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Original article

Oestrus and LH reponses to oestradiol during lactational anoestrus in Chinese Meishan and Large White sows

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Summary — To investigate endocrine mechanisms associated with the occasional occurrence of fertile oestrus during lactation in the high prolific Chinese Meishan (MS) breed, the incidence of oestrus and changes in plasma luteinizing hormone (LH) levels before and after oestradiol benzoate (OB, 15 μg/kg body weight) administration on day 22 was compared in 4 MS and 6 Large White (LW) sows. All sows exhibited oestrus in response to OB. Only 1 sow (MS) ovulated in response to OB, became pregnant and farrowed. Mean plasma LH levels before OB were low (MS: 0.38 ± 0.06 ng LH/ml, LW: 0.29 ± 0.04 ng LH/ml, ns). LH levels above 2 ng/ml (surge) occurred in 2/4 MS and 2/6 LW sows at 60 ± 5 h after OB. The MS sow that ovulated had an LH surge level of 4.5 ng/ml plasma at 40 h after OB. These results indicate minor breed differences in the control of LH secretion during lactational anoestrus.

LH / lactational anoestrus / oestrogen feedback / high prolificacy / Meishan pig

Résumé — Effet de l'administration de benzoate d'œstradiol durant la lactation sur la venue en œstrus et les niveaux de LH des truies Chinoises Meishan et Large White. *Il semble que l'anœstrus de lactation soit faiblement marqué chez les femelles de race Meishan (MS). L'objectif de cette étude est de comparer chez des truies allaitantes MS (n = 4) et Large-White (LW, n = 6) l'effet d'une administration de benzoate d'œstradiol (BO, 15 μg/kg de poids vif) à 22 j de lactation sur l'apparition de l'œstrus et l'évolution de la LH. Toutes les truies sont venues en œstrus après l'injection de BO. Seule une truie (MS) a ovulé à la suite de ce traitement et elle est devenue gravide. Les niveaux moyens de LH étaient faibles avant l'administration de BO (MS : 0,38 ± 0,06 ng LH/ml; LW : 0,29 ± 0,04 ng LH/ml; ns). Les concentrations de LH supérieures à 2 ng/ml ne sont observées que chez 2 truies MS et 2 truies LW à 60 ± 5 h après BO. La femelle MS qui a ovulé, avait une teneur maximale de LH de 4,5 ng/ml à 40 h après BO. Les résultats ne suggèrent que de faibles différences entre races du système de rétrocontrôle durant l'anœstrus de lactation.*

rétrocontrôle de LH / anœstrus de lactation / œstradiol / hyperprolificité / porc chinois

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INTRODUCTION

In recent years, Chinese pig breeds of the Taihu strain such as the Meishan have gained considerable interest, both for breeding purposes as well as scientifically, due to their significantly high prolificacy (Legault, 1985; Molénat and Legault, 1990). Puberty occurs at about 3 months, thus much earlier than in European pig breeds (6–7 months). Average litter sizes are 30–40% higher than those of contemporary Large White pigs (Bolet *et al,* 1986; Haley and Lee, 1990). The predominant cause of the high fertility in Meishan pigs appears to be an increased prenatal survival (Bidanel and Legault, 1986; Bazer *et al,* 1988; Haley *et al,* 1990). Another interesting feature of the exceptional prolificacy of the Meishan breed is the unusual occasional incidence of fertile oestrus during lactation (13%; Caritez and Legault, personal communication), whereas sows of European origin generally remain in anoestrus during the suckling period.

The purpose of this study was to investigate endocrine mechanisms responsible for the occurrence of fertile oestrus occasionally observed in Meishan sows during lactation. It is known that plasma LH levels are depressed during lactation with inhibition of pulsatile LH secretion (Parvizi *et al,* 1976; Stevenson *et al,* 1981), the suckling being the major factor controlling LH secretion, apparently by limiting secretion of GnRH (Cox and Britt, 1982). In a study carried out several years ago, an inhibition of the positive oestrogen feedback mechanism during early lactation and a partial recovery of this LH control system during late lactation was demonstrated, by evaluating the LH response to a single im injection of oestradiol benzoate (Elsaesser and Parvizi, 1980). These findings suggest that blockade of the positive oestrogen feedback is a cause of lactational anoestrus in the sow. In this study, we therefore compared the incidence of oestrus and patterns of LH before and after an oestradiol benzoate administration at 3/4 lactation in Meishan and Large White sows.

