Effect of mimicking prepartum concentration of estradiol-17β on the inflammatory response to endotoxin in gilts

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Summary

The effect of mimicking prepartum concentration of estradiol-17β on the inflammatory response to endotoxin in gilts was studied. The study was performed in a split-litter design and comprised 5 pairs of littermates. A catheter was inserted into the jugular vein 2 days prior to the start of the study. In each pair, 1 littermate was treated IM with 2.5 mg of estradiol-17β/75 kg of body weight, and the other littermate was given peanut oil IM as a control. The day after treatment, all gilts were challenge-exposed with a *Salmonella typhimurium*-derived endotoxin (1 μg/kg, IV) and the inflammatory response to challenge exposure was monitored. There was no effect of estradiol treatment on the transient clinical signs of endotoxemia or on the increase in rectal temperature. The increase in blood concentrations of prostaglandin $F_{2\alpha}$ metabolite and cortisol after endotoxin challenge exposure was not affected by estradiol. Decrease in number of circulating blood mononuclear cells and polymorphonuclear leukocytes was not changed by estradiol treatment. Taken together, mimicking prepartum concentration of estradiol did not affect either the magnitude or the kinetics of the inflammatory response to endotoxin in gilts. Relevance of these findings to development of endotoxin-mediated diseases, such as the postpartum agalactia syndrome, needs further study.

Mastitis caused by coliform bacteria is a predominant finding in sows that develop the postpartum agalactia syndrome.[1-3] Absorption of endotoxin from these coliforms into the circulation is regarded as an important cause of this syndrome.[4] It is also noteworthy that several signs of disease in sows with postpartum agalactia syndrome[1] correspond to those mediated by cytokines in the inflammatory response to endotoxemia.[5]

Several studies in sows[6-9] have focused on putative changes in the immune capacity around parturition as an explanation for development of the postpartum agalactia syndrome. Some of the data in these studies actually indicate periparturient immune suppression, but there are some opposing data.

During the weeks before parturition, plasma concentration of estrogen increases markedly in sows.[10,11] This hormone has, in addition, been documented to be immunomodulating in other species.[12] Therefore, estrogen has become a candidate for mediating a putative periparturient immune suppression. Experimental data from studies in which estradiol has been administered in vitro indicate that estradiol impairs phagocytosis by porcine polymorphonuclear leukocytes (PMNL), but enhances concanavalin-A-induced lymphocyte blastogenesis and interleukin 2 production.[13] On the other hand, studies in which estradiol has been administered IM to gilts indicate that estradiol enhances phagocytosis by porcine PMNL, but reduces concanavalin-A-induced lymphocyte blastogenesis.[14,15] Thus, some of these data oppose the hypothesis of a periparturient immune suppression attributable to high estrogen concentration.

Interestingly, estradiol has been reported to enhance secretion of the cytokine interleukin 1 (IL-1) by rat macrophages in vitro.[16] Interleukin 1 is a key mediator of the inflammatory response[5]; thus, this finding may indicate that high estradiol concentration can enhance the inflammatory response.

There is evidence, in rodents, that regulation of the inflammatory response may be limited by glucocorticoids released in a neuroendocrine feedback loop triggered by the response itself.[17,18] Whether other steroid hormones, such as estradiol, interfere in this neuroendocrine regulation of the immune response is not known.

The overall objective of the study reported here was to test an alternative hypothesis of an estrogen-induced immune suppression in sows at parturition; does the high prepartum concentration of estrogen in plasma enhance the inflammatory response to endotoxin from coliform bacteria to such magnitude that it appreciably contributes to development of the postpartum agalactia syndrome? This study was performed in a split-litter design to reduce genetic influence on the outcome. The inflammatory response to

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endotoxin was measured as clinical signs of disease, rectal temperature, plasma concentrations of cortisol and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) metabolite, as well as leukocyte count in estradiol-treated and control gilts.

