# EFFECT OF NALOXONE AND MORPHINE ON THE ESTRADIOL-INDUCED LH SURGE IN GILTS

## EFECTO DE NAXOLONE - MORFINA SOBRE EL ESTRADIOL EN LA INDUCCION DE LA CURVA DE LH EN GANADO PORCINO

Ziecik*, A.J., H. A. Yearwood* and F. España España**

* Division of Reproductive Endocrinology and Pathophysiology, Centre for Agrotechnology and Veterinary Sciences, Polish Academy of Sciences, 10-718 Olsztyn, Poland.

** Agrotechnology-Research Centre (CIDA). Department of Animal Production. Apdo. 4240. Córdoba. España.

### Additional Keywords

LH. Opioids. Pig.

### Palabras clave adicionales

LH. Opioides. Cerdo.

## SUMMARY

This study examines the role of endogenous opioids in control of the estradiol-induced luteinizing hormone (LH) surge. In the experiment (Exp.) 1, fifteen Duroc gilts were ovariectomized at the age of 5 to 6 months and one month later challenged with estradiol benzoate (EB; 25 μg/kg i.m.) at 0 h of the experiment. Control animals (n=5) received continuos i.v. infusion of saline solution (5 ml/h) from 30 to 34 h and from 60 to 64 h preceded by single i.v. injection of 10 ml of saline in each case. The naloxone group (n=5) received naloxone (1 mg/kg) continuously between 30 to 34 h after injection of EB, preceded by a single i.v. administration of the same amount of the drug. The morphine group (n=5) was given morphine (1 mg/kg) continuously from 60 to 64 h after EB preceded by a bolus i.v. injection of the same dose of opioid. In Exp.2 ten Duroc gilts ovariectomized at the age of 6 months were prepared and challenged with EB in the same way as in Exp.1. The control group (n=5) received saline infusion from 54 to 60 h after the EB treatment while the naloxone group (n=5) received naloxone between 54 to 60 h.

In Exp.1 EB alone suppressed LH values (pmol/l) from 28.1 - 36.9 to 2.2 - 4.4 during 6 to 48 h in all groups (negative feedback phase). In controls and NAL groups LH increased to 67.7±6.7 and 56.7±7.3 between 54 to 96 h (positive feedback phase), respectively ($p>0.05$). Hovewer, the beginning of LH surge was delayed for 6 h in naloxone given animals. LH in morphine treated gilts was lower between 54 to 96 h than in controls and naloxone gilts ($p<0.05$).

These data show that while endogenous opioid peptides may not be involved in EB inhibition of LH secretion, exogenous opioids suppresses the EB-induced LH surge in gilts.

## RESUMEN

Este estudio examina el papel de los opioides endógenos en el control del estradiol en la inducción de la curva de la hormona luteinizante (LH) en cerdas.

En el experimento 1 (Exp.1), quince cerdas

nulíparas púberes, raza Duroc, fueron ovariectomizadas a los 5-6 meses de edad. Un mes más tarde (a la hora 0 del experimento), se les administró benzoato de estradiol ( EB; 25 mg/kg i.m.) y se asignaron tres grupos. El control recibió (n=5) en forma continua una infusión salina i.v. ( 5 ml/h), entre las 30-34 h y 60-64 h de iniciado el Exp., precedida en ambos casos por una inyección (i.v.) de 10 ml de solución salina. El grupo tratado con naloxona (NAL; n=5 ), recibió NAL (1 mg/kg ) contínuamente entre las 30-34 h después de la inyección i.m. de estradiol, precedida por una dosis única ( 1 mg/kg ) de NAL. El tercer grupo (n=5) se inyectó contínuamente con morfina (1 mg/kg ) entre las 60-64 h a partir de la 0 h, precedida por una dosis i.v. del mencionado opioide. En el Exp.2, diez cerdas nulíparas púberes, raza Duroc, ovariectomizadas y tratadas con EB en la misma forma que en el Exp.1, se agruparon en hembras control (n=5), que recibieron solución salina de 54 a 60 h después de la dosis de EB y un grupo tratado con naloxona (n=5) entre las 54-60 h después de la dosis de EB .

