

Cortisol and Postpartum Luteal Function in Cattle

Research Article

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ABSTRACT

The present study investigated serum cortisol in response to postpartum ovarian resumption and luteal function. Postpartum ovarian resumption was detected based on a rise in progesterone (P_4) concentrations during three weeks after calving. Twenty two Holstein cows in a semi-closed dairy farm in Qena city, Egypt were used. The study was carried out from late March to early June; the period characterized by a good temperature humidity index (THI) and absence of heat stress. According to the concentrations of P_4 in serum ($P_4 > 1.0$ ng/mL), 11 of the 22 cows resumed the ovarian activity within three weeks after calving (ovarian resumption cows). No effect of THI was found during the postpartum period of study on the concentrations of P_4 , cortisol and cholesterol in the ovarian resumption or non-ovarian resumption group. In the former, cholesterol showed a significant correlation with both P_4 ($r=0.405$, $P<0.05$) and cortisol ($r=0.393$, $P<0.05$). However, in the latter, cholesterol had a significant correlation with P_4 ($r=0.289$, $P<0.05$) but showed no correlation with cortisol. In addition, cortisol showed a significant correlation with P_4 ($r=0.329$, $P<0.05$) in the ovarian resumption group but showed no correlations in the non-resumption group. Our study suggests that cortisol plays a role in the function of corpus luteum and maintenance of luteal life span during this period.

KEY WORDS cattle, cholesterol, cortisol, luteal function, postpartum.

INTRODUCTION

Recovery of function after pregnancy and parturition of both the hypothalamic-pituitary-gonadal (HPG) axis and the genital tract is an important event for resumption of ovarian activity (Malven, 1984). It occurs during the first three weeks after parturition in cattle (Savio *et al.* 1990). The return to ovarian function of early postpartum cows requires optimal conditions for several metabolic and endocrine factors. Progesterone (P_4) is considered a marker of luteal function, since the formation of a corpus luteum is considered a sign of ovarian resumption (Roche *et al.* 2000). The concentration of P_4 remained minimal until after formation of the first postpartum corpus luteum

(Lewis, 1997), and increased to more than 1.0 ng/mL blood within 2-3 days after the first ovulation (Stevenson and Britt, 1979).

Environmental stress was believed to be the main factor associated with the delayed ovarian resumption (Schopper *et al.* 1989; Opsomer *et al.* 1996; Breen and Karsch, 2004). However, many papers have investigated the effect of cortisol on the HPG axis through studying its effect on the pulsatility of gonadotropin-releasing hormone (GnRH) (Oakley, 2009) and / or the secretory function of the pituitary gonadotrophs (Daley *et al.* 1999; Breen and Karsch, 2006; Oakley, 2009). Effect of stress-induced rise of cortisol on the follicular (Breen *et al.* 2005; Oakley, 2009) and luteal activities (Rueda *et al.* 2000; Andersen, 2002; Myers

et al. 2007) were also studied. However, the question remains: in absence of stress, what is the role of cortisol in postpartum cyclic cows after ovulation? Rueda *et al.* (2000) suggested that cortisol may play a role in the corpus luteum as an anti-apoptotic factor in the bovine luteal cells. Furthermore, the corpus luteum was thought to have the potential to respond to a locally generated cortisol (Michael *et al.* 2003).

Several studies have believed in the luteotropic role of cortisol in the regulation and remodelling of the corpus luteum (Tetsuka *et al.* 2003; Myers *et al.* 2007) and stimulation of P_4 secretion (Kawate *et al.* 1993). The present study aims to determine the profiles of cortisol in postpartum resumption and non-resumption cows based on a rise of P_4 concentrations in blood. Furthermore, cholesterol profiles were also studied as a precursor for all steroids including P_4 and cortisol.

MATERIALS AND METHODS

Animals

Twenty two healthy Holstein pluriparous cows (age 2-5 years, mean body weight (BW), 250 kg) were used. The cows were housed on a semi-closed dairy farm provided with shadows in Qena city, Egypt. They were fed enough hay and concentrates from 08:30 to 09:30 am and from 16:30 to 17:30 pm daily. The concentrates were given according to the BW of each cow and the residuum of hay was removed at each feeding time. Water was available continuously. The cows delivered from March 18 to April 20. All cows delivered normally without intervention and body condition scores averaged 2.5 from the day of calving and throughout the postpartum period of study. The animals were artificially milked twice daily at 06:00 and 18:00. The milk yield per cow averaged 2500 kg in the lactation period (305 days). The cows were clinically examined once a week up to 45 days after calving by visual inspection, rectal palpation and vaginoscopy.

