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# Daily Cortisol Profile in Lactating and Non-Lactating Dairy Goats (*Capra hircus*)

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**Abstract:** The objective of the present study was to determine the daily plasma cortisol pattern in domestic lactating and non-lactating goats. In a total of 12 multiparous goats (6 lactating, 6 non-lactating), 4 daily blood samples were taken over the course of 12 days, beginning at different times, in order to cover two, 24 h periods. Lactating goats were at their 4th month of lactation and were hand-milked daily at 07:30-08:30 h since parturition. Repeated measures ANOVA, Tukey-Kramer and student's t-tests were used, considering diurnal (07:00-18:00 h) and nocturnal hours (19:00-06:00 h) as well as the condition of lactation. Higher levels of cortisol (45 nmol $L^{-1}$) were found during diurnal hours, followed by important and sustained decreases (6-16 nmol $L^{-1}$). A pair of smaller peaks appeared between 23:00-24:00 h, followed by a decrease that was maintained until 06:00 h, at which time high levels of cortisol returned (26 nmol $L^{-1}$). Daily average cortisol values were similar between lactating (16.6±1.8) and nonlactating goats (16.5±1.8; nmol $L^{-1}$; $p>0.05$). Diurnal cortisol was higher ($p = 0.003$) than nocturnal (19.6±1.9vs. 13.5±1.1 nmol $L^{-1}$). Daily cortisol levels are similar between lactating and non-lactating domestic goats at their fourth month of lactation and cortisol levels undergo significant changes during the day, with higher concentrations in diurnal compared to nocturnal hours.

**Key words:** Cortisol, goats, cortisol daily profile, diurnal, nocturnal

## INTRODUCTION

The daily activity of the Hypothalamic-Pituitary-Adrenocortical axis (HPA) is associated with elevations in Adrenocorticotropic Hormone (ACTH) and cortisol at determined times of day (Desir *et al*., 1980). In primates (Schmidt-Reinwald *et al*., 1999), cows (Thun *et al*., 1981; Lefcourt *et al*., 1993), ewes (Fulkerson and Tang, 1979; Lincoln *et al*., 1982; Przekop *et al*., 1985) and pigs (Ekkel *et al*., 1997; Ruis *et al*., 1997), the cortisol concentrations tend to be greater during diurnal than nocturnal times, describing a circadian rhythm that is sometimes affected by certain activities, such as feeding (Murayama and Sasaki, 1987) or milking (Negrão and Marnet, 2003) in ewes. In animals with nocturnal habits such as the rat, the peak reaches its maximum after the beginning of the dark time, when the animal demonstrates its greatest activity (Morin and Dark, 1992).

Given that the adrenal response to any stimulus may be different depending on the time of day in which the stimulus is applied (Janssens *et al*., 1995), daily fluctuations in the concentrations of corticosteroid hormones need to be considered in studies on the adrenal response to stressful situations (Ader and Friedman, 1968; Janssens *et al*., 1995) and in studies on the effect of adrenal hormones in other physiological systems. In ruminants other than goats, machine and hand-milking increases cortisol (Gorewit *et al*., 1992; Negrão and Marnet, 2003; Negrão *et al*., 2004) and noradrenaline levels (Lefcourt *et al*., 1997). This should also be considered when assessing the daily activity of the HPA axis or its response to a given stimulus. Information regarding these parameters in goats is currently not available.

Furthermore, when studying the well-being of housed goats, it is important to know the levels of cortisol at each hour of the day as a reference value; however, information on adrenal activity in goats is not abundant.

The objective of the present study was to determine the daily plasmatic cortisol profile in lactating and non-lactating domestic goats in housed conditions.

## MATERIALS AND METHODS

**Location, animals and design:** The study was carried out on an experimental intensive farm located south of Mexico

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Table 1: Time of sampling during the experiment. Samples were taken every hour of the day and time indicated in order to obtain data for a complete day without continuous sampling

| Day of the experiment | Time of sampling |
|---|---|
| 1, 7 | 08:00-11:00 h |
| 2, 8 | 12:00-15:00 h |
| 3, 9 | 16:00-19:00 h |
| 4, 10 | 20:00-23:00 h |
| 5, 11 | 00:00-03:00 h |
| 6, 12 | 04:00-07:00 h |