En el Exp.1, el tratamiento sólo con EB suprime los valores (pmol/l) de LH de 28,1- 36,9 a 2,2-4,4, durante 6-48 h en todos los grupos (fase *feedback* negativo). En el grupo control y tratado con NAL los valores de LH aumenta a 67,7±6,7 y a 56,7±7,3 entre las 54 a 96 h (fase de *feedback* positivo), respectivamente ($p>0,05$). Sin embargo, el comienzo de la curva de LH fue retrasado por 6 horas en el grupo tratado con NAL. En las cerdas tratadas con morfina los valores de LH fueron más bajos entre las 54-96 h, comparados con el grupo control y las tratadas con NAL ( $p<0,05$).

Estos datos muestran que mientras los péptidos-opioides endógenos pueden no estar involucrados en la acción inhibitoria del EB en la secreción de la LH, los opioides exógenos suprimen la curva de LH inducida por EB en cerdas.

## INTRODUCTION

The increasing serum concentration of estradiol secreted by developing preovulatory follicles stimulates the preovulatory luteinizing hormone (LH) surge and subsequent ovulation. Estradiol exerts biphasic actions of LH in the ovariectomized female pig (Dial et al; Foxcroft *et al.*, 1985). Initially there is a period of negative feedback followed by an LH surge that is indistinguishable from the natural one (Foxcroft *et al.*, 1985; Stevenson *et al.*, 1981). Estrogen inhibits or greatly reduces the release of LH for a period of approximately 48 to 60 hours. Apart from the initial 12 h of this period, the pituitary is able to respond to LH releasing hormone (LHRH) during the negative phase (Britt et al, 1991). It is suggested than the negative and positive feedback effect of estrogen in pigs is predominantly at the hypothalamus rather that at the pituitary gland (Britt et al, 1991; Kesner, 1988; Kesner *et al.* 1987; Ziezic *et al.*, 1988). The fact that only a few LHRH neurons have estrogen receptors suggests that estrogen does not act directly on LHRH neurons but rather inhibits or triggers LHRH release by acting on other types of neurons which terminate on and then affect the activity of LHRH neurons (Shivers *et al.*, 1983).

Naloxone, an endogenous opioid peptides (EOP) antagonist, stimulated LH release in the luteal phase of the pig estrous cycle when progesterone secretion was high but not during follicular phase of the estrous cycle (Barb *et al.*, 1986). Secretion of LH was suppressed after the injection of the EOP

agonist morphine into the lateral cerebral ventricle of both ovariectomized prepubertal and ovariectomized mature gilts (Barb *et al.*, 1989; Estienne *et al.*, 1990). However, there are not many reports on the effects of EOP antagonist and agonist on the estrogen induced preovulatory - like surge in pigs and other species. This study examines LH secretion in ovariectomized, estradiol-primed gilts when EOP activity is suppressed during the negative and positive feedback phase by infusion of naloxone during positive feedback or when opioid activity is enhanced by exogenous opioid agonist-morphine delivered during positive feedback.

## MATERIALS AND METHODS

### ANIMALS AND EXPERIMENTAL PROCEDURE

*Experiment 1.* Experiment 1 was designed to examine LH secretion when endogenous opioids are suppressed by naloxone during negative feedback and when opioids activity is increased by morphine during positive feedback. In this experiment fifteen prepubertal Duroc gilts weighing about 75 kg were ovariectomized at the age of 5 to 6 months and challenged with estradiol benzoate (EB; Polfa; Kutno, Poland 25μg/kg i.m.) one month later at 0 h of the experiment. Two days before the injection of EB each gilt was fitted under general anaesthesia with an indwelling catheter in the anterior vena cava via a cephalic vein (Kotnica *et al.*, 1978). It was exteriorized by a passage under the skin to the back to facilitate blood collection and infusion of an agonist and an antagonist of opioids. Gilts were assigned to three treatment groups. Control animals (n=5) received continuous pomp i.v. infusions of saline solution (5 ml/h) from 30 to 34 h and from 60 to 64 h after injection of EB preceded by single i.v. injection of 10 ml saline in each case. The naloxone (NAL) group (n=5) received single 10 ml injection of NAL (1 mg/kg; Sigma Chemicals., St.Louis, MO, USA) at 30 h and then NAL (20 ml) was infused by pomp continuously at rate 1 mg/kg/4 h until 34 h after the EB treatment. An intention was to maintain naloxone in circulation during part the negative feedback phase.