Materials and Methods

Gilts—The study comprised of 5 pairs of littermates of crossbred (Swedish Landrace × Swedish Yorkshire) gilts, weighing between 66 and 88 kg. Gilts were born at the Department of Obstetrics and Gynaecology and housed in individual pens throughout the study. They were fed twice a day with 1.0- to 1.5-kg pellets (energy content, 12.0 MJ/kg), and clinical signs of disease were not observed before endotoxin administration. Two days before treatments, gilts were anesthetized by IV administration of pentothal sodium at a guideline dosage of 11 mg/kg of body weight, and a silastic catheter was inserted in the jugular vein.[19]

Experimental design and blood sample collection—The experimental design was approved by The Ethical Committee for Animal Experiments, Uppsala, Sweden. At 8 AM, 2 days after jugular vein catheterization (day 1), 1 littermate in each pair was given a single injection of 2.5 mg of estradiol-17β benzoate (EB) in 1 ml of peanut oil[a]/75 kg, IM. At the same time, the other littermate was given 1 ml of peanut oil[b]/75 kg, IM as control. At 8 AM, the day after EB or oil administration (day 2), all gilts were given highly purified endotoxin from *Salmonella typhimurium* (1 μg/kg, IV). The endotoxin was phenol-extracted and further purified as described[20] and was dissolved in physiologic saline solution (5 μg/ml) before injection. During days 1 and 2, blood samples were collected through the jugular vein catheter every 15 minutes from 7:45 AM to 10:00 AM, then every 30 minutes to 12 noon and thereafter, every other hour to 8 PM. Samples were collected in evacuated tubes containing appropriate additives (ie, EDTA for cell counts and heparin for other assays).

Clinical signs of disease and measurement of rectal temperature—At all blood sample collections, gilts were observed for clinical signs of disease, such as vomiting and lethargy. Body temperature was measured at various occasions by use of an electronic thermometer placed in the rectum (Fig 2).

Hormonal assays—Heparinized blood samples for hormonal analysis were immediately centrifuged, and the plasma was saved and stored at −20 C until assay. Plasma concentration of estradiol-17β and $PGF_{2\alpha}$ metabolite (15-ketodihydro-$PGF_{2\alpha}$) were determined by use of luminescence immunoassay[21] and radioimmunoassay,[22] respectively.

Plasma concentration of cortisol was determined by luminescence immunoassay.[c] Standards and controls were provided in human serum. Before assay, 250-μl aliquots of standards, controls, and samples were extracted in 2.5 ml of diethyl ether and washed twice with 1 ml of distilled water. The solvent was removed by evaporation in dry air, and the residue was dissolved in 250 μl of phosphate buffer and left overnight at 4 C. One hundred microliters of horseradish peroxidase-labeled hormone and 100 μl of antiserum were pipetted into wells containing duplicate 50 μl-aliquots of extracted standards, controls, and plasma samples. The wells were covered with a lid and placed in a shaking incubator at 37 C. After 1 hour ± 5 minutes, well contents were aspirated and the empty wells were washed. Immediately after washing, 250 μl of signal reagent was dispensed into all wells. Luminescence of the wells was read in a luminometer 5 minutes after addition of signal reagent. Analysis of serial dilutions of porcine plasma containing high concentration of cortisol produced displacement curves parallel to the standard curve. The intra-assay coefficient of variation, calculated from the precision profiles of 5 assays, was < 11.1% for concentration of cortisol between 11.6 and 1,125.0 nmol/L. The corresponding interassay coefficients of variation for 3 quality-control samples were 28.7% (28 ± 9 nmol/L), 8% (128 ± 11 nmol/L), and 9% (367 ± 38 nmol/L). Calibration curves, precision profiles (intra-assay variation), and interassay coefficients of variation were calculated.[d]

Blood cell counts—Total WBC count was determined, using an automatic cell counter.[e] Blood smears were stained with May-Grünwald and Giemsa solutions for differential counting of 200 WBC. The WBC were categorized as blood mononuclear cells (BMC) and PMNL.

