Partial recovery of the stimulatory oestrogen feedback action on LH release during late lactation in the pig

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Summary. The functioning of the stimulatory oestrogen feedback mechanism during lactational anoestrus of the sow was evaluated by determining the LH response to a single i.m. injection of oestradiol benzoate. Oestradiol-17 β and progesterone levels were also measured following treatment and following weaning. On Day 5 of lactation there was no LH response to oestradiol benzoate but on Day 35 of lactation a small but significant ($P \leq 0.05$) increase in plasma LH occurred, and this was followed by a rise in plasma progesterone in 4 out of 9 animals 8 or 13 days later. It is concluded that blockade of the stimulatory oestrogen feedback mechanism is one cause of lactational anoestrus in the sow.

Introduction

Apart from a non-ovulatory oestrus that occurs in a proportion of sows within 5 days of parturition (Warnick, Casida & Grummer, 1950; Baker, Woehling, Casida & Grummer, 1953; Heitman & Cole, 1956), oestrus is inhibited during lactation in the sow. Lactational anoestrus is maintained until weaning, provided that more than one sucking piglet is continuously present (Parvizi, Elsaesser, Smidt & Ellendorff, 1976) and that lactation is not extended unusually. Under certain conditions temporary separation of the sow and litter results in termination of lactational anoestrus (Crighton, 1970; Parvizi *et al.*, 1976).

The underlying endocrine mechanism is only partly understood. It is known that ovarian and pituitary activity are suppressed during lactation: follicular growth and corpora lutea are absent during the entire lactation (Crighton & Lamming, 1969), plasma progesterone values are consistently low and plasma oestrogen levels are nearly undetectable (Ash & Heap, 1975; Baldwin & Stabenfeldt, 1975; Parvizi *et al.*, 1976). Although high levels of pituitary FSH have been reported, pituitary LH content was low (Crighton & Lamming, 1969) and plasma LH levels are reduced with depression of the pulsatile pattern of LH secretion (Parvizi *et al.*, 1976). Ovariectomy before or during lactation fails to increase pituitary LH content (Crighton & Lamming, 1969) or plasma LH levels (Parvizi *et al.*, 1976) until weaning, thus indicating that the negative feedback mechanism of gonadal steroids is overruled by an unknown inhibitory effect on LH secretion as long as suckling is maintained.

Nothing is known about the functioning of the stimulatory oestrogen feedback during lactational anoestrus in the sow, e.g. whether an LH surge can be induced by oestrogen treatment. Single i.m. injections of oestradiol benzoate induce LH surges in peripubertal female Landrace pigs (Elsaesser & Foxcroft, 1978; Elsaesser & Parvizi, 1979) and the purpose of this study was to examine the LH response to oestradiol benzoate during early and late lactation. In addition, luteal activity after oestrogen treatment and after weaning was evaluated by means of plasma progesterone measurements.

Materials and Methods

Experiments

Lactating German Landrace sows were kept under natural lighting conditions and received standard pig chow and water. On Day 3 of lactation (Day 0 = day of parturition) the number of piglets was adjusted to 9–10. The piglets were weaned on Day 49 of lactation.

The animals were allocated to 4 groups of 9 sows each. At 10:00 h on Day 5 Group 1 received an i.m. injection of 60 µg oestradiol benzoate (Progynon B Oleosum: Schering)/kg body weight and Group 2 received 0.012 ml sesame oil/kg body weight. On Day 35 of lactation sows in Groups 3 and 4 were treated like those in Groups 1 and 2 respectively. Blood samples (1 or 5 ml) for LH and oestradiol-17β determinations were taken from an ear vein immediately before and at 24, 48, 60 and 72 h after injection as described by Hultsch (1979). Oestradiol-17β concentrations were measured only at 0, 24 and 72 h. Blood samples (1 ml) for determination of progesterone were collected, at 10:00 h, 8 and 13 days after injection and at 10 and 17 days after weaning. Heparinized plasma was stored at –20°C. The treatment effects on farrowing intervals and litter size were recorded.

Hormone analyses

Plasma LH was determined as described previously by Pomerantz, Ellendorff, Elsaesser, König & Smidt (1974). Sensitive and specific porcine LH antiserum (a gift from Dr G. D. Niswender) was diluted to 1:20 000 and 100 µl bound about 40% of LH in the absence of unlabelled LH. The LH standard preparation (LER-786-3, a gift from Dr L. E. Reichert, Jr) was equal to 0.65 NIH-LH-S1/mg. The intraassay coefficient of variation was 3.5% and at least 0.3 ng LH/ml could be detected.

Plasma oestradiol-17β and progesterone concentrations were measured by specific radio-immunoassay without prior chromatography as described previously by Elsaesser, Parvizi & Ellendorff (1978) and Elsaesser (1980), respectively, with the following modification. The oestradiol antiserum (E 12) had been raised against oestradiol-17β-6-carboxy-O-methyl-oxime-bovine serum albumin by intradermal immunization of a rabbit and was diluted 1:100 000. The antibody cross-reacted 1.5% with oestradiol benzoate, 0.6% with oestrone, 0.1% with oestriol and <0.1% with progesterone, testosterone and hydrocortisone. The inter- and intra-assay coefficients of variation were: 13.3 and 9.0% respectively for the determination of oestradiol-17β, and 14.1 and 9.9% respectively for the determination of progesterone.

Statistical analyses

The significance of differences in LH and oestradiol-17β concentrations between treatment groups was determined by one-way analysis of variance for samples of unequal size (Snedecor, 1956). Because of the variability in the time required for the attainment of peak concentrations of LH (Elsaesser & Foxcroft, 1978) the maximal LH value for each animal in the period 48–72 h after treatment was used to detect significant differences between groups.