The morphine (MORPH) group (n=5) were given MORPH (1 mg/kg) in 10 ml bolus injection at 60 h and then continuously (20 ml) from 60 to 64 h in dose of 1 mg/kg/4 h. The application design was intended to maintain MORPH in the circulation during the first part of expected LH surge. NAL or MORPH was dissolved in 0.9 p. 100 saline solution and continous infusions were performed through PVC extension tubings (2.5 to 3 m in length) at a rate of 5 ml/h. Heparinized blood samples (5 ml) for analyses of LH were collected every 6 h from 0 h, i.e. immediately before EB injection, to 96 h after EB, with hourly collection between 30 to 42 h for NAL and control groups and from 60 to 72 h for the MORPH and

control groups.

*Experiment 2.* Experiment 2 was designed to study LH secretion when endogenous opioids are suppressed by naloxone during a positive feedback phase. Ten Duroc gilts weighing about 80 kg and ovariectomized at the age of 6 months were prepared and challenged with EB in the same way as in Experiment 1 and assigned into two equal groups (n=5). The control group received continuous i.v. infusion of saline solution (5 ml/h) from 54 to 60 h after the injection of EB while the NAL group received i.v. infusion of NAL (1 mg/kg/6 h) continuously between 54 to 60 h after EB preceded by bolus i.v. injection of 1 mg/kg of this EOP antagonist. Blood samples for radioimmunoassay of LH were collected every 6 h from 0 to 96 h after EB with an hourly collection between 54 to 60 h for the control and NAL group. In both experiments blood samples were centrifuged for 15 min at 1000 x g immediately after the collection and the plasma stored at -20°C until analysis.

**HORMONE ANALYSIS**

Serum concentrations of LH were determined by a homologous radioimmunoassay (Ziezic *et al.*, 1992). Purified porcine LH (USDA-pLH-1-1) was used for preparation of the radio-iodinated antigen and USDA-pLH-B-1 as a standard. The assay sensitivity at 95 p. 100 binding was 1.7 pmol/l. Samples were quantified in two assays with average intra and inter-assay coefficients of variation of 8 and 14 p. 100 respectively. Gilts from different treatments were represented in each assay.

**STATISTICAL ANALYSIS**

Data was analysed by analyses of variance for repeated measures (Gill and Hafs, 1971). To facilitate comparison of LH responses to EB, the period during which suppression of LH occurred, was defined as the negative feedback phase (period 1; 0 to 48 h), and the period during which LH secretion patterns changed to produce an increase in LH levels in individual gilts, was defined as the positive feedback phase (period 2; 54 to 96 h). Sources of variation included treatment (control, morphine, naloxone), period (negative feedback phase, positive feedback phase) and interaction of treatment and period. The treatment by period interaction was significant, so subsequent analyses were performed separately for periods 1 and 2. To characterize an estradiol induced LH surge the following parameters were established: a) time to emergence of LH surge was estimated by the LH surge initiating rise (LH - SIR) parameter proposed by Testart *et al.* (1982). In our studies, LH - SIR corresponded to the time when the plasma LH amplitude was three times the mean of four preceding values and the surge had to encompass at least 3 samples collected at 6 h intervals. To be defined as a surge of LH, the change in LH during the period of positive feedback had to meet the following criteria: 1) the peak of LH had to be

