44 and a minor peak at fraction 4 (7.2%) (Fig. 1b).

Uterine content of ir-BEND in Large White and Meishan gilts

The ir-BEND in uterine fluids was affected by the interaction of breed and day ($P < 0.001$), but was independent of status when analysed as pg per uterus or pg mg^{-1} protein in uterine fluid. Data from cyclic and pregnant gilts of each breed were therefore combined within day for further analyses.

In Large White gilts, ir-BEND concentrations were higher ($P < 0.01$) on days 10 and 11 than on day 8, but by days 12 and 14 values were similar to those for day 8 (Fig. 2a). In

Fig. 1. Reverse phase HPLC (RP-HPLC) chromatographic profiles of (a) immunoreactive β -endorphin (ir-BEND) and (b) ir-GnRH from pig uterine fluids. Ir-BEND of uterine fluid contained both BEND and N-acetylated BEND (Nac-BEND) in a ratio of approximately 1.8:1, whereas 83.6% of total ir-GnRH co-eluted with synthetic GnRH. Arrows indicate the fractions at which the void volume (V_0) and synthetic peptides eluted.

Meishan gilts, ir-BEND decreased from day 8 to days 11, 12 and 14 ($P < 0.02$). Consequently, the amount of ir-BEND in the uterine fluid of Large White gilts was greater ($P < 0.02$) than that of Meishan gilts on days 10, 11, 12 and 14 (asterisks in Fig. 2a). Similar results were obtained when ir-BEND was expressed as pg mg^{-1} protein in uterine fluid (Fig. 2b). A breed by day interaction ($P < 0.001$) was detected since ir-BEND was higher for Large White gilts on day 11, but greater in Meishan gilts on day 8.

Uterine content of ir-GnRH in cyclic and pregnant gilts

Differences in total ir-GnRH content in uterine fluid (pg per uterus) were influenced by interaction of breed, status and day ($P < 0.02$). Total ir-GnRH content was similar on days 8, 10, 11 and 12 for cyclic and pregnant Large White gilts, but increased markedly ($P < 0.0001$) only for pregnant gilts on day 14 (Fig. 3a). For pregnant Meishan gilts, total ir-GnRH content was higher on day 8 ($P < 0.03$), day 12 ($P < 0.001$) and day 14 ($P < 0.01$), compared with that of cyclic Meishan gilts (Fig. 3b).

Concentration of ir-GnRH (pg mg^{-1} protein in uterine fluid) was affected by the interaction of status and day ($P < 0.04$), but not by breed. A status by day interaction indicated that ir-GnRH concentrations were highest ($P < 0.01$) on days 8 and 14 for pregnant gilts (Table 1).

Discussion

The study reported here characterized ir-BEND and ir-GnRH in pig uterine fluids using RP-HPLC. Results of chromatographic

Fig. 2. (a) Total content of immunoreactive β -endorphin ir-BEND and (b) ir-BEND concentration (pg mg^{-1} protein) in uterine fluids of (□) Large White and (■) Meishan gilts. Data from cyclic and pregnant gilts of the same breed were combined as there was no effect of status, status by day, or breed by status by day. *Significant differences between Large White and Meishan gilts on the same day of oestrous cycle or pregnancy ($P < 0.03$). Within Large White gilts, $b > a$ ($P < 0.01$). Within Meishan gilts, $c > d$ ($P < 0.02$). Overall effects: breed by day ($P < 0.001$).

Fig. 3. Total content of ir-GnRH in uterine fluids from cyclic (□) and pregnant (■) (a) Large White and (b) Meishan gilts. *Significant differences between cyclic and pregnant gilts ($P < 0.02$). Overall effects: breed by status by day ($P < 0.03$).

