EFFECT OF CANNULATION AND ENVIRONMENTAL TEMPERATURE ON THE CONCENTRATION OF SERUM CORTISOL IN PREGNANT SOWS

L. A. BATE and R. R. HACKER

Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario N1G 2W1. *Received 9 July 1984, accepted 27 Nov. 1984.*

BATE, L. A. AND HACKER, R. R. 1985. Effect of cannulation and environmental temperature on the concentration of serum cortisol in pregnant sows. Can. J. Anim. Sci. **65**: 399–404.

Serum concentrations of cortisol were monitored in four 100-day-old pregnant sows during and following cannulation through an ear vein and thereafter for 11.5 consecutive days. During this period, the sows were exposed to the following environmental temperatures: 2 days at 18°C and 3 days at 2, 18 and 32°C, respectively. Serum concentrations of cortisol increased significantly during and after cannulation, but returned to basal levels within 4 h indicating that blood samples can be taken subsequently without stressing the animal. The serum concentration of cortisol followed a circadian rhythm when the sows were kept at 18°C. This daily variation was characterized by maximum and minimum concentrations during the morning and evening, respectively. When the temperature was lowered to 2°C, the circadian rhythm of serum cortisol was maintained, but the daily mean levels were higher. On return to 18°C, the circadian rhythm was temporarily disrupted and the mean daily serum cortisol concentration was lowered. Exposure of sows to 32°C increased the daily mean serum cortisol concentration and restored the circadian rhythm but with an inverted pattern. It is concluded that exposure to different environmental temperatures can affect the daily mean level and circadian pattern of serum cortisol of pregnant sows.

Key words: Sows, temperature, cannulation, cortisol, stress

[Effet d'une canule et de la température ambiante sur la teneur en cortisol du sérum chez les truies gravides.]

Titre abrégé: Effet d'une canule et de la température sur la teneur en cortisol du sérum des truies.

Nous avons mesuré les teneurs en cortisol du sérum chez quatre truies en gestation depuis 100 jours, pendant la pose d'une canule dans une veine de l'oreille et après, pendant 11,5 jours consécutifs. Pendant l'expérience, les truies ont été soumises aux conditions suivantes: 2 jours à 18°C et 3 jours à 2, 18 et 32°C, respectivement. Les teneurs en cortisol du sérum ont augmenté sensiblement pendant la pose de la canule et après, mais sont redevenues normales moins de quatre heures après l'opération, ce qui laisse à penser qu'on peut par la suite prélever des échantillons de sang sans causer de stress pour l'animal. Les teneurs en cortisol du sérum ont varié selon un cycle circadien lorsque les truies étaient gardées à 18°C. Elles atteignaient leur maximum le matin et leur minimum le soir. Lorsque la température a été abaissée à 2°C, le cycle circadien s'est maintenu mais les teneurs quotidiennes moyennes étaient plus élevées. Le retour à 18°C a provoqué une perturbation temporaire du cycle circadien et une baisse de la teneur quotidienne moyenne en cortisol du sérum. Le passage à une température de 32°C a provoqué une augmentation de la teneur quotidienne moyenne en cortisol du sérum et le rétablissement du cycle circadien, mais ce dernier était inversé. Il semble donc que les conditions de température ambiante peuvent influer sur la teneur quotidienne moyenne en cortisol du

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sérum et sur le cycle circadien de la variation de cette teneur chez les truies en gestation.

Mots clés: Truies, température, canule, cortisol, stress

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Animal handling is one of many factors which can affect glucocorticoid concentration in domestic species (Dantzer et al. 1983). In swine, the introduction of a cannula into the ear vein for frequent blood sampling is a research technique which requires handling and physical restraint of the pig for short periods of time. Furthermore, the presence of a cannula may itself be a stressor (Selye 1973).