MATERIAL AND METHODS

Experiments were carried out in February 1990 at the Station de Physiologie de la Reproduction in Nouzilly with lactating sows, 4 of the prolific Chinese Meishan (MS) breed and 6 Large White (LW) sows. Two MS sows were primiparous, the other 2 farrowed for the 5th and 6th time, respectively. Of the LW sows, 2 were primiparous, 3 farrowed for the 3rd time and 1 for the 5th time. The mean body weight for the Meishan was 162 kg (range 110–211), that for Large Whites was 197 kg (range 158–220). The animals were kept with their litters in farrowing crates under natural lighting conditions and received standard pig chow and water. On day 1 of lactation the number of piglets was adjusted to 10–12. The piglets were weaned on day 29 of lactation.

All sows were fitted surgically with indwelling jugular catheters 2–3 days before the start of the experiments, as described previously (Ellendorff *et al,* 1977). To protect the catheters from destruction by the piglets, the ends of the catheters were secured in purpose-built small tissue bags, stitched to the back of the animals. In addition, the bags were impregnated once or twice daily with a hot spice mixture (tabasco).

On day 20 of lactation, blood samples (3 ml) were taken for 6 h at 15-min intervals for analysis of LH. On day 22 all sows received an im injection of 15 μg oestradiol benzoate (OB, Progynon B Oleosum, Schering)/kg body weight. Blood samples (3 ml) were obtained every 4 h for analysis of LH during 24 h before and 120 h after OB injection at 11 00 h (time 0). In addition, blood samples (6 ml) for determination of oestradiol-17β were drawn at 12-h intervals. To monitor occurrence of ovulation, blood (3 ml) was sampled for determination of progesterone at 2-day intervals for 3 weeks after OB. During dark phases red light forehead torches were used. Blood samples were immediately heparinized, spinned and the plasma was stored at –20 °C until analysis.

Following OB injection, onset and duration of oestrus was checked twice daily (8 00 and 16 00 h) by standing to a boar. All sows were in-

seminated or mated at least twice during induced oestrus. Pregnancy was tested by echography 4 weeks after insemination or mating (Martinat-Botté *et al*, 1988).

Plasma LH was determined by a homologous radioimmunoassay (Ponzilius *et al*, 1986). A specific antiserum (UCB Porcine-Anti LH, UCB; Brussels, Belgium), which had been raised in rabbits against porcine LH, was diluted 1:80 000. Highly purified porcine LH (LER-786-3) had a biological activity of 0.65 NIH-LH-SI U/mg and was used for both the standard stock solution and for ^{125}I-labeled hormone tracer. The sensitivity of the LH assay was 0.2 ng/ml plasma. The intra- and interassay coefficient of variation was 3.5 and 6.0%, respectively. Samples were analyzed in duplicate using 100 µl plasma. Samples from the same animal were analyzed in the same assay.

Plasma oestradiol levels were measured with a sensitive RIA validated for pig plasma as described by Cox *et al* (1987). The antibody (E2- 6 ≠ 3) was kindly provided by NR Mason, Eli Lilly Corporation and ^{125}I-oestradiol (Amersham) was used as a tracer. Plasma volume for analysis was 0.5 ml. The sensitivity of the assay, defined as the smallest amount detectable from 0, was 2.5 pg/ml plasma. Intra- and interassay coefficients of variation were 11.3 and 19.7%, respectively.

All data were analyzed using the SPSS/PC+ programme and expressed as means ± SEM. For each animal the duration of oestrus, mean levels of LH before OB, maximal LH levels after OB and time to maximal LH levels were determined. The significance of differences in these parameters between breeds was calculated using Student's-t-test for samples of unequal size.