Statistical analysis—Data from days 1 and 2 were analyzed separately, using a computerized statistical program.[f] Normality of distributions for all data sets was assessed, and rectal temperature and $PGF_{2\alpha}$ metabolite values were found to require $\log_2$ transformations before further statistical analysis. For clarity, these data were converted to antilog$_2$ before graphic presentation. A general linear model was developed, and ANOVA[23] was used to evaluate the effect of estradiol treatment on the traits. The model was:

$$Y_{ijl} = \mu + TREATMENT_i + SAMPLE_j + ANIMAL(TREATMENT)_{ik} + TREATMENT \times SAMPLE_{ij} + ERROR_{ijk}$$

where

Y_{ijl} = an observed value for a trait measuring the inflammatory response;

μ = population mean for the trait;

$TREATMENT_i$ = a fixed effect attributable to treatment regime (estradiol/oil);

$SAMPLE_j$ = a fixed effect attributable to sample collection time;

$ANIMAL(TREATMENT)_{ik}$ = a random effect,

[a] Ovex B Vet, AB Leo, Malmö, Sweden.
[b] Apoteksbolaget AB, Stockholm, Sweden.
[c] Amerlite, Kodak Clinical Diagnostics Ltd, Amersham, England.
[d] Multicalc system, Wallac Oy, Turku, Finland.
[e] Sysmex F 800 System, Toa Medical Electronics (Europe) GmbH, Hamburg, Germany.
[f] GLM, PC-SAS Version 6.04, SAS Institute Inc, Cary, NC.

attributable to animal nested within treatment regime;

$TREATMENT \times SAMPLE_{ij}$ = a fixed effect attributable to the interaction between treatment regime and sample collection time;

$ERROR_{ijk}$ = a random residual error term.

The random effect of animal nested within treatment regime was used as an error term when testing treatment effects. The residual error term was used as the denominator to test the remaining effects. Unless indicated otherwise, data reported are least-square mean values obtained from ANOVA.

Results

Estradiol concentration in plasma—In the EB-treated gilts, plasma concentration of estradiol-17β increased rapidly after treatment on day 1, from 25 to 26 pmol/L at 8 AM to 684 pmol/L at 9 AM (Fig 1). The highest concentration (1,200 pmol/L) was recorded in the afternoon of day 1. On day 2, plasma concentration of estradiol-17β decreased from 791 pmol/L in the morning to 300 pmol/L in the evening (Fig 1). In control gilts, plasma concentration of estradiol-17β remained between 20 and 25 pmol/L throughout the study.

Clinical signs of disease—There were no clinical signs of disease on day 1. On day 2, most of the pigs vomited 15 to 45 minutes after endotoxin administration. All pigs became lethargic and lay down within 30 to 90 minutes of the injection. Gilts recovered and did not manifest signs of disease 8 to 12 hours after endotoxin administration. It was not possible to detect any difference in clinical signs of disease between the 2 groups of pigs.

Overall effects of the estradiol treatment—There were no significant overall effects of EB treatment on recorded variables, on day 1 or 2 (Table 1).

Rectal temperature—On day 1, rectal temperature fluctuated between 39.1 and 39.6 C in both groups of pigs (Fig 2). On day 2, rectal temperature increased ($P < 0.001$) in both groups from 39.0 C before endotoxin administration, to peak temperature between 41.4 and 41.5 C at 4 hours after endotoxin administration (Fig 2). Eight to 10 hours after endotoxin was administered, rectal temperature had reverted to original values. There was no difference in rectal temperature between the 2 groups of pigs on any of the measurement occasions.