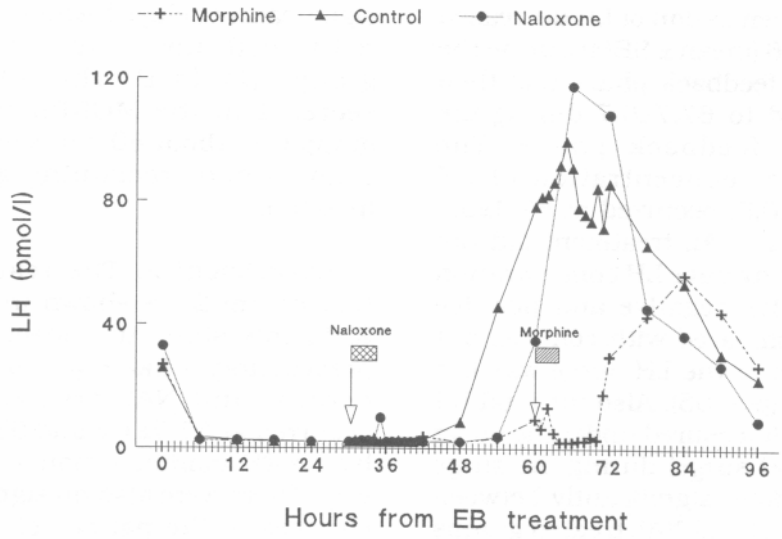


***Figure I.*** *Concentrations of LH (pmol/l) in plasma of gilts given naloxone (n=5) during negative feedback, morphine (n=5) during positive feedback and saline (control; n=5). Standard errors for period of negative and positive feedback are in* ***table I****. Morphine inhibited LH release p<0.05) during the positive feedback phase.* (Concentraciones plasmáticas de LH (pmol/l), en hembras nulíparas púberes tratadas con naloxona (n=5) durante la fase de *feedback* negativo, morfina (n=5) durante la fase de *feedbak* positivo y solución salina (control; n=5). Los errores típicos en los periodos de *feedback* negativo y positivo se señalan en la **tabla I**. La morfina inhibió la secreción de la LH (p<0,05) durante la fase de *feedback* positivo).

at least fivefold the value calculated for SIR and duration of LH release was longer than 12 h and 2) the increasing and decreasing phases had to occur during 54 to 96 h. The criteria were similar to those described previously (Ziezic *et al.*, 1987; Britt *et al.*, 1991); b) maximum serum LH concentration after EB (amplitude of LH surge); c) time to the maximum plasma LH concentration after EB injection (time of greatest surge); d) total LH secreted during the surge. The total amount of LH released was calculated by measuring, with a planimeter the total area under the LH curve during the surge. Analyses not involving repeated measures e.i. concerning parameters a, b, c and d were performed utilizing one way analysis of variance (ANOVA).

## RESULTS

*Experiment 1.* In Experiment 1 oestradiol benzoate caused suppression of LH values (pmol/l) from 28.1 - 36.9 to 2.2 - 4.4 within the first 6 h after the administration (**figure1**). In control females the ave-

rage concentration of LH decreased to 6.5±0.6 (mean ± SEM) during the negative feedback phase and then increased to 67.7±6.7 during the positive feedback phase. The maximum concentration of LH (132.2±20.5) occurred at 67.4±3.1 h (**table I**). NAL treatment did not affect the average LH concentration during the negative and positive phase compared with controls but the onset of the LH surge was 6 h delayed ($p<0.05$). Also the total LH secreted (measured under the curve for the LH surge) during the surge did not differ significantly between the control and NAL treated groups (**table I**). In contrary LH concentrations (26.0±1.9) in MORPH treated gilts were lower between 54-96 h than in controls and NAL treated gilts ($p<0.05$). The time to emergence of the LH surge and the time of the greatest surge were also significantly delayed when compared to both the control and NAL groups (**table I**). The total LH secreted in the MORPH treated group was about 60 per cent lower than in both remaining groups ($p<0.05$).

*Experiment 2.* The results of Experiment 2 are shown in **figure 2**. In this study the onset of the preovulatory - like surge of LH in control and NAL treated gilts occurred at 57.5±1.2 and 53.5±3.2 h after EB administration, respectively. There were also no significant changes in the pattern of the LH surges during the positive phase of feedback. The mean time of the greatest surge was registered at 66.3±5.6 h in the control and at 64.2±1.7 h in the NAL treated group. The peak level of the NAL treated group (184.4±23.8) was slightly

***Table I.*** *Concentrations of LH (mean±SEM) during the periods of negative (0-48 h) and positive (54-96 h) feedback phase, time to emergence of LH surge, time and amplitude of the greatest surge and total LH secreted during surge.*
(Concentraciones plasmáticas de LH (X ±ETX), durante la fase de feedback negativo (0 - 48 h) y positivo (54 - 96 h), tiempo de aparición de la curva de LH, tiempo y amplitud de la curva y total de LH secretada durante la curva).