City (2760 m above sea level, 19°13'N, 99°8'W) using a protocol that was approved by the Internal Animal Ethics Committee of the Faculty of Veterinary Medicine and Zootechnics. During the month of July, a total of 12 adult multiparous goats (2.5-3 years age; 45-53 kg body weight; 6 lactating, 6 non-lactating) in housed conditions were used. Lactating goats were at their fourth month of lactation and were hand-milked daily since parturition, beginning at 07:30 h. Blood samples were taken hourly from all animals by venipuncture from the jugular vein. Four jugular blood samples were taken per day over the course of 12 days at different times (Table 1) and in random order. This allowed us to have 2 blood samples for each hour of the day; the cortisol levels of both samples were averaged to calculate the value for a given hour of the day. Blood samples were obtained using tubes with heparin (BD Vacutainer®) and sterile needles (PrecisionGlide®, 22G×1.1/2) and the procedure had a duration of 10 sec per animal. Samples were immediately centrifuged and plasma was frozen until it was processed by radio immunoanalysis with a commercial kit (Diagnostic Products Corporation, Los Angeles Cal., USA). The sensitivity of the assay was 5.5 nmol $L^{-1}$ and within- and between-assay coefficients of variation were 2.3 and 5.0%, respectively. Feeding time was 08:00 (oat hay and silage) and 14:00 h (concentrated grain and alfalfa), according to the National Research Council (NRC, 1981). Access to water was free.

Since inherent stress in response to the sampling could alter the obtained results (Säkkinen *et al.*, 2004), all goats were exposed to an adaptation program to the respective handling for 2 weeks prior to the beginning of the study. In this process, the sampling procedure was simulated by holding the animal gently and injecting 2 m of saline solution via the jugular. The person handling the goats was always the same.

**Data analysis:** The cortisol concentration over the course of 24 h was divided equally into two periods of 12 h each (diurnal; 07:00-18:00 h and nocturnal; 19:00-06:00 h). The cortisol values in each sample were added to calculate the area under the curve. The basal levels of each animal were calculated as the average of the five lowest measurements in all samplings (Claus *et al.*, 1990). The data were analyzed using descriptive statistics and compared considering the lactation condition (lactating vs. non-lactating) and periods (diurnal vs. nocturnal) as the between subjects factors; time of day was considered as the within subjects factor. Analysis was made by repeated-measures ANOVA (GLM procedure); the student's t-test and Tukey-Kramer test were used for comparisons of the means at a significance level of 0.05 (SAS, 1999).

## RESULTS AND DISCUSSION

The average daily values of cortisol were similar between lactating and non-lactating goats (16.6±2 and 16.5±2 nmol $L^{-1}$, respectively; $p>0.05$; Fig. 1 and 2).

In both lactating and non-lactating goats, the highest levels of cortisol (up to 45 nmol $L^{-1}$) were found during diurnal times (between 10:00 and 14:00 h), followed by a significant decrease to basal levels (between 6 and 16 nmol $L^{-1}$) between 15:00 and 22:00 h. A pair of smaller elevations was observed between 23:00 and 24:00 h, followed by a sustained diminution until 06:00 h, at which time it returned to high levels (26 nmol $L^{-1}$, Fig. 1).

The average cortisol values between nocturnal and diurnal hours were different ($p = 0.003$), with higher levels observed in the diurnal period (19.6±1.9 nmol $L^{-1}$) compared to the nocturnal period (13.5±1.9 nmol $L^{-1}$; Fig. 2). The average cortisol values during the 24 h period in all the goats are shown in Fig. 3.

The area under the cortisol curve over the course of 24 h was similar between lactating and non-lactating goats (399.2±44.9vs. 397.2±44.9 nmol $L^{-1}$, respectively, $p>0.05$), but was higher during diurnal than nocturnal times (236±23.5 vs. 162.1±13.3 nmol $L^{-1}$, respectively, $p<0.05$).

The basal levels of the corticoid were higher in non-lactating than in lactating goats (4.94±0.67 vs. 2.97±0.67, nmol $L^{-1}$, respectively, $p = 0.04$). Eighty-six percent of the basal values occurred at times between 15:00-22:00 h and 01:00-05:00 h.