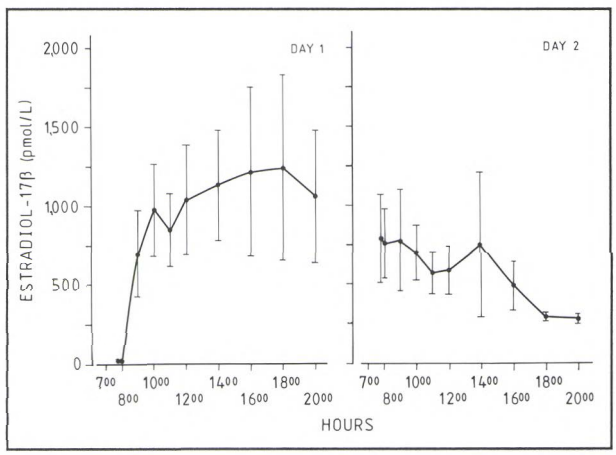

Figure 1—Mean ± SEM concentration of estradiol-17β in plasma from gilts treated with estradiol-17β benzoate (●—●). Time is represented by a 24-hour clock.

Table 1—Analysis of variance for variables reflecting the inflammatory response in gilts

Variable	Day before endotoxin challenge			Day of endotoxin challenge		
	R^2*	CV†	Effect of estradiol‡	R^2	CV	Effect of estradiol
Rectal temperature	0.59	0.16	1.0	0.93	0.20	0.54
$PGF_{2\alpha}$ metabolite in plasma	0.88	−14	1.0	0.94	67	0.45
Cortisol in plasma	0.68	32	1.0	0.93	22	0.63
No. of BMC	0.84	11	1.0	0.94	18	0.38
No. of PMNL	0.82	14	1.0	0.83	37	0.72

* Coefficient of determination; † Coefficient of variation; ‡ *P* value. $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$; BMC = blood mononuclear cells; PMNL = polymorphonuclear leukocytes.

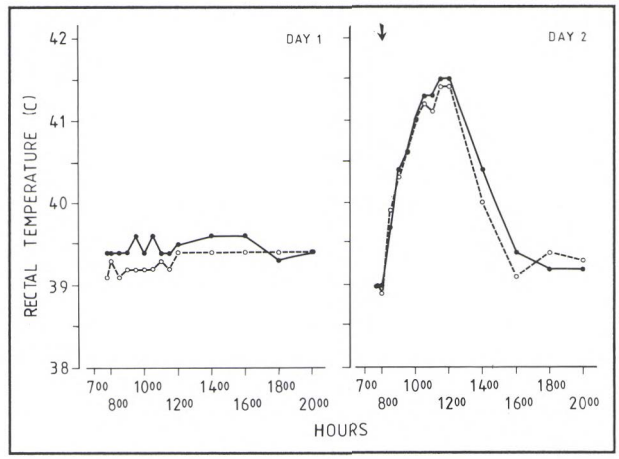

Figure 2—Rectal temperature of gilts treated with estradiol-17β benzoate (●—●) or peanut oil vehicle (○---○) and subsequently challenge-exposed with endotoxin. (Arrow indicates endotoxin administration.) *See* Figure 1 for key.

Prostaglandin $F_{2\alpha}$ metabolite concentration in plasma—On day 1, plasma concentration of $PGF_{2\alpha}$ metabolite varied between 0.30 and 0.44 nmol/L in both groups of pigs (Fig 3). On day 2, plasma concentration of the $PGF_{2\alpha}$ metabolite increased markedly ($P < 0.001$) in both groups of pigs immediately after endotoxin administration, from 0.33 nmol/L before the injection to peak values between 42 and 43 nmol/L at 30 minutes after endotoxin administration (Fig 3). Six to 8 hours after endotoxin administration, plasma concentration of the $PGF_{2\alpha}$ metabolite had returned to preinjection values. Difference in $PGF_{2\alpha}$ metabolite concentrations between the 2 treatment regimens was not recorded at any of the sample collection times.