| Treatment group | n | Negative feedback concentrations (pmol/l) | Positive feedback concentrations (pmol/l) | Time to emergence of LH surge (h) | Time of greatest surge (h) | Amplitude of greatest surge (pmol/l) | Total LH secreted (arbitary units) |
|---|---|---|---|---|---|---|---|
| Control | 5 | 6.5±0.6 | 67.7±6.7 | 54.0±1.8a | 67.4±3.1a | 132.2±20.5 | 3.5±0.7a |
| Naloxone | 5 | 6.5±0.6 | 56.7±7.3 | 60.0±0.0b | 69.0±1.7a | 130.7±32.9 | 3.8±1.0a |
| Morphine | 5 | 5.4±0.6 | 26.0±1.9b | 71.0±0.9c | 82.4±3.3b | 70.7±8.8 | 1.4±0.2b |

a, b, c - Means with a different superscripts within a column are different ($p<0.05$)

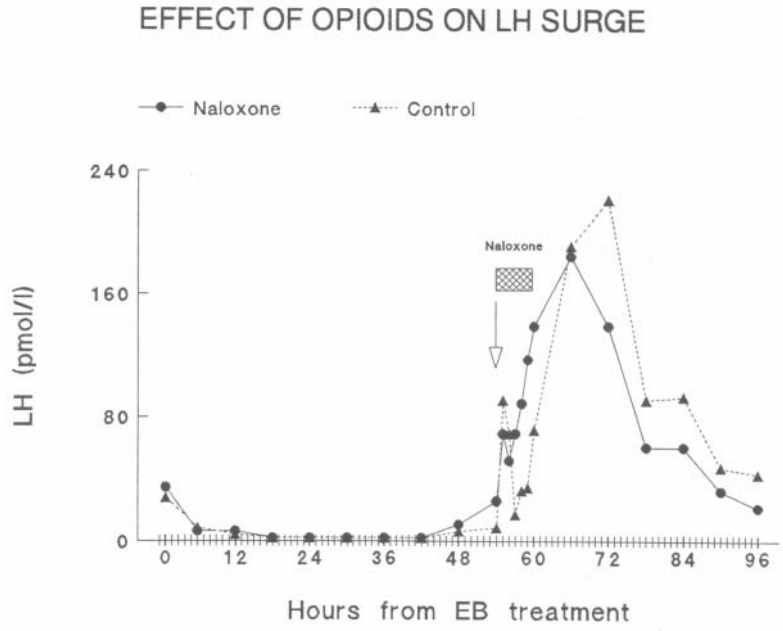


***Figure 2.*** *Concentrations of LH (pmol/l) in plasma of gilts given naloxone (n=5) and saline (control; n=5) during the positive feedback phase. Standard errors were generally proportional to the mean. Naloxone did not affect (p>0.05) LH release during the positive feedback phase.* (Concentraciones plasmáticas de LH (pmol/l en hembras nulíparas púberes tratadas con naloxona (n=5) y solución salina (control; n=5) durante la fase de *feedback* positivo. Los errores típicos fueron generalmente proporcionales a la media . La naloxona no afectó (p>0.005) la secreción de LH durante la fase de *feedback* positivo).

lower when compared with controls (221.3±23.1). The total LH under the curve for the LH surge (arbitrary units) was similar in the control and NAL treated groups (5.3±0.9 vs 5.0±0.8) respectively.

## DISCUSSION

Endogenous opioid peptides (Kraeling *et al.*, 1992) are strongly involved in the regulation of LHRH and consequently LH secretion in the pig. The experiments presented in this paper have asked two questions - is the estrogen-induced negative feedback of LH secretion caused by a high opioid tone?; can increased OR decreased opioid activity during the positive feedback phase affect the LH surge?

In our study the endogenous peptide antagonist - NAL applied from 30 to 34 h after EB injection did not influence LH secretion during the negative feedback phase but did influence the timing of the LH surge onset during the positive phase. It is interesting that Kraeling *et al.* (1992) have shown that intravenous infusion of NAL 42 to 66 hours after estradiol injection also delayed the emergence of the

LH surge in ovariectomized gilts. Such a long term effect of naloxone found in our study is difficult to explain. It may mean that opioid receptors are present during negative feedback but the inhibition of LHRH by EOP is overridden by other neurochemical regulators. Alternatively, EOP do not participate in the inhibition of LHRH/LH release during the negative feedback phase in gilts.