The effect of temperature on serum cortisol concentration has been studied in neonatal pigs (Zamora et al. 1975), growing pigs (Blatchford et al. 1974), and boars (Larsson et al. 1983). Growing pigs between 50 and 130 days showed changes in mean daily concentration of cortisol when exposed to different temperature regimens (Rafai and Fodor 1980b). Daily variations in serum corticosteroids have been demonstrated in cattle (Fulkerson et al. 1980), rats (Wong et al. 1983) and also swine (Whipp et al. 1970; Bottoms et al. 1972). To the best of our knowledge, no study has been carried out to investigate the effect of temperature on the circadian rhythm of glucocorticoids in swine.

This study was designed to determine the duration of the stress effect of ear vein cannulation and to determine the effects of different environmental temperatures on serum cortisol concentrations of pregnant sows.

MATERIALS AND METHODS

Four Yorkshire sows, 100 days pregnant, were cannulated and housed in 0.65 × 2.1-m dry stalls with solid metal sides in an environmental chamber. The sows were kept in the chamber for 11.5 days and were bled through the cannula every 2 h. Sows had continuous access to water and were fed 2.5 $kg \cdot day^{-1}$ of a 14% protein, corn and soybean meal diet. The light at eye level was 120.75 lx between 0600 and 2000 h and reduced to 2.82 lx between 2000 and 0600 h.

The cannulation procedure was a modification of a method described by Anderson and Elsley (1969). To facilitate the procedure, the sows were restrained in a squeeze cage with the head partially immobilized. One ear, the forehead and the back of the sow was shaved and disinfected with 70% ethanol. A sterilized 3.75-cm × 14-gauge needle punctured the ear vein and 35–45 cm of a 2-m length of PE 90 tubing ((Clay Adams N.J.) 0.86 mm i.d., 1.27 mm o.d.) previously disinfected with zepharin chloride solution (Winthrop Laboratories, Aurora, Ont.) was introduced through the needle. Following needle removal the free end of the tubing was fitted with a blunt, stoppered 20-gauge needle. At this time, the first blood sample was taken, and the cannula was flushed with heparinized saline solution (0.9% sodium chloride solution USP (Travenol Laboratories, Malton, Ont.) and 100 $IU \cdot mL^{-1}$ of heparine sodium USP (Allen and Hanburys, Toronto, Ont.)). The cannula was sutured to the skin at the entrance to the ear vein and to the forehead and the back of the sow. The exposed portion of the cannula on the ear was attached to the skin with KaMar glue (Steamboat Springs, Col.) and protected with elastoplast adhesive tape (Smith and Nephew Inc., Lachine, Que). The free end of the cannula was connected with fishing nylon to a two-pulley system located directly above the stall. The entire cannulation procedure took approximately 20 min. The second blood sample was taken immediately upon completion of the cannulation procedure.

The sows were sequentially exposed to the following temperature regimens: Two days at 18°C and 40% RH, 3 days at 2°C and 58% RH, 3 days at 18°C and 40% RH and 3 days at 32°C and 40% RH.

After collection the blood samples were cooled to 4°C within 30 min, allowed to clot for 6–10 h, centrifuged and the serum was separated into plastic vials which were stored at −17°C until analyzed for cortisol using the solid phase RIA developed by Micromedic System Ind. (Horsham, Pa.). The RIA had an intra- and inter-assay coefficient of variation of 5.41 and 6.23%, respectively.

The data were analyzed by ANOVA, using temperature as treatments. Comparisons were

made between the following: temperatures, the different times within a day, and days within a temperature regimen. Tukeys multiple range test was used to compare the daily means (Steel and Torrie 1980).

RESULTS

The concentration of circulating cortisol during and immediately following cannulation of an ear vein of pregnant sows was higher ($P<0.05$) than the basal levels. Within 4 h of insertion of the cannula the concentration of cortisol had returned to basal levels and the circadian rhythm was re-established within 24 h (Fig. 1).