Cortisol concentration in plasma—On day 1, cortisol concentration in plasma was highest in the morning (38 to 57 nmol/L) in both groups of pigs and decreased to lower values in the afternoon (13 to 17 nmol/L; Fig 4). On day 2, plasma concentration of cortisol increased markedly ($P < 0.001$) in both

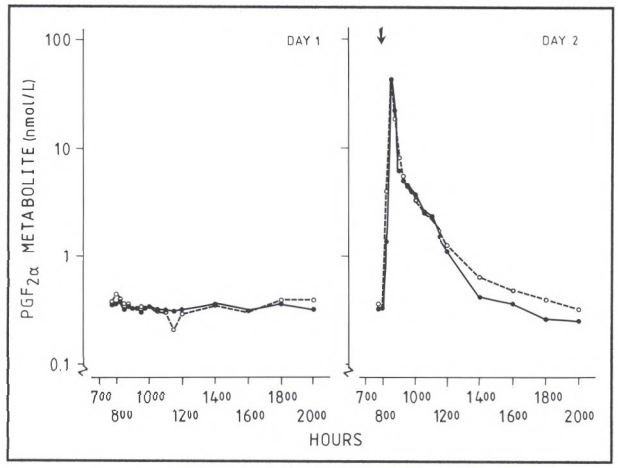

Figure 3—Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) metabolite concentration in plasma from gilts treated with estradiol-17β benzoate (●—●) or peanut oil vehicle (○---○) and subsequently challenge-exposed with endotoxin. (Arrow indicates endotoxin administration; note the logarithmic scale on the y-axis.) *See* Figure 1 for key.

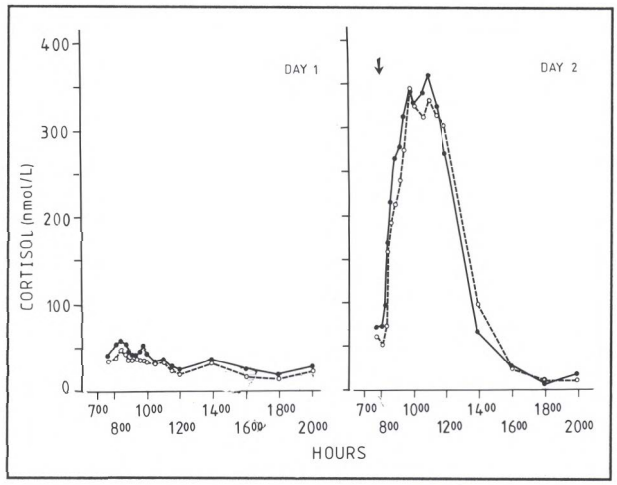

Figure 4—Cortisol concentration in plasma from gilts treated with estradiol-17β benzoate (●—●) or peanut oil vehicle (○---○) and subsequently challenge-expcsed with endotoxin. (Arrow indicates endotoxin administration.) *See* Figure 1 for key.

groups of pigs after endotoxin administration, from about 60 nmol/L before the injection to approximately 350 nmol/L at 2 hours after the injection (Fig 4). This higher concentration lasted for 2 hours, when it started to decrease. An effect of EB treatment on cortisol concentration in plasma was not detected on any of the sample collection occasions.

Numbers of BMC—On day 1, BMC count varied between 10.0 and 15.3 × 10^9 cells/L (Fig 5). On day 2, there was a marked decrease ($P < 0.001$) in BMC count after endotoxin administration in both groups of pigs, from 9 to 11 × 10^9 cells/L before the injection to about 2 × 10^9 cells/L at 4 hours after the injection (Fig 5). Twelve hours after the injection, BMC count had almost returned to preinjection values. There was no difference between EB-treated and control gilts in BMC count at any of the blood sample collection times.