RESULTS

All Meishan and Large White sows displayed distinct oestrus in response to OB treatment. The onset of oestrus was highly synchronized. In 3 out of 4 Meishan and 5 out of 6 Large White sows the beginning of oestrus was observed 72 h after OB. Duration of oestrus was 75 ± 8 h in Meishan and unusually long in Large White (116 ± 24 h compared to 66 ± 12 h after weaning). Only in 1 Meishan sow did ovulation in response to OB occur, as indicated by a rise in progesterone levels. Pregnancy was confirmed by echography in this animal on day 30 after insemination. This animal farrowed 16 live piglets.

Plasma oestradiol levels before OB treatment were low (< 2.5 pg/ml) except for the Meishan sow which ovulated (14 pg/ml plasma). The pattern of oestradiol levels in response to OB treatment appeared dissimilar between breeds (fig 1); however, there was no significant difference.

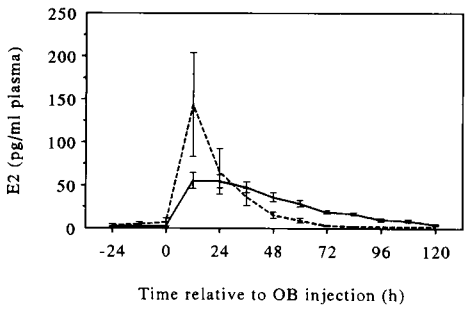

Fig 1. Plasma oestradiol levels (m ± SEM) in lactating Meishan (------) (n = 4) and Large White (——) (n = 6) sows before and after im administration of oestradiol benzoate (15 µg/kg body weight on day 22 of lactation). The lower limit of sensitivity was 2.5 pg oestradiol/ml plasma.

During the 6-h intensive sampling periods plasma LH levels were low in both breeds (MS: 0.38 ± 0.06 ng LH/ml, LW: 0.29 ± 0.04 ng LH/ml; ns). One or 2 distinct LH pulses were observed during the sampling period in 2 MS and 3 LW sows; the other sows did not display any obvious sign of pulsatile LH release. The highest mean pretreatment LH level (0.51 ng/ml) was recorded in the Meishan sow that ovulated in response to OB.

Negative feedback action of oestradiol on LH secretion was not apparent in any of the breeds, and positive feedback responses of LH in response to OB were modest (figs 2, 3). LH levels above 2 ng/ml

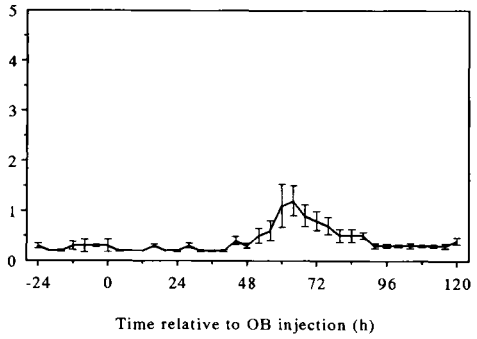

Fig 2. Plasma LH levels (m ± SEM) in lactating Large White sows before and after im administration of oestradiol benzoate (15 μg/kg body weight on day 22 of lactation; $n = 6$).

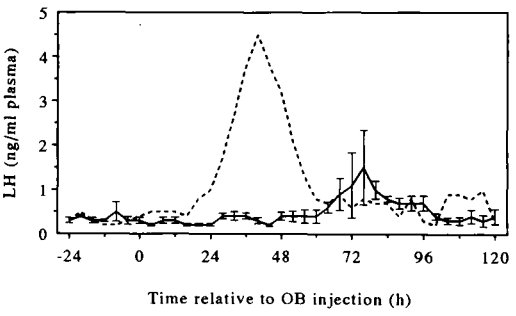

Fig 3. Plasma LH levels (m ± SEM) in one ovulating Meishan sow (------) and in 3 non-ovulating lactating Meishan sows (——) before and after im administration of oestradiol-benzoate (15 μg/kg body weight on day 22 of lactation).