The examination covered the animal's general condition, uterine involution, and vaginal secretions. Expression of oestrus signs during the postpartum period were believed to be declined (Lucy *et al.* 2007; Peter *et al.* 2009) mainly during the transition period (first three weeks). Therefore, detection of oestrus was not involved in our study. However, most cows passed the postpartum period without any gross abnormalities in the reproductive system. The twenty two cows were divided into two groups; ovarian resumption and non-resumption group according to serum P_4 concentrations during the first three weeks post-calving. The former group was characterized by elevated serum P_4 concentrations ($P_4 > 1.0$ ng/mL), however, those in the latter group remained low ($P_4 < 1.0$ ng/mL). Thus, fluctuations in

serum P_4 levels could reveal the state of postpartum ovarian activity during the period of our study. Because of metabolic disorders, lameness and mastitis that could be predisposing factors for stress during the postpartum period, the number of cows used decreased from 22 to 19 at 31 days after parturition, and to 18 and 16 at 38 days and 45 days after parturition, respectively.

Experimental design

The study of postpartum cortisol profile in our experiment is based on excluding the stress factors that increase the levels of cortisol in blood, mainly heat stress factor and the postpartum metabolic disorders. Data on ambient temperature and humidity were obtained from the meteorology station of Qena city located near the farm. A maximum temperature humidity index (THI) was calculated from the ambient temperature and humidity using an equation reported previously (Thom, 1959; Hahn, 1969; Mc Dowell *et al.* 1979). Postpartum luteal function and ovarian resumption was diagnosed based on a rise in the serum P_4 concentration ($P_4 > 1.0$ ng/mL) during the postpartum transition period (first three weeks after calving). However, blood samples were collected throughout the 45-day postpartum period on days 1, 6, 12, 18, 31, 38, and 45 after calving to follow up the hormonal profiles. Three millilitres of jugular venous blood was collected into a vacutainer tube at 08:00 each day. The blood samples were left to coagulate for 30 minutes and centrifuged at 3000 rpm. (2500×G) to obtain serum. The sera were stored at -20 °C until assayed. The concentrations of P_4 , cortisol and cholesterol in the serum were measured.

Measurement of P_4 , cortisol and cholesterol concentrations P_4 and cortisol were extracted from serum with ethyl ether, and their concentrations in the extracted fraction were measured using commercially available enzyme immunoassay kits (Biocheck, Inc 837 Cowan Rd, Burlingame, CA 94010 and Human Gesellschaft für Biochemica und Diagnostica mbH Max-Planck-Ring 21, D-65205, Germany, respectively). Cholesterol was assayed by the spectro-photometer using a commercial kit (Biomerix, cholesterol RTU, 07986 D-GB-05/2031, France). All samples were assayed in a single run, and the intra-assay coefficient of variation was less than 10.

Statistical analysis

All data are presented as the mean $\pm$ SEM. The statistical significance of differences in serum P_4 , cortisol and cholesterol concentrations between the ovarian resumption and non-resumption groups was determined with Student's t-test. Correlations among data on THI, P_4 , cortisol and cholesterol throughout the postpartum period were also calculated. All data were analyzed using graph pad prism (graph

pad software, San Diego, CA, USA). Results were considered significant at $P < 0.05$

RESULTS AND DISCUSSION

Temperature humidity index (THI)

A record of THI during the period of study from March to June covers the dates of parturition from March to April, in the 22 Holstein cows studied, and the subsequent 45 days of exposure to environmental conditions.

The mean ambient temperature in March, April and May was 20.6 °C, 20.9 °C and 25 °C, respectively, while mean humidity was 37.4%, 31.6% and 25.4%, respectively. However, the THI averaged 66.6 throughout the period of study.

Postpartum P_4 profile and luteal function

The postpartum P_4 profile for 45 days after parturition is shown in Figure 1. The twenty two cows were divided into two groups according to serum P_4 concentrations at the 18th days post-calving. Serum P_4 concentrations in 11 of the 22 cows were elevated ($P_4 > 1.0$ ng/mL), however, P_4 concentrations in the other 11 cows remained low ($P_4 < 1.0$ ng/mL). A significant difference in P_4 concentrations between resumption and non-resumption cows at the 18th days after calving was found (3.1 ± 0.7 vs. 0.6 ± 0.05 ng/mL, respectively) ($P < 0.05$). Thus, according to the fluctuations in serum P_4 levels, half of the 22 cows were considered to have a resumed ovarian activity during the postpartum period.