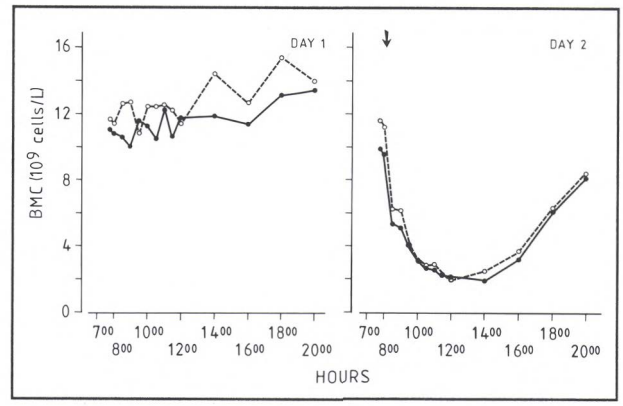

Figure 5—Blood mononuclear cells (BMC) in gilts treated with estradiol-17β benzoate (●—●) or peanut oil vehicle (○---○) and subsequently challenge-exposed with endotoxin. (Arrow indicates endotoxin administration.) *See* Figure 1 for key.

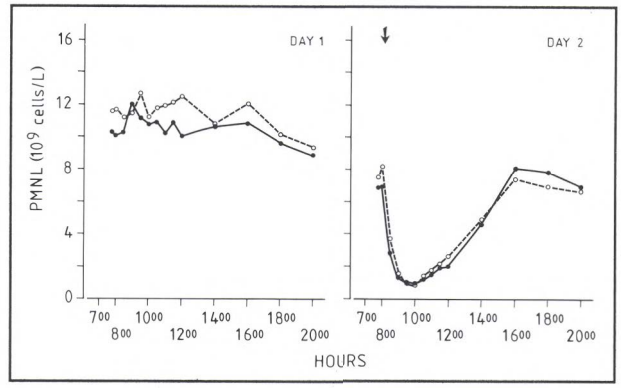

Figure 6—Polymorphonuclear leukocytes (PMNL) in gilts treated with estradiol-17β benzoate (●—●) or peanut oil vehicle (○---○) and subsequently challenge-exposed with endotoxin. (Arrow indicates endotoxin administration.) *See* Figure 1 for key.

Numbers of PMNL—On day 1, PMNL count varied between 8.9 and 12.7 × 10^9 cells/L in both groups of pigs (Fig 6). On day 2, PMNL count decreased markedly ($P < 0.001$) in both groups of pigs, from 7 to 8 × 10^9 cells/L before endotoxin administration to about 10^9 cells/L at 2 hours after the injection (Fig 6). Eight hours after endotoxin administration, PMNL count had reverted to preinjection values. There was no effect of EB treatment on PMNL count at any of the blood sample collections.

Discussion

The gilts of this study had clear, but transient, signs of endotoxemia, as reflected by several variables. This inflammatory response to endotoxin was not affected by plasma concentration of estradiol-17β, comparable to that recorded on the days before parturition.[10,11] Lack of effect was obvious in overall analysis of the data and the time-point by time-point analysis.

Clinical signs of disease, increase in rectal temperature, increase in plasma concentration of $PGF_{2\alpha}$ metabolite, and reduction in BMC and PMNL after endotoxin administration were in accordance with results of earlier studies on endotoxin challenge ex-

posure of pigs.[24,25] The rapid increase in plasma concentration of cortisol after endotoxin administration was consistent with findings in other species.[26]

There was no difference in transient clinical signs of disease or in the increase in rectal temperature between the 2 groups of pigs after endotoxin administration. The described increase in rectal temperature (ie, fever) is induced by cytokines, such as IL-1 and tumor necrosis factor.[5] These results, thus, oppose the suggestion that estradiol may enhance IL-1 production.[16]

Endotoxins are potent activators of the arachidonic acid cascade, in which $PGF_{2\alpha}$ is one of the products formed.[27] Formation of circulating metabolites (eg, 15-ketodihydro-$PGF_{2\alpha}$) is a variable that reflects arachidonic acid release during an inflammatory reaction. Magnitude of this release can serve as an indicator of severity of the inflammatory reaction.[28] Interestingly, it has been reported that estradiol-17β can suppress prostaglandin release in the porcine uterus.[29] In this study, however, there was no significant effect on $PGF_{2\alpha}$ metabolite concentration in plasma by EB treatment.