(surge) occurred in 2 out of 4 MS and 2 out of 6 Large White sows. Mean maximal LH levels after OB were 2.4 ± 0.9 ng/ml for MS sows and 1.6 ± 0.4 ng/ml for LW sows (ns). Time to maximal LH levels was 73 ± 11 h for non ovulating MS sows and 66 ± 3 h for LW sows (ns). The Meishan sow that ovulated had a clear LH surge with a maximal level of 4.5 ng/ml at 40 h after OB. The changes in LH release for those MS sows which did not ovulate were similar to those observed in the Large White breed. Parity did not affect changes in LH secretion.

DISCUSSION

This study extends our earlier findings that the functioning of the positive oestrogen feedback on LH release is inhibited during early lactation (day 5, Elsaesser and Parvizi, 1980) by demonstrating an inhibition of this LH control system during the fourth week of lactation in both Large White and Meishan sows (with the exception of 1 animal). Similar observations have been made by Cox *et al* (1988) in crossbred suckled sows.

The endocrine mechanisms underlying the lactational anoestrus in pigs and the failure to activate the positive feedback are not fully understood. Apparently, the suckling-induced suppression of GnRH synthesis and release and the inhibition of episodic LH release are the main cause of lactational anoestrus in the sow (Britt *et al,* 1985; Varley and Foxcroft, 1990). The well-known negative feedback of oestradiol observed after injection of oestradiol in non-lactating sows (Elsaesser and Parvizi, 1979; Stevenson *et al,* 1981) was not apparent in this study or in other studies (Cox *et al,* 1988). However, the sampling frequency could have been inadequate to detect an unequivocal negative feedback response to oestradiol, since in a detailed study (De Rensis *et al,* 1991) inhibition of LH secretion by oestradiol during lactation was apparent.

A clear LH surge followed by ovulation was induced in only 1 Meishan sow, supporting to a certain degree the hypothesis of this study, that blockade of the stimula-

tory oestrogen feedback on LH release during lactation is less rigid in Meishan than in Large White sows. The Meishan sow that ovulated in response to OB displayed the highest LH levels before treatment. The maximal level of the LH surge (4.5 ng/ml) in this animal was similar to that normally observed in our laboratory for non-lactating Landrace or Göttingen miniature sows; however, it was much lower than the peak magnitude recently described for cyclic Meishan gilts (Ellendorff *et al,* 1988). Apparently, a high number of mature follicles ready to ovulate was present in the ovaries of this Meishan sow, as indicated by the elevated oestradiol levels before OB treatment and the number of piglets born (16) which was similar to the size of the previous litter (18). These data also suggest that unusually high preovulatory secretion of LH, as observed in cyclic Meishan gilts (Ellendorff *et al,* 1988), is not an absolute prerequisite for high ovulation rates or normal embryonic development in Meishan sows.

There was a clear dissociation between the induction of oestrus and the stimulation of an ovulatory LH surge by OB, indicating that the control centers responsible for oestrous behaviour are less susceptible to the effects of lactation than the control centers involved in the generation of an LH surge. A differential sensitivity to endogenous opioids might be involved in this dissociation, since endogenous opioids have been shown to mediate the suckling-induced suppression of LH release (Barb *et al,* 1986, Mattioli *et al,* 1986) and since morphine, an endogenous opioid agonist, is able to shorten the duration of oestrus (Esbenshade and Huff, 1989). In the Meishan, the duration of OB-induced oestrus was similar to that described by Ellendorff *et al* (1988) in cyclic Meishan gilts. In Large White sows, however, the duration of OB-induced oestrus was much longer than that of the following post-weaning oestrus. It is possible that the delayed disappearance of oestradiol from the blood circulation observed in Large White sows after OB is responsible for the long duration of oestrus in this breed.

In conclusion, the results of this study indicate minor breed differences in the control of LH secretion and the functioning of the positive oestrogen feedback mechanism during lactation. Since a clear LH surge followed by ovulation was induced in only 1 out of 4 MS sows, we cannot unequivocally conclude that blockade of the stimulatory oestrogen feedback action on LH release during lactational anoestrus is less rigid in Meishan than in Large White sows.

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