The average circadian patterns of plasma cortisol following exposure of the pregnant sows to different environmental temperatures are summarized in Fig. 2. During the first period of exposure to thermoneutral temperature (18°C), the serum cortisol levels showed a consistent circadian rhythm ($P<0.05$), peaking between 0400 and 0600 h and reaching the lowest levels 12 h later. When the temperature was decreased to 2°C, the daily mean concentration of cortisol increased ($P<0.05$) as compared to concentrations found in the sows when exposed to the 18°C temperature. Under cold conditions, the circadian rhythm was maintained, with large differences ($P<0.05$) between morning and evening. Moreover, the daily mean value of cortisol increased ($P>0.05$) with time of exposure. During the subsequent 3 days, when the sows were again exposed to a temperature of 18°C, the daily pattern was temporarily disrupted and the daily mean level of cortisol decreased with time of exposure. Under heat stress (32°C) the daily mean level of cortisol increased progressively ($P<0.05$). A circadian rhythm was re-established, but in a re-

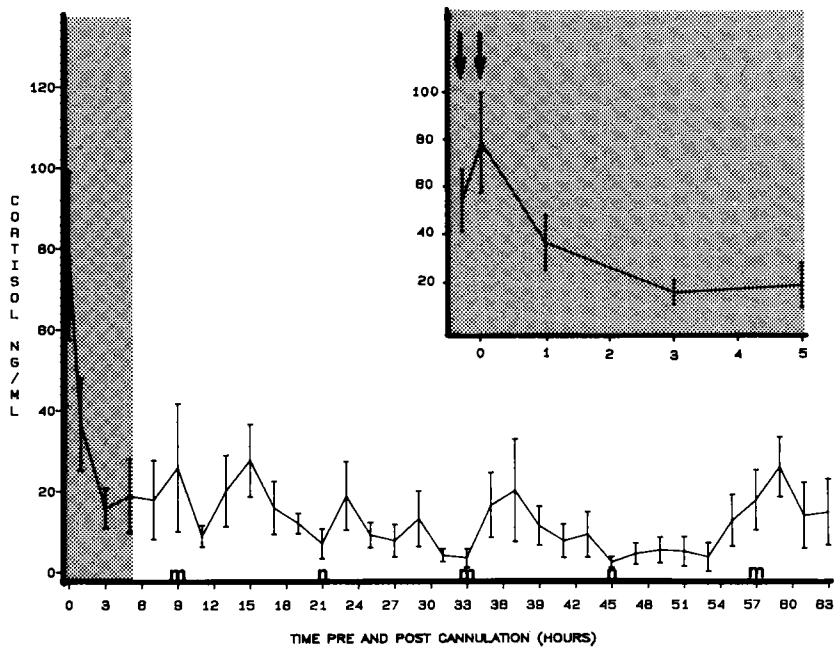

Fig. 1. Concentration of serum cortisol in four pregnant sows during and until 63 h after insertion of a cannula into the ear vein. Midnight and noon are indicated with 'm' and 'n', respectively. Insert is a magnification of the shaded area and the arrows mark the beginning and the end of the cannulation procedure. Vertical bars indicate SD.

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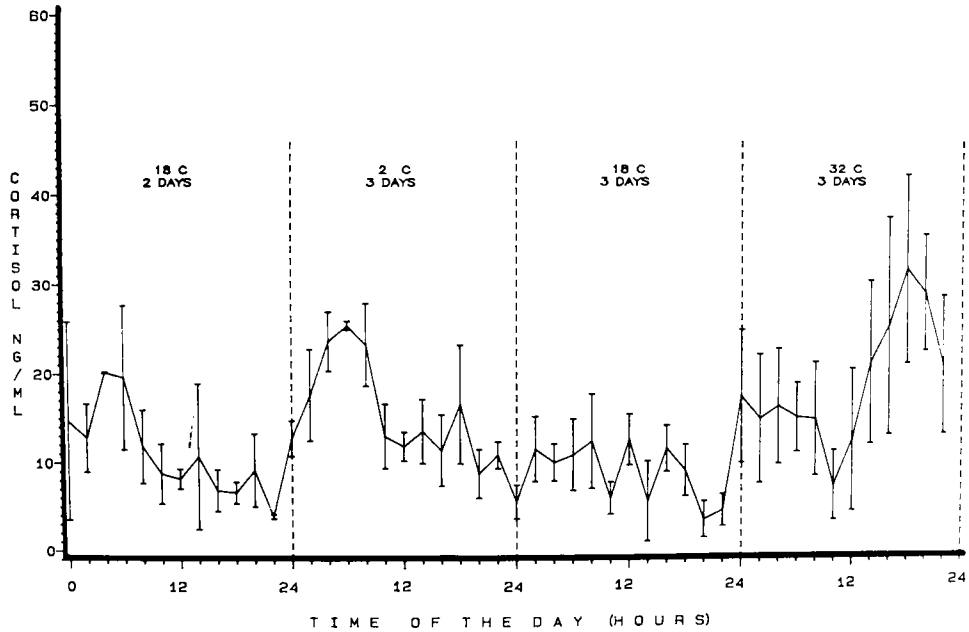