Barb *et al.* (1988) were able to stimulate LH secretion by NAL in ovariectomized and progesterone treated mature but not prepubertal gilts. On the other hand, in an in vitro study examining LHRH release from the median eminence of ovariectomized gilts primed with estradiol benzoate or progesterone or estradiol benzoate and progesterone, the highest LHRH release was recorded after the estrogen treatment (Okrasa *et al.*, 1990). These data also indicated that the inhibition of endogenous opioid action at the median eminence stimulated LHRH release depended on both progeste-rone and estradiol milieu.

Infusion of MORPH during 60-64 hours after EB in our study not only delayed the estradiol-induced surge but also decreased LH secretion for 60 percent during the positive feedback phase. A delay in the emergence of the estradiol-induced LH surge in prepubertal ovariectomized gilts was also found where MORPH was intracerebroventricularly injected at 40 and 48 hours after estradiol but magnitudes of the LH surge and total LH secreted were not different among MORPH and saline-treated pigs (10). In contrast, MORPH given s.c. at 8 h intervals was unable to affect the characteristics of the estradiol induced LH surge in ovariectomized miniature pigs as reported by Kuneke *et al.* (1993) although the doses used were adequate enough to delay the onset of estrus after weaning (Armstrong *et al.*, 1988). The differences between the experiments relating to the effect of morphine on the estradiol-induced LH surge in ovariectomized pigs may be explained by the manner of MORPH administration (intracerebroventricularly, s.c. and i.v.) or time of opioid agonist infusion or injections (40 to 48, 0 to 72 and 60 to 64 hours after estradiol injection, respectively).

The administration of naloxone during the beginning of the positive feedback phase did not affect the amount of LH released during the infusion period. There is an explanation that EOP activity is suppressed during the positive feedback phase so that LHRH release from the median eminence during the positive feedback surge has an *all or none* effect on LH secretion. In the study presented by Kuneke *et al.* (1993) when immature 60 - day -old gilts were challenged with NAL for 6 to 48 h during the expected estradiol-induced LH surge NAL also did not influence LH secretion. However, in these animals LH concentrations did not decrease to negative feedback levels in the post surge period.

Collectively, this paper demons-

trates that while opioids may not be involved in the EB inhibition of LH secretion during the negative feedback phase, exogenous opioids suppresses the EB-induced LH surge during the positive phase in gilts.

## ACKNOWLEDGEMENTS

We thank the USDA Hormone Program for porcine pituitary hormones and CSIC for supporting A.J.Ziecik and F.España. We also thank J.Klos and S.J.Rzucidlo for the technical assistance. Some of the results were presented in a preliminary form at the Winter Meeting of the Society for the Study of Fertility, Paris, 13-14 December, 1991.

## REFERENCES

***Armstrong, J.D, R.R. Kraeling and Jh. Britt.1988.*** Morphine suppresses luteinizing hormone concentra-tions in transiently weaned sows and delays onset of estrus after weaning. *J. Anim. Sci.* 66: 2216-2223.

***Barb, C.R, R.D. Kineman, J.S. Kesner, G.B. Rampacek and R.R. Kraeling. 1989.*** Luteinizing hormone secretion following intracerebroven-tricular administration of morphine in the prepubertal gilt. *Life Sci.* 45: 691-696

***Barb, C.R, R.R. Kraeling, G.B. Rampacek and C.S. Whisnant 1986.*** Influence of the stage of the estrous cycle on endogenous opioid modula-tion of luteinizing hormone, prolactin and cortisol secretion in the gilt. *Biol. Reprod.* 35: 1162-1167.

***Barb, C.R, G.B. Rampacek, R.R. Kraeling, E. Estienne and C.E. Taras. 1988.*** Estienne *et al*. Absence of brain opioid peptide modulation of luteinizing hormone secretion in the prebpubertal gilts. *Biol. Reprod.* 39: 603-609.

***Britt, J.H, K.L. Esbenshade, A.J. Ziecik. 1991.*** Roles of estradiol on gonadotropin-releasing hormone in controlling negative and positive feedback associated with the luteinizing hormone surge in ovariectomized pigs. *Biol. Reprod.* 45: 478-485.