The present study shows that the cortisol pattern in the domestic goat undergoes significant changes during the day, suggesting greater adrenal activity during the diurnal hours, which is a common characteristic of several species (Fulkerson and Tang, 1979; Ingram *et al.*, 1999; Kolevská *et al.*, 2003). Similar average cortisol values during the day were found by Kokkonen *et al.* (2001) in adult Finnish landrace goats, however, the highest levels found during diurnal times in our study differed with those found by these authors. Kokkonen *et al.* (2001) found that the main elevations in cortisol occurred during nocturnal times, immediately after midnight; a similar

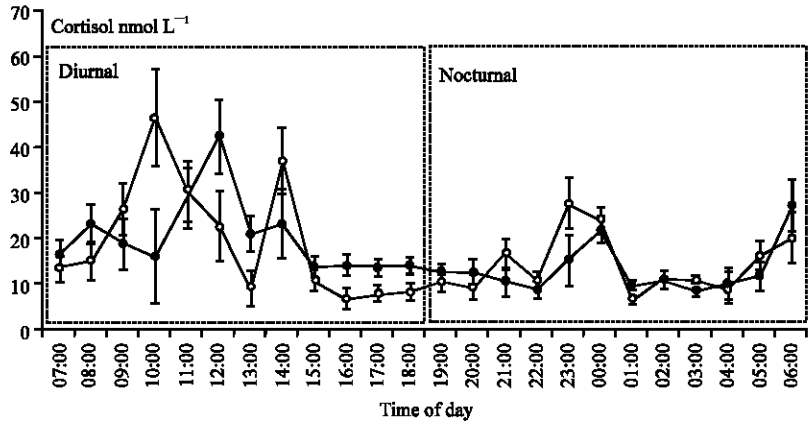


Fig. 1: Average cortisol values per hour (±se) in lactating (empty circle) and non-lactating (black circle) housed goats during 24 h

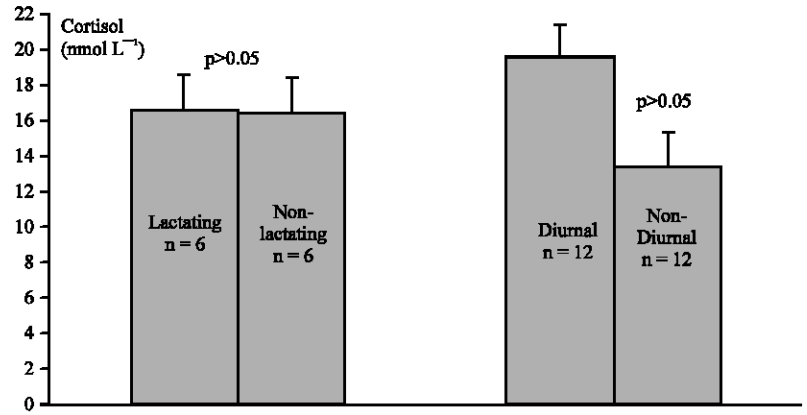


Fig. 2: Mean cortisol levels per hour in lactating and non-lactating goats and diurnal (07:00-18:00h) and nocturnal (19:00-06:00h) h

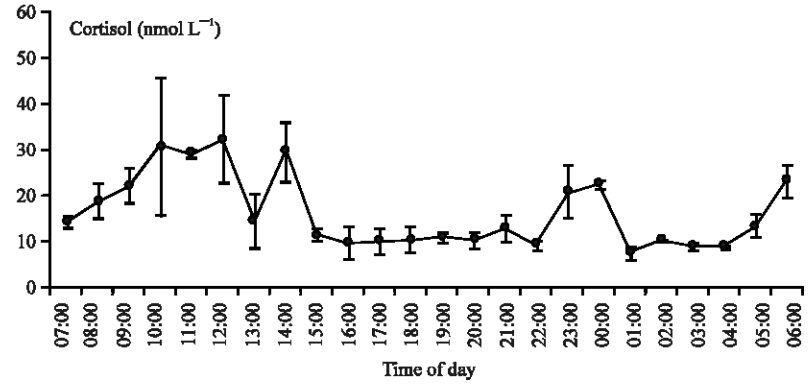


Fig. 3: Average cortisol values per hour (±se) in twelve goats during 24 h

isolated elevation was found in our study between 23:00 and 24:00 h, although it was not the highest one observed during the day and there was no clear explanation for it.

The highest cortisol levels appeared during the first part of the day (10:00-14:00 h), after a gradual increase that began at 05:00 h. After its highest concentrations were

observed, the corticoid diminished to basal values from 15:00 h until 22:00 h. Basal values resembled findings by Galicia (2005) in the same type of goats and Kannan *et al.* (2000) in Spanish goats prior to stressful situations.