Increased plasma concentration of cortisol follows an inflammatory response to endotoxin challenge exposure,[26] and down-regulates this response.[30] Despite the fact that the 2 steroid hormones, cortisol and estrogen, have several metabolic pathways in common,[31] there were no signs of interaction by estrogen in the inflammation-cortisol feed-back loop in our gilts.

The leukopenia associated with the early inflammatory response to endotoxins is attributable to margination of the leukocytes.[32] Endotoxin-induced margination is attributable to expression of various adhesion molecules on PMNL and BMC, as well as on endothelial cells.[33,34] This process does not seem to be affected by estradiol, even though decreased numbers of circulating BMC after EB treatment of healthy gilts have been reported.[14] However, in that report, the decrease was observed 3 days after treatment.

In conclusion, mimicking prepartum concentration of estrogen in gilts for 2 days, neither affected the overall magnitude nor kinetics of the inflammatory response to endotoxin given the second day. Future studies on possible effects of estrogen on the inflammatory response may involve longer exposure to estrogen.

References

1. Ringarp N. Clinical and experimental investigations into a post-parturient syndrome with agalactia in sows. *Acta Agric Scand* 1960; Suppl 7.

2. Armstrong CH, Hooper BE, Martin CE. Microflora associated with agalactia syndrome of sows. *Am J Vet Res* 1968;29:1401–1407.

3. Ross RF, Orning AP, Woods RD, et al. Bacteriological study of sow agalactia. *Am J Vet Res* 1981;42:949–955.

4. Elmore RG, Martin CE. Mammary glands. In: Leman AD, Straw B, Glock RD, et al, eds. *Diseases of swine*, 6th ed. Ames, Iowa: The Iowa State University Press, 1986;168–183.

5. Dinarello CA. Role of interleukin-1 and tumor necrosis factor in systemic response to infection and inflammation. In: Gallin JI, Goldstein IM, Snyderman R, eds. *Inflammation: Basic principles and clinical correlates*, 2nd ed. New York: Raven Press Ltd, 1992;211–232.

6. Löfstedt J, Roth JA, Ross RF, et al. Depression of polymorphonuclear leukocyte function associated with experimentally induced *Escherichia coli* mastitis in sows. *Am J Vet Res* 1983;44:1224–1228.

7. Magnusson U, Fossum C. Variations in number and functional capacity of blood mononuclear cells during the peripartal period in the gilt. *J Vet Med [B]* 1988;35:570–578.

8. Magnusson U, Fossum C. Numerical variations among blood mononuclear cells during the peripartal period in the gilt. *J Vet Med [B]* 1990;37:459–467.

9. Tsuma VT, Magnusson U. Opsonic activity in mammary secretion and serum of gilts during the lactation period. *J Vet Med [B]* 1992;39:285–289.

10. Robertson HA, King GJ. Plasma concentrations of progesterone, oestrone, oestradiol-17β and oestrone sulphate in the pig at inplantation, during pregnancy and at parturition. *J Reprod Fertil* 1974;40:133–141.

11. Baldwin DM, Stabenfeldt GH. Endocrine changes in the pig during late pregnancy, parturition and lactation. *Biol Reprod* 1975;12:508–515.

12. Grossman CJ. Regulation of the immune system by sex steroids. *Endocr Rev* 1984;5:435–455.

13. Magnusson U. In vitro effects of prepartum concentrations of oestradiol 17β on cell-mediated immunity and phagocytosis by porcine leukocytes. *Vet Immunol Immunopathol* 1991;28:117–126.