Results

Treatment with oestradiol benzoate on Day 5 had no effect on plasma LH concentrations, but after injection on Day 35 there was a slight (at least twice as high as pretreatment LH levels) but significant increase in the LH levels (Table 1). Values in the control animals were low with only small variation and were unaffected by the stage of lactation (Days 5–8, 0.68 ± 0.06 (s.e.m.) ng/ml, $n = 43$; Days 35–38, 0.71 ± 0.08 ng/ml, $n = 27$). Plasma concentrations of

oestradiol-17 β at 24 and 72 h after treatment with oestradiol benzoate were 563 ± 127 and 198 ± 20 pg/ml respectively in Group 1 and 302 ± 83 pg/ml and 239 ± 71 pg/ml respectively in Group 3, while values before treatment and in the control animals in Groups 2 and 4 ranged between ≤ 20 and 58 pg/ml plasma.

Table 1. Effect of oestradiol benzoate treatment on plasma LH levels and luteal activity in lactating sows

Group	Mean $\pm$ s.e.m. maximal LH levels (ng/ml) 48–72 h after treatment	Percentage of animals with luteal activity [†]			
		After treatment		After weaning on Day 49	
		8 days	13 days	10 days	17 days
I	0.73 ± 0.13	0.0	0.0	62.5	83.3
II	0.83 ± 0.16	0.0	0.0	80.0	100.0
III	$1.75 \pm 0.27^*$	22.2	22.2	14.3	33.3
IV	0.95 ± 0.19	0.0	0.0	80.0	100.0

* $P \leq 0.05$ compared with Group IV.

[†] Plasma progesterone levels ≥ 5 ng/ml.

Elevated plasma progesterone levels, which would have suggested luteal activity, were not found during lactation in any of the animals of Groups 1, 2 and 4, either before or after treatment (Table 1). Of the 9 animals treated on Day 35 of lactation with oestradiol benzoate, 2 (22.2%) responded with a rise in progesterone levels 8 days later (i.e. about 5–6 days after the induced LH surge) and 2 others showed elevated progesterone levels 13 days following injection, suggesting that ovulation had occurred (Table 1). The rise in LH levels in these sows was not significantly different from the increase observed in those which did not ovulate. In Group 3 sows, resumption of luteal activity after weaning was postponed in 6 of the 9 animals. No significant effects on farrowing interval and litter size were observed for any of the 4 groups.

Discussion

The important finding of this study is that the stimulatory oestrogen feedback mechanism is not capable of operating during early lactation. From the oestradiol-17 β concentrations following oestradiol benzoate injections it can be concluded that sufficient oestrogen was circulating to trigger an LH surge. Treatment and bleeding schedules identical to those used in this study revealed a clear surge of LH (mean: 5 ng LH/ml plasma) in peripubertal (160-day-old) female Landrace pigs (Elsaesser & Parvizi, 1979). The possibility exists, therefore, that the pituitary is unable to release LH during lactation. Pituitary LH content (Crighton & Lamming, 1969) and plasma LH levels (Parvizi *et al.*, 1976) are very low and an inability of the lactating sow to increase LH synthesis has been suggested (Crighton & Lamming, 1969). However, it is claimed that LH-RH injections during lactation resulted in 'normally' increased LH levels (van de Wiel, van Landeghem, Bevers & Willemse, 1978). Moreover, the fact that an LH surge can be induced by oestradiol benzoate on Day 35 of lactation, when pituitary LH content and plasma LH levels are still low, would also argue against the hypothesis that the blockade takes place at the pituitary level.

It is likely that a prolactin release induced by suckling plays an important role in the mechanism of the blockade of the stimulatory oestrogen feedback action. The antagonistic role of prolactin is well established in many species (Lu *et al.*, 1976; Kann, Martinet & Schirar, 1976) and lactating sows have high prolactin levels compared with values in the post-weaning period (van Landeghem & van de Wiel, 1978; Bevers, Willemse & Kruip, 1978). An impairment of LH release following oestrogen administration to hyperprolactinaemic ewes has

been reported and does not seem to be caused by a reduced responsiveness of the pituitary to LH-RH (Kann *et al.*, 1976). If prolactin also plays an important role in lactational anoestrus of the sow, the recovery of the oestrogen-induced LH release during late lactation could be explained on the basis of the progressive decrease of plasma prolactin levels in the period between parturition and weaning (van Landeghem & van de Wiel, 1978). Studies in the rat suggest that the suckling stimulus rather than high circulating concentrations of prolactin is mainly responsible for the inhibition of LH release during lactation (Lu *et al.*, 1976). The reduced frequency of suckling during late lactation, caused by increased food intake of piglets fed *ad libitum*, would then account for the partial recovery of the stimulatory oestrogen feedback mechanism.

Oestrogen induction of LH release at Day 35 of lactation caused an increase in plasma progesterone levels in only 4 out of 9 animals at 8 or 13 days after injection, suggesting that luteal activity had been induced. However, none of these animals showed elevated plasma progesterone levels at both time points, indicating disturbed luteal function. Among other possible explanations, the reason for this finding could be the small magnitude of the induced rise in LH (not comparable with the preovulatory LH surge during the oestrous cycle) and/or lack of follicular growth during lactation (Crighton & Lamming, 1969). The delay in the resumption of luteal activity after weaning in Group 3, which was neither related to the magnitude of the induced LH increase nor to the induction of luteal activity immediately before weaning, cannot be explained on the basis of our present knowledge.

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