In adult sheep and the new-born lambs, cortisol levels are higher during diurnal than nocturnal times (Lincoln *et al.*, 1982). When deprivation of natural photoperiod is applied and the light time is kept constant during the day, synchronization of the daily pattern of hormone secretion remains, suggesting that the light-dark cycle is not the cause ("zeitgeber") of the variations in its levels (Kennaway *et al.*, 1981; Lincoln *et al.*, 1982). Even pinealectomized ewes maintain the cortisol pattern, with the highest levels observed during morning hours (Kennaway *et al.*, 1981), as in our study.

In cows, authors such as Negrão *et al.* (2004) and Gorewit *et al.* (1992) have suggested that increases in the corticoid during the day are related to handling practices such as milking, since machine and hand-milking have both shown to increase cortisol levels (Wagner and Oxemeider, 1972; Gorewit *et al.*, 1992). In the present study, the daily average levels of cortisol were similar in goats and were independent of their lactation condition (lactating or not); in addition, the highest levels of hormone observed during the day did not correspond with the times of milking (07:30-08:30 h). Apparently, the association of high cortisol levels with milking occurs in primiparous females (Negrão and Marnet, 2003) and possibly in multiparous females during their first visits to the milking area (Van Reenen *et al.*, 2002). In Holstein cows, cortisol levels during early lactation are higher than at other stages (Fukasawa *et al.*, 2008), suggesting that the beginning of lactation might be a strong stressor. In preliminary observations, multiparous goats with experience in the handling of milking are less agitated than primiparous goats. In our study, lactating goats were multiparous and well adapted to milking management.

The design of the present study did not allow us to establish the causes of corticoid elevation and reduction during certain times of the day. The endogenous origin of the daily pattern of the hormone has been proven in many animal species and it is accepted that it is the result of an endogenous rhythm "put on time" by external factors such as environmental light and, probably, handling routines (Piccione and Giovanni, 2002).

Cortisol levels can be affected by the sampling method; blood sampling itself is disturbing and the use of previous catheterization to minimize this disturbance is not always possible (Cook, 2002; Möstl and Palme, 2002); in red deers (*Cervus elaphus*), Ingram *et al.* (1999) found that the basal concentration of the hormone is lower when a remote sampling device is used than when the procedure requires physical containment. This has been confirmed in reindeer (*Rangifer tarandus*) (Säkkinen *et al.*, 2004). Although, it is expected that physical containment causes activation of the HPA axis, there is no available information regarding the use of such sampling devices in domestic goats. In the present study, cortisol levels were similar to those obtained by Galicia (2005) previous to situations of acute stress in the same type of goats and to those found by Hydbring *et al.* (1999) in milk goats prior to the onset of parturition. In the pigmy goat, similar basal levels have been reported at different times of the year (Howland *et al.*, 1985). In our study, most of the cortisol values were within the physiological range reported for goats (Eriksson and Teräväinen, 1989; Engelbrecht *et al.*, 2000) and were similar to levels previously found during and after stress by electro-ejaculation (Ortiz-de-Montellano *et al.*, 2007) and without any treatment (Aoyama *et al.*, 2007).

## CONCLUSION

It is our conclusion that in the domestic housed goat: The daily cortisol levels are similar between lactating and non-lactating goats at an advanced stage of lactation; and cortisol levels undergo significant changes during the day, with higher concentrations during diurnal than nocturnal times.

## REFERENCES

Ader, R. and S.B. Friedman, 1968. Plasma corticosterone response to environmental stimulation: Effects of duration of stimulation and the 24 h adrenocortical rhythm. Neuroendocrinology, 3: 378-386.

Aoyama, M., A. Negishi, A. Abe, R. Yokoyama, T. Ichimaru and S. Sugita, 2007. Physiological and behavioural effects of an intracerebroventricular injection of corticotropin releasing hormone in goats. Vet. J., doi:10.1016/j.tvjl.2007.04.002 (in Press).

Claus, R., R. Over and M. Dehnhard, 1990. Effect of male odour on LH secretion and the induction of ovulation in seasonally anoestrous goats. Anim. Reprod. Sci., 22: 27-38.