Table 1. Concentrations of ir-GnRH (pg mg^{-1} protein) in uterine fluids of gilts during the oestrous cycle and early pregnancy. Data from both Meishan and Large White gilts of the same reproductive status were combined

Day	Oestrous cycle	Pregnancy
8	70.0 ± 13.4	149.9 ± 30.6**
10	48.6 ± 9.3	69.9 ± 16.8
11	45.5 ± 4.7	61.8 ± 13.6
12	73.2 ± 9.5	61.2 ± 11.3
14	44.1 ± 18.3	98.0 ± 21.7*
Overall	56.4 ± 5.6	87.2 ± 9.4*

Values are expressed as means $\pm$ SEM. Overall: effects of status ($P < 0.01$), day ($P < 0.01$), breed ($P < 0.58$), status by day ($P < 0.04$) were detected. Significant difference between oestrous cycle and pregnancy of the same day (* $P < 0.01$ ** $P < 0.001$).

profiles showed that ir-BEND co-eluted with BEND and Nac-BEND in a ratio of approximately 1.8:1, similar to the ratio of 2:1 for BEND and Nac-BEND in porcine endometrial tissues reported by Li *et al.* (1992a). The BEND antiserum used in this study, which detects BEND and Nac-BEND, has a 5% cross-reactivity with BLPH on a mass basis. A substance(s) with a molecular weight similar to BLPH has been identified in pig uterine fluids (Li *et al.*, 1987). The presence of BLPH, BEND and Nac-BEND in uterine fluids appears to differ from their distribution in regions of the pig pituitary. The main products found in the pig anterior pituitary are BLPH and BEND, whereas in the intermediate lobe main products include the BLPH C'-fragment and its *N*-acetyl derivative (Smyth and Zakarian, 1980). The similarity in ir-BEND chromatographic profiles of pig uterine fluid and endometrial tissues suggests that ir-BEND in uterine fluids is probably a secretory product of the porcine endometrium. Our previous identification of ir-BEND in porcine endometrial tissues by immunocytochemical analysis (Li *et al.*, 1992a) and in a porcine endometrial cell line by RP-HPLC (Li *et al.*, 1992b) further support this speculation.

RP-HPLC analysis of ir-GnRH in uterine fluids revealed a major peak eluting at the same fraction as authentic GnRH and a small peak eluting at V_0 , suggesting that the majority of ir-GnRH in uterine fluids was GnRH. The peak of ir-GnRH at V_0 is probably due to degradation or association of ir-GnRH with unknown compounds. Similar artefacts have been noted during the extraction of ir-GnRH from the hypothalamus of sheep, rats and humans (King *et al.*, 1988), as well as the hypothalamus and uterus of rabbits (Li *et al.*, 1990) and pigs (Li *et al.*, 1993). Although the bioactivity of ir-GnRH was not examined in the current study, binding of ir-GnRH of uterine origin to pituitary GnRH receptors has been reported (Li *et al.*, 1990, 1993).

This study also compared differences in the uterine secretion of ir-BEND and ir-GnRH between Large White and Meishan gilts in an attempt to link these differences with their contrasting reproductive performance. Chinese Meishan pigs are noted for their exceptional prolificacy compared with Large White pigs (Yun, 1988). Studies comparing the reproductive biology

of Meishan and Large White gilts indicated that Meishan gilts reach puberty at an earlier age, expressed a longer period of oestrus, and had slightly shorter oestrous cycles than did Large White gilts (Bazer *et al.*, 1988b). In pregnant Meishan gilts, total glucose, fructose, sodium, potassium, immunoglobulin A and prostaglandins were greater in uterine fluids (Bazer *et al.*, 1991), and uterine fluids of Meishan gilts at day 14 of pregnancy contained higher antiviral activities, as indicated by interferon titres (La Bonnardiére *et al.*, 1991). Large White gilts secrete more luminal fluid mitogen than do Meishan gilts from day 10 to day 14 of the oestrous cycle and during pregnancy (Simmen *et al.*, 1989). Results of the present study indicated a significant difference between Meishan and Large White gilts in the pattern of ir-BEND secretion; in Meishan gilts, ir-BEND secretion was highest on day 8 and then declined to day 14 of the oestrous cycle and pregnancy, whereas ir-BEND secretion in Large White gilts reached peak values at day 10 to day 11 and subsequently declined. Meishan gilts demonstrated faster and more uniform conceptus development between days 8 and 12 of pregnancy and higher embryonic survival through day 30 of pregnancy than did Large White gilts. Meishan conceptuses elongated on days 11 and 12, compared with days 12 and 14 for Large White conceptuses (Bazer *et al.*, 1988a). However, on the basis of the present study, the higher embryonic survival rates in Meishan gilts cannot be ascribed to the earlier peak of ir-BEND and the overall lower amounts of ir-BEND.