Fig. 2. Average circadian pattern of serum cortisol concentrations in four pregnant sows following exposure to different environmental temperatures. Vertical bars indicate SD for two or three consecutive days.

verse manner. The lowest levels of cortisol were observed during the morning and the highest levels during the evening ($P<0.05$).

DISCUSSION

The results clearly indicate that the procedure used for cannulation is a potent stressor. Restraint, shaving, puncture of the ear vein and introduction of the cannulae were responsible for a large portion of the increase in cortisol concentration. The stress may have been further magnified by the continuous restraint and the attachment of the cannula. The rapid return of serum cortisol to basal levels, after the sows were allowed to rest, is reasonable considering that the half-life of cortisol is about 80–110 min (Goldfien 1982). The pattern of serum cortisol resulting from the stress of the total procedure is comparable to the pattern of total corticosteroids in boars subjected to 5 min of restraint by using a wire snare (Juniewicz and Johnson 1984). This suggests that neither the housing nor the presence of the cannula were as stressful as the process of cannulation. These findings indicate that acutely stressed animals can be used for experimental purposes involving corticosteroid measurements 6 h following cannulation. The data also indicate that the circadian rhythm of serum cortisol concentration is reestablished soon after the acute stressor has been removed. The peaks and valleys in the circadian rhythm of cortisol serum concentration during normal or cold temperature are consistent with the findings in growing pigs (Rafai and Fodor 1980a), although the net values in our sows were lower.

The response of the animal to a sustained stress is an increase in pituitary ACTH output with the consequent growth of the adrenal gland (Chart et al. 1958), which increases glucocorticoid secretion. A reversal of this condition could be responsible for the disruption of the circadian rhythm of

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cortisol found in sows after exposure to cold stress. The daily increase in serum concentration of cortisol in sows under heat stress agrees with the finding of others (Aberle et al. 1974; Rafai and Fodor 1980b), but the modification of the circadian pattern of cortisol concentration observed in the sows under heat stress was not discussed by these authors. Under heat stress, the increase in the daily mean level of cortisol was a consequence of an upward shift in the entire profile.

It has been established that the suprachiasmatic nuclei of the hypothalamus is the "pacemaker" of circadian rhythms in the rat. Furthermore, bilateral lesions of this area eliminate the circadian rhythmicity of adrenal corticosterone (Moore and Eichler 1972). While there is lack of information on the control of circadian rhythms, and the effects of circadian rhythms on physiological events in swine, it is apparent, from the present study, that environmental temperature is capable of altering the settings of the region in charge of circadian rhythms, with the consequent modification of the pattern observed. The mechanism by which environmental temperature affects circadian rhythms deserves further investigation.

In summary, this study has shown that exposure of pregnant sows to different environmental temperatures can dramatically affect the circadian rhythm and the daily mean levels of cortisol. Thus, environmental temperature should be considered when interpreting the influence of adrenal activity on physiological events in pigs.