Cook, C.J., 2002. Rapid noninvasive measurement of hormones in transdermal exudate and saliva. Physiol. Behav., 75: 169-181.

Desir, D., E. Van Cauter, J. Golstein, V.S. Fang, R. Leclercq, S. Refetoff and G. Copinschi, 1980. Circadian and ultradian variations of ACTH and cortisol secretion. Horm. Res., 13: 302-316.

Ekkel, E.D., B. Savenije, W.G.P. Schouten, V.M. Wiegant and M.J.M. Tielen, 1997. The effects of mixing on behavior and circadian parameters of salivary cortisol in pigs. Physiol. Behav., 62: 181-184.

Engelbrecht, Y., T. Herselman, A. Louw and P. Swart, 2000. Investigation of the primary cause of hypoadrenocorticism in South African angora goats (*Capra aegagrus*): A comparison with Boer goats (*Capra hircus*) and Merino sheep (*Ovis aries*). J. Anim. Sci., 78: 371-379.

Eriksson, L. and T.L. Teräväinen, 1989. Cyrcadian rhythm of plasma cortisol and blood glucose in goats. Aus. J. Applied Sci., 2: 202-203.

Fukasawa, M., H. Tsukada, T. Kosako and A. Yamada, 2008. Effect of lactation stage, season and parity on milk cortisol concentration in Holstein cows. Livest. Sci., 113: 280-284.

Fulkerson, W.J. and B.Y. Tang, 1979. Ultradian and circadian rhythms in the plasma concentration of cortisol in sheep. J. Endocrinol., 81: 135-141.

Galicia, L.L., 2005. Efecto del transporte por tres horas sobre la secreción de cortisol, LH y la presencia de estro y ovulación en cabras con ciclo sincronizado. Bachelors Thesis, Escuela de Medicina Veterinaria y Zootecnia, Benemérita Universidad Autónoma de Puebla, Puebla, México.

Gorewit, R.C., K. Svennersten, W. R. Butler and K. Uvnas-Moberg, 1992. Endocrine responses in cows milked by hand and machine. J. Dairy Sci., 75: 443-448.

Howland, B.E., L.M. Sanford and W.M. Palmer, 1985. Changes in serum levels of LH, FSH, prolactin, testosterone and cortisol associated with season and mating in male pygmy goats. J. Androl., 6: 89-96.

Hydbring, E., A. Madej, E. MacDonald, G. Drugge-Boholm, B. Berglund and K. Olsson, 1999. Hormonal changes during parturition in heifers and goats are related to the phases and severity of labour. J. Endocrinol., 160: 75-85.

Ingram, J.R., J.N. Crockford and L.R. Matthews, 1999. Ultradian, circadian and seasonal rhythms in cortisol secretion and adrenal responsiveness to ACTH and yarding in unrestrained red deer (*Cervus elaphus*) stags. J. Endocrinol., 162: 289-300.

Janssens, C.J. J.G., F.A. Helmond and V.M. Weigant, 1995. The effect of chronic stress on plasma cortisol concentrations in cyclic female pigs depends on the time of day. Domest. Anim. Endocrinol., 12: 167-177.

Kannan, G., T.H. Terrill, B. Kouakou, O.S. Gazal, S. Gelaye, E.A. Amoah and S. Samake, 2000. Transportation of goats: Effects on physiological stress responses and live weight loss. J. Anim. Sci., 78: 1450-1457.

Kennaway, D.J., J.M. Obst, E.A. Dunstan and H.G. Friesen, 1981. Ultradian and seasonal rhythms in plasma gonadotropins, prolactin, cortisol and testosterone in pinealectomized rams. Endocrinology, 108: 639-646.

Kokkonen, U.M., P. Riskila, M.T. Roihankorpi and T. Soveri, 2001. Circadian variation of plasma atrial natriuretic peptide, cortisol and fluid balance in the goat. Acta Physiol. Scan., 171: 1-8.

Kolevská, J., V. Brunclík and M. Svoboda, 2003. Circadian rhythm of cortisol secretion in dogs of different daily activities. Acta Vet. Brno, 72: 599-605.

Lefcourt, A.M., G. Paul, H. Mayer, D. Schams and R.M. Bruckmaier, 1997. Response of catecholamines to manual teat stimulation or machine-milking of Lacaune and Friesen dairy ewes. J. Dairy Sci., 80: 3205-3211.