In both breeds, ir-BEND secretory profiles for cyclic and pregnant gilts were comparable. This is in agreement with results from a previous study of ir-BEND content in the endometrium of cyclic and pregnant gilts in which no difference was detected in endometrial ir-BEND concentration (Li *et al.*, 1992a). In the same report, an upward trend in endometrial ir-BEND concentration during days 14 and 15 was also observed. The increase in endometrial content of ir-BEND on days 14–15 of the oestrous cycle or pregnancy was at least partially a result of the decline in uterine secretion of ir-BEND.

Uterine secretion of ir-GnRH reached peak values from days 12 to 14 of pregnancy, but remained unchanged between day 8 and day 14 in cyclic gilts. The increase in ir-GnRH in uterine fluids from days 12 to 14 of pregnancy may be a result of increased uterine secretion of ir-GnRH during early pregnancy, or secretion by the conceptus. It is not known whether pre-implantation pig conceptuses synthesize and secrete ir-GnRH; however, ir-GnRH has been reported in placental tissues of pigs (Li *et al.*, 1993) and other species (Gibbons *et al.*, 1975; Khodr and Siler-Khodr, 1980; Gautron *et al.*, 1981; Nowak *et al.*, 1984; Rice and Smirnis, 1990).

Although the biological role of uterine ir-BEND and uterine ir-GnRH remains to be elucidated, there are many possibilities. Uterine opiate receptors have been identified, and their binding capacity for opiate ligands has been shown to change during pregnancy (Baraldi *et al.*, 1985; Vertes *et al.*, 1986). The presence of BEND and opiate receptors suggests that uterine EOP may interact with uterine opiate receptors through an autocrine or paracrine mechanism to modulate uterine function, as well as embryonic growth and development (Sandman *et al.*, 1990; Vernadakis *et al.*, 1990). It has been shown that there are two classes of GnRH receptor in the human uterine leiomyomata, and the growth of leiomyomata may be blocked by a direct action of GnRH (Wiznitzer *et al.*, 1988). In normal uterine

tissues, it is unclear whether endometrial ir-GnRH can exert paracrine action on the myometrium. It is also possible that secretion of endometrial ir-GnRH into the uterine lumen potentiates placental ir-GnRH activity and assists in regulating the synthesis and secretion of steroid hormones during pregnancy, such as oestrogens and progesterone (Siler-Khodr *et al.*, 1986). Alternatively, BEND has been found to modulate immune responses *in vivo* and *in vitro* (Fischer, 1988; Blalock, 1989). Immunoreactive-GnRH, GnRH mRNA, and GnRH receptors have been demonstrated in the immune system (Marchetti *et al.*, 1989a; Emanuele *et al.*, 1990; Azad *et al.*, 1991), and GnRH agonists can modulate immune activities (Marchetti *et al.*, 1989a, b). Thus, another possible function for the decline in ir-BEND secretion and considerable increase of ir-GnRH in uterine fluids during days 12 to 14 of pregnant gilts may be the modulation of local immune responses and enhancement of the implantation process.