14. Magnusson U, Einarsson S. Effects of exogenous oestradiol on the number and functional capacity of circulating mononuclear and polymorphonuclear leukocytes in the sow. *Vet Immunol Immunopathol* 1990;25:235–247.

15. Magnusson U, Fossum C. Effect of estradiol-17β treatment of gilts on blood mononuclear cell functions in vitro. *Am J Vet Res* 1992;53:1426–1430.

16. Hu S-K, Mitcho YL, Rath NC. Effects of estradiol on interleukin 1 synthesis by macrophages. *Int J Immunopharmacol* 1988;10:247–252.

17. Mason D. Genetic variation in the stress response: susceptibility to experimental allergic encephalomyelitis and implications for human inflammatory disease. *Immunol Today* 1991;12:57–60.

18. Munck A, Guyre PM, Holbrook NJ. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocr Rev* 1984;5:25–44.

19. Rodriguez H, Kunavongkrit A. Chronical venous catheterization for frequent blood sampling in unrestrained pigs. *Acta Vet Scand* 1983;24:318–320.

20. Lindberg AA, Greisman SE, Svenson SB. Induction of endotoxin tolerance with nonpyrogenic O-antigenic oligosaccharide-protein conjugates. *Infect Immun* 1983;41:888–895.

21. Forsberg M, Linde-Forsberg C, Karlsson Å, et al. Progesterone and oestradiol in canine plasma monitored by luminescence immunoassays. *J Reprod Fertil* 1993;(Suppl 47):127–132.

22. Granström E, Kindahl H. Radioimmunoassay of the major plasma metabolite of $PGF_{2\alpha}$, 15-keto-13,14-dihydro-$PGF_{2\alpha}$. *Methods Enzymol* 1982;86:320–339.

23. SAS Institute Inc. *SAS/STAT Guide for personal computers*, Version 6 Edition. Cary NC: SAS Institute Inc, 1987;549–640.

24. Yagoda C, Bylund-Fellenius AC, Kindahl H. Effects of oral and intravenous administration of endotoxin in prepubertal gilts. *J Vet Med [A]* 1993;40:33–44.

26. Massart-Leen AM, Burvenich C, Vandeputte-Van Messom G, et al. Partial prostaglandin-mediated mechanism controlling the release of cortisol in plasma after intravenous administration of endotoxins. *Domest Anim Endocrinol* 1992;9:273–283.

27. Roberts JS, Barcikowski B, Wilson L, et al. Hormonal and related factors affecting the release of prostaglandin $F_{2\alpha}$ from the uterus. *J Steroid Biochem* 1975;6:1091–1097.

28. Fredriksson G, Kindahl H, Edqvist LE. Endotoxin induced prostaglandin release and corpus luteum function in goats. *Anim Reprod Sci* 1985;8:109–121.

29. Bazer FW, Marengo SR, Geisert RD, et al. Exocrine versus endocrine secretion of prostaglandin $F_{2\alpha}$ in the control of pregnancy in swine. *Anim Reprod Sci* 1984;7:115–132.

30. Quinn PJ. Mechanism of action of some immunomodulators used in veterinary medicine. *Adv Vet Sci Comp Med* 1990;35:43–99.

31. Hsu WH, Crump MH. The adrenal gland. In: McDonald LE, Pineda MH, eds. *Veterinary endocrinology and reproduction*, 4th ed. Philadelphia: Lea & Febiger, 1989;202–230.

32. Goodman ML, Way BA, Irwin JW. The inflammatory response to endotoxin. *J Pathol* 1979;128:7–14.

33. Zimmerman GA, Prescott SM, McIntyre TM. Endothelial cell interactions with granulocytes: tethering and signaling molecules. *Immunol Today* 1992;13:93–100.

34. Shimizu Y, Newman W, Tanaka Y, et al. Lymphocyte interaction with endothelial cells. *Immunol Today* 1992;13:106–112.