Lefcourt, A.M., J. Bitman, S. Kahl and D.L. Wood, 1993. Circadian and ultradian rhythms of peripheral cortisol concentrations in lactating dairy cows. J. Dairy Sci., 76: 2607-2612.

Lincoln, G.A., O.F.Z. Almeida, H. Klandorf and R.A. Cunningham, 1982. Hourly fluctuation in the blood levels of melatonin, prolactin, luteinizing hormone, follicle-stimulating hormone, testosterone, tri-iodothyronine, thyroxine and cortisol in rams under artificial photoperiods and the effects of cranial sympathectomy. J. Endocrinol., 92: 237-250.

Morin, L.P. and J. Dark, 1992. Hormones and Biological Rhythms. In: Behavioural Endocrinology, Becker, J.B., S.M. Breedlove and D. Crews (Eds.). MIT Press, USA., pp: 473-566.

Möstl, E. and R. Palme, 2002. Hormones as indicators of stress. Domest. Anim. Endocrinol., 23: 67-74.

Murayama, S. and Y. Sasaki, 1987. Circadian variation in the blood cortisol level formed by feeding sheep. Jpn. J. Zootech. Sci., 58: 333-340.

National Research Council, 1981. Nutrient requirements of goats: angora, dairy and meat goats in temperate and tropical countries. National Academy Press, Washington, D.C.

Negrão, J.A., M.A. Porcionato, A.M. de Passille and J. Rushen, 2004. Cortisol in saliva and plasma of cattle after ACTH administration and milking. J. Dairy Sci., 87: 1713-1718.

Negrão, J.A. and P.G. Marnet, 2003. Cortisol, adrenalin, noradrenalin and oxytocin release and milk yield during first milkings in primiparous ewes. Small Rumin. Res., 47: 69-75.

Ortiz-de-Montellano, M., F. Galindo-Maldonado, E.O. Cavazos-Arizpe, A.M. Aguayo-Arceo, J.F.J. Torres-Acosta and A. Orihuela, 2007. Effect of electro-ejaculation on the serum cortisol response of Criollo goats (*Capra hircus*). Small Rumin. Res., 69: 228-231.

Piccione, G. and C. Giovanni, 2002. Biological rhythm in livestock. J. Vet. Sci., 3: 145-157.

Przekop, F., E. Stupnicka, E. Wolinska-Witort, K. Mateusiak, B. Sadowski and E. Domanski, 1985. Changes in circadian rhythm and suppression of the plasma cortisol level after prolonged stress in the sheep. Acta Endocrinol. Cop., 110: 540-545.

Ruis, M.A.W., J.H.A. Te Brake, B. Engel, E.D. Ekkel, W.G. Buist, H.J. Blokhuis and J.M. Koolhaas, 1997. The circadian rhythm of salivary cortisol in growing pigs: Effects of age, gender and stress. Physiol. Behav., 62: 623-630.

Säkkinen, H., J. Tornbeg, P.J. Goddard, E. Eloranta, E. Ropstad and S. Saarela, 2004. The effect of blood sampling method on indicators of physiological stress in reindeer (*Rangifer tarandus tarandus*). Domest. Anim. Endocrinol., 26: 87-98.

Schmidt-Reinwald, A., J.C. Pruessner, D.H. Hellhammer, I. Federenko, N. Rohleder, T.H. Schurmeyer and C. Kirschbaum, 1999. The cortisol response to awakening in relation to different challenge tests and a 12 h cortisol rhythm. Life Sci., 64: 1653-1660.

SAS, 1999. SAS Statistics users´ guide. Cary, N.C., USA.

Thun, R., E. Eggenberger, K. Zerobin, T. Luscher and W. Vetter, 1981. Twenty-four-hour secretory pattern of cortisol in the bull: evidence of episodic secretion and circadian rhythm. Endocrinology, 109: 2208-2212.

Van Reenen, C.G., J.T.N. Van der Werf, R.M. Bruckmaier, H. Hopster, B. Engel, J.P.T.M. Noordhuizen and H.J. Blokhuis, 2002. Individual differences in behavioral and physiological responsiveness of primiparous dairy cows to machine milking. J. Dairy Sci., 85: 2551-2561.

Wagner, W.C. and S.L. Oxemeider, 1972. Adrenal function in the cow. Diurnal changes and the effects of lactation and neurohypophyseal hormones. J. Anim. Sci., 34: 630-635.