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References

- Azad N, Emanuele NV, Halloran MM, Tentler J and Kelley MR (1991) Presence of luteinizing hormone-releasing hormone (LHRH) mRNA in rat spleen lymphocytes *Endocrinology* **128** 1679–1681
- Baraldi M, Giarre G, Santi M, Facchinetti F, Petraglia F and Genazzani AR (1985) Pregnancy related changes of opiate receptors identified in rat uterine membranes by ³H-naloxone binding *Peptides* **6** 971–974
- Bazer FW, Roberts RM and Sharp DC, III (1978) *Methods in Mammalian Reproduction – Collection and Analysis of Female Genital Tract Secretions*. Academic Press, New York
- Bazer FW, Thatcher WW, Martinat-Botte F and Terqui M (1988a) Conceptus development in Large White and prolific Chinese Meishan pigs *Journal of Reproduction and Fertility* **84** 37–42
- Bazer FW, Thatcher WW, Martinat-Botte F and Terqui M (1988b) Sexual maturation and morphological development of the reproductive tract in Large White and prolific Chinese Meishan pigs *Journal of Reproduction and Fertility* **83** 723–728
- Bazer FW, Thatcher WW, Martinat-Botte F, Terqui M, Lacroix MC, Bernard S, Revault M and Dubois DH (1991) Composition of uterine flushings from Large White and prolific Chinese Meishan gilts *Reproduction, Fertility and Development* **3** 51–60
- Blalock JE (1989) A molecular basis for bidirectional communication between the immune and neuroendocrine systems *Physiological Reviews* **69** 1–32
- Bryant-Greenwood GD (1991) The human relaxin: consensus and dissent *Molecular and Cellular Endocrinology* **79** C125–C132
- Bryant-Greenwood GD, Tashima L, Greenwood FC, Taylor E and Peaker M (1991) Endometrial relaxin: effects of mastectomy in the cyclic and pregnant guinea pig *Endocrinology* **129** 2119–2125
- Emanuele NV, Emanuele MA, Tentler J, Kirsteins L, Azad N and Lawrence AM (1990) Rat spleen lymphocytes contain an immunoreactive and bioactive luteinizing hormone-releasing hormone *Endocrinology* **126** 2482–2486
- Fischer EG (1988) Opioid peptides modulate immune functions. A review. *Immunopharmacology and Immunotoxicology* **10** 265–326
- Gautron JP, Pattou E and Kordon C (1981) Occurrence of higher molecular forms of LHRH in fractionated extracts from rat hypothalamus, cortex and placenta *Molecular and Cellular Endocrinology* **24** 1–5
- Gibbons JM, Mitnick M and Chieffo V (1975) In vitro biosynthesis of TSH- and LH-releasing factors by the human placenta *American Journal of Obstetrics and Gynecology* **121** 127–131
- Hartert DE and Ramirez VD (1980) The effects of ions, metabolic inhibitors, and colchicine on luteinizing hormone-releasing hormone release from superfused rat hypothalami *Endocrinology* **107** 375–382