***Dial, G.D, O.K. Dial, G.W. Beaver, S.D. Glenn, P.J. Dziuk. 1983.*** Estrous behavior and circadian discharge of luteinizing hormone in the prepubertal gilt in response to exogenous estrogen. *Biol. Reprod.* 29: 1047-1056.

***Estienne, M.J, J.S. Kesner, C.R. Barb, R.R. Kraeling, G.B. Rampacek and C.E. Estienne. 1990.*** Gonadotropin and prolactin secretion following intraventricular administration of morphine in gilts. *Proc. Soc. Exp. Biol. Med.* 193: 192-7

***Foxcroft, G.R, F. Elsaesser, K. Stickney, N.B. Haynes, H.L. Back. 1985.*** Ovarian estrogen-dependent maturation of the LH/FSH surge mechanism during prepubertal development in the gilt. *J. Endocrinol.* 101: 371-380.

***Gill, J.L and H. Hafs. 1971.*** Analysis of repeated measurements of animals. *J. Anim. Sci.* 33: 331-336.

***Kesner, J.S. 1988.*** Site of action of estradiol-induced luteinizing hormone surge in farm animals and primates. *Domest. Anim. Endocrinol.* 5: 265-281.

***Kesner, J.S, R.R. Kraeling, G.B. Rampacek, B. Johnson. 1987.*** Absence of an estra-diol-induced surge of luteinizing hormone in pig receiving unvarying pulsatile gonadotropin releasing hormone stimulation. *Endocrinology*. 121: 1862-1869.

***Kotwica, J, T. Krzymowski J. Debek. 1978.*** Kaniulizowanie naczyñ zylnych do badañ endokrynologicznych. *Med.Wet*. 34: 118-120.

***Kraeling, R.R, C.R. Barb, L.S. Leshin and G.B. Rampacek. 1992.*** Central nervous system peptide and amino acid modulation of luteinizing hormone and prolactin secretion in the pig. *J. Physiol. Pahrmacol*. 43 (Suppl 1): 79-103

***Kuneke, G, N. Parvizi, F. Elsaesser. 1993.*** Effect of naloxone and pulstaile luteinizing-hormone - releasing hormone infusions on oestradiol-induced luteinizing hormone surges in immature gilts. *J. Reprod. Fertil.* 97: 395-401.

***Okrasa, S, R. Weigl, J. Mah and J. Tilton. 1990.*** Concentration of prolactin, LH and FSH after naloxone administration in follicular phase gilts. *Anim. Reprod. Sci*. 22: 49-56.

***Shivers, B.D, R.E. Harhan, J.I. Morell and D.W. Pfaff. 1983.*** Absence of oestradiol concentration in cell nuclei of LHRH - immunoreactive neurons. *Nature* 304: 345-347.

***Stevenson, J.S, N.M. Cox, J.H. Britt. 1981*** Role of ovary in controlling luteinizing hormone, follicle stimulating hormone, and prolactin secretion during and after lactation in pigs. *Biol. Reprod*. 24: 341-353.

***Testar, J, R. Frydman, K. Nahoul, J. Gremier, M.C. Feinstein, M. Roger*** *et al.* ***1982.*** Steroids and gonadotropins during the late preovulatory phase of the menstrual cycle. Time relation-ships between plasma hormones levels and luteinizing hormone surge onset. *J. Steroid. Biochem*. 17: 675-682.

***Ziecik, A.J, J.H. Britt and K.L. Esbenshade. 1988.*** Short-loop feedback control of the estrogen induced luteinizing hormone surge in pigs. *Endocrinology* 122: 1658-1662.

***Ziecik, A.J, M. Jedlinska, J.S. Rzucidlo. 1992.*** Effect of estradiol and progestero-ne on myometrial LH/hCG receptors in pig. *Acta Endocrinol*. (Copenh) 127: 185-188.

***Ziecik, A, J.E. Tilton, F. Espana and R. Weigl. 1987.*** Effect of human chorionic gonadotropin on preovulatory luteinizing hormone surge and ovarian hormone secretion in gilts. *J. Anim. Sci.* 64: 1134-1143.

*Recibido: 18-X-93. Aceptado: 24-V-94.*