ACKNOWLEDGMENTS

The assistance of Mrs. M. Kocher and Mr. D. Wey in data collection and laboratory analysis is acknowledged. The support of Natural Sciences and Engineering Research Council of Canada Grant no. 82-143. Ontario Pork Producer's Marketing Board and the Ontario Ministry of Agriculture and Food is acknowledged.

Aberle, E. D., Merkel, R. A., Forrest, J. C. and Alliston, C. W. 1974. Physiological responses of the stress susceptible and stress resistant pigs to heat stress. J. Anim. Sci. **38**: 954–959.

Anderson, D. M. and Elesley, F. W. H. 1969. A note on the use of indwelling catheters in conscious pigs. J. Agric. Sci., Camb. **72**: 669–673.

Blatchford, D. R., Buckingham, J. C., Hodges, J. R., Holtzbauer, M., Ingram, D. L., Legge, K. L. and Rees, L. H. 1974. The effect of thermal stress and frustration on the pituitary-adrenal axis of the young pig. J. Physiol. **242**: 18P–19P.

Bottoms, G. D., Roesel, O. F., Rausch, F. D. and Akins, E. L. 1972. Circadian variation in plasma cortisol and corticosterone in pigs and mares. Am. J. Vet. Res. **33**: 785–790.

Chart, J. J., Sheppard, H., Allen, M. J., Bencze, W. L. and Gaunt, R. 1958. New amphenone analogs as adrenocortical inhibitors. Experientia **14**: 151–152.

Dantzer, R., Mormade, P., Bluthe, R. M. and Soissons, J. 1983. The effect of different housing conditions on behavioural and adrenocortical reactions in veal calves. Reprod. Nutr. Develop. **23**: 501–508.

Fulkerson, W. J., Sawyer, G. J. and Gow, C. B. 1980. Investigations of ultradian and circadian rhythms in the concentration of cortisol and prolactin in the plasma of dairy cattle. Aust. J. Biol. Sci. **33**: 557–561.

Goldfien, A. 1982. Basic and clinical pharmacology. B. G. Katzung, ed. LMP Los Altos, California. 420 pp.

Juniewicz, P. E. and Johnson, B. H. 1984. Ability of cortisol and progesterone to mediate the stimulatory effect of adrenocorticotropic hormone upon testosterone production by the porcine testis. Biol Reprod. **30**: 134–142.

Larsson, K., Einarsson, S., Lundstrom, K. and Hakkarainen. J. 1983. Endocrine effects of heat stress in boars. Acta Vet. Scand. **24**: 305–314.

Moore, R. and Eichler, V. 1972. Loss of circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Res. **42**: 201–206.

Rafai, P. and Fodor, E. 1980a. Studies on porcine adrenocortical function I. Circadian variations in, and effect of ACTH treatment on, the peripheral plasma cortisol level. Acta Vet. Acad. Sci. Hung. **28**: 433–441.

Rafai, P. and Fodor, E. 1980b. Studies on porcine adrenocortical function. II. Effect of the ambient temperature and restricted feeding on

the peripheral cortisol level. Acta Vet. Acad. Sci. Hung. **28**: 443–454.

Selye, H. 1973. The evolution of the stress concept. Am. Sci. **61**: 692–699.

Steel, R. G. and Torrie, J. H. 1980. Principles and procedures of statistics. 2nd ed. McGraw-Hill Book Co., New York.

Whipp, S. C., Wood, R. L. and Lyon, N. C. 1970. Diurnal variation in concentrations of hydrocortisone in plasma of swine. Am. J. Vet. Res. **31**: 2105–2107.

Wong, C. C., Dohler, K. D., Geerlings, H. and v.z. Muhlen, A. 1983. Influence of age, strain and season on circadian periodicity of pituitary, gonadal and adrenal hormones in the serum of male laboratory rats. Hormone Res. **17**: 202–215.

Zamora, C. S., Kowalczyk, T., Hoekstra, W. G., Grummer, R. H. and Will, J. 1975. Plasma concentration of pepsinogen and corticosteroid in relation to gastric lesions in swine. Am. J. Vet. Res. **36**: 1327–1331.

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