- Khodr GS and Siler-Khodr TM (1980) Placental luteinizing hormone-releasing factor and its synthesis *Science* **207** 315–317
- King JA, Hassan MF, Mehl AEI and Millar RP (1988) Gonadotropin-releasing hormone molecular forms in mammalian hypothalamus *Endocrinology* **122** 2742–2752
- La Bonnardiere C, Martinat-Botte F, Terqui M, Lefevre F, Zouari K, Martal J and Bazer FW (1991) Production of two species of interferon by Large White and Meishan pig conceptuses during the peri-attachment period *Journal of Reproduction and Fertility* **91** 469–478
- Li WI and Chen CL (1987) Running and shipping elevate plasma levels of β -endorphin-like substance (B-END-LI) in Thoroughbred horses *Life Sciences* **40** 1411–1421
- Li WI, Chen CL, Hansen PJ and Bazer FW (1987) β -Endorphin in uterine secretions of pseudopregnant and ovariectomized, ovarian steroid-treated gilts *Endocrinology* **121** 1111–1115
- Li WI, Chen CL and Chou JY (1989) Characterization of a temperature-sensitive β -endorphin-secreting transformed endometrial cell line *Endocrinology* **125** 2862–2867
- Li WI, Chin MP and Wu HX (1990) Synthesis and secretion of immunoreactive GnRH-like substances (ir-GnRH-LI) from rabbit uterus in vivo and in vitro *Biology of Reproduction* **42** (Supplement 1) 95
- Li WI, Sung LC and Bazer FW (1991a) Immunoreactive methionine-enkephalin (ir-MENK) secretion by porcine uterus *Endocrinology* **128** 21–26
- Li WI, Wu H and Kumar AM (1991b) Synthesis and secretion of immunoreactive-methionine-enkephalin from rabbit reproductive tissues in vivo and in vitro *Biology of Reproduction* **45** 691–697
- Li WI, Wu H and Chen CL (1992a) Endometrial immunoreactive- β -endorphin increases during mid-estrous cycle and early pregnancy in gilts *Biology of Reproduction* **46** 740–746
- Li WI, Wu H, Chin MP and Wu G (1992b) Porcine endometrial epithelial cells immortalized by transfection with origin-defective, temperature-sensitive simian virus 40 DNA *Life Sciences* **51** 1969–1979
- Li WI, Chin MP and Jiao S (1993) Immunoreactive gonadotropin-releasing hormone (GnRH) in the porcine reproductive tissues *Peptides* **14** 543–549
- Lowry OH, Rosebrough NJ, Farr AL and Randall RJ (1951) Protein measurement with the folin phenol reagent *Journal of Biological Chemistry* **193** 265–275
- Lundin S, Forman A, Rechberger T, Svane D and Andersson KE (1989) Immunoreactive oxytocin and vasopressin in the non-pregnant human uterus and oviductal isthmus *Acta Endocrinologica* **120** 239–244
- Marchetti B, Guarcello V, Morale MC, Bartoloni G, Farinella Z and Scapagnini U (1989a) Luteinizing hormone-releasing hormone (LHRH) binding sites in the rat thymus: characteristics and biological functions *Endocrinology* **125** 1025–1036
- Marchetti B, Guarcello V, Morale MC, Bartoloni G, Raiti F, Palumbo G, Farinella Z, Cordaro S and Scapagnini U (1989b) LHRH agonist restoration of age-associated decline of thymus weight, thymic LHRH receptors, and thymocyte proliferative capacity *Endocrinology* **125** 1037–1045
- Nowak RA, Wiseman BS and Bahr JM (1984) Identification of a gonadotropin-releasing hormone-like factor in the rabbit fetal placenta *Biology of Reproduction* **31** 67–75
- Petraglia F, Facchinetti F, M'Futa K, Ruspa M, Bonavera JJ, Gandolfi F and Genazzani AR (1986) Endogenous opioid peptides in uterine fluid *Fertility and Sterility* **46** 247–251
- Rice GE and Smirnis M (1990) Identification of luteinizing hormone releasing hormone-like immunoactivity in ovine cotyledons *Regulatory Peptides* **27** 51–59
- Sandman CA, Barron JL, Demet EM, Chicx-Memet A, Rothenberg SJ and Zea FJ (1990) Opioid peptides and perinatal development: is β -endorphin a natural teratogen? *Annals of the New York Academy of Science* **579** 91–108
- SAS Institute Inc. (1988) SAS/STAT User's Guide. SAS Institute Inc., Cary, NC
- Siler-Khodr TM, Khodr GS, Valenzuela G and Rhode J (1986) Gonadotropin-releasing hormone effects on placental hormones during gestation II. Progesterone, estrone, estradiol and estriol *Biology of Reproduction* **34** 255–262
- Simmen RCM, Simmen FA, Ko Y and Bazer FW (1989) Differential growth factor content of uterine luminal fluids from large white and prolific Meishan pigs during the estrous cycle and early pregnancy *Journal of Animal Science* **67** 1538–1545
- Smyth DG and Zakarian S (1980) Selective processing of β -endorphin in regions of porcine pituitary *Nature* **288** 613–615
- Vernadakis A, Sakellaridis N, Geladopoulos T and Mangoura D (1990) Function of opioids early in embryogenesis *Annals of the New York Academy of Science* **579** 109–122
- Vertes M, Pamer Z and Garai J (1986) On the mechanism of opioid-oestradiol interactions *Journal of Steroid Biochemistry* **24** 235–238
- Wahlstrom T, Laatikainen T, Salminen K and Leppaluoto J (1985) Immunoreactive β -endorphin is demonstrable in the secretory but not in the proliferative endometrium *Life Sciences* **36** 987–990
- Wiquist I, Norstrom A and Wiquist N (1984) Effects of oxytocin on cervical and uterine connective tissue *Acta Endocrinologica* **106** 271–276
- Wiznitzer A, Marbach M, Hazum E, Insler V, Sharoni Y and Levy J (1988) Gonadotropin-releasing hormone specific binding sites in uterine leiomyomata *Biochemical and Biophysical Research Communications* **152** 1326–1331
- Yun WL (1988) Pig breeds in China *Pig News and Information* **9** 407–413