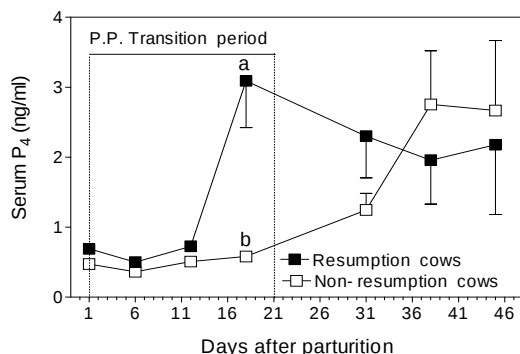


Figure 1 Serum progesterone (P_4) profile from calving to 45 days postpartum

Ovarian activity was evaluated using P_4 concentrations during the 21 days after parturition (P.P. transition period)

Cows with P_4 concentrations of more than 1.0 ng/mL were placed in the ovarian resumption group ($n=11$) and those of less than 1.0 ng/mL were placed in the non-ovarian resumption group ($n=11$)

a is significantly different from b ($P < 0.05$)

Each value during the 1st, 6th, 12th and 18th days after calving represents the mean $\pm$ SEM for 11 cows. Other values from 31 to 45 days after calving represent the mean $\pm$ SEM for 7 to 11 cows

Postpartum cortisol and cholesterol profiles

The postpartum cortisol and cholesterol profiles throughout the 45 days after parturition are shown in Figure 2 and Fig-

ure 3 respectively. Serum cortisol concentrations were significantly higher ($P < 0.05$) in the cows in which luteal function resumed, on days 12, 18, 31 and 38 after calving (56.9 ± 9.0 ($n=11$) vs. 20.4 ± 3.8 ($n=11$), 63.0 ± 15.4 ($n=11$) vs. 26.5 ± 4.9 ($n=11$), 97.1 ± 24.3 ($n=8$) vs. 43.6 ± 7.4 ($n=11$), 110.3 ± 12.2 ($n=7$) vs. 54.3 ± 14.9 ($n=11$), respectively). Serum cholesterol concentrations were significantly lower ($P < 0.05$) in the animals in which luteal function resumed, on days 1, 6, 38 and 45 after parturition (92.4 ± 10.5 ($n=11$) vs. 150.9 ± 17.2 ($n=11$), 82.8 ± 8.1 ($n=11$) vs. 137.1 ± 19.1 ($n=11$), 134.4 ± 13.0 ($n=7$) vs. 185.0 ± 12.0 ($n=11$), 156.0 ± 13.7 ($n=7$) vs. 219.6 ± 13.2 ($n=9$), respectively).

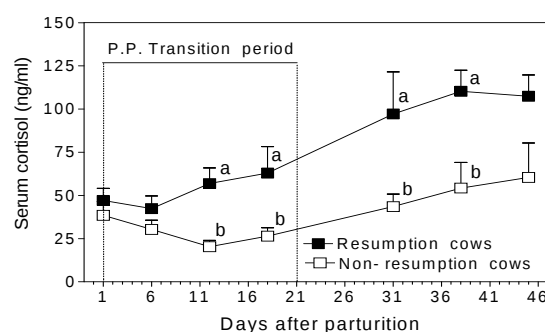


Figure 2 Serum cortisol profile from calving to 45 days after calving in ovarian resumption and non-resumption cows

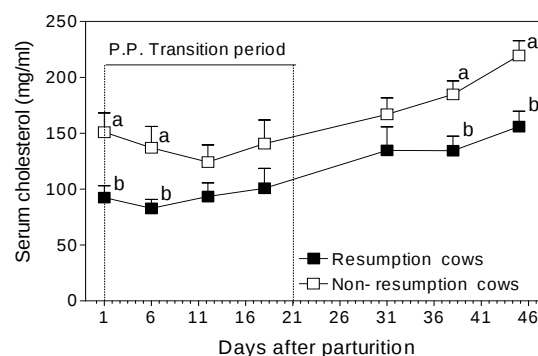


Figure 3 Serum cholesterol profile from calving to 45 days after calving in ovarian resumption and non-resumption cows

Mean serum cortisol concentrations during the postpartum transition period were significantly higher ($P < 0.05$) in the resumption than non-resumption (52.3 ± 5.1 ($n=11$) vs. 29.0 ± 2.9 ($n=11$)); in contrast, mean serum cholesterol concentrations were significantly lower in the resumption group (52.3 ± 5.1 vs. 92.4 ± 6.2 , $n=11$) (Figure 4).

THI, P_4 , cortisol and cholesterol-linear regression

Linear of regression (r^2) between the parameters (THI, P_4 , cortisol and cholesterol) in the ovarian resumption and non-resumption groups during 45 days after parturition are shown in the Table 1. Concerning environmental THI from late March to early June, neither group of cows showed a significant correlation between THI and the other parame-

ters (P_4 , cortisol and cholesterol), excluding the effect of THI fluctuations on the studied profiles. In the ovarian resumption group, cholesterol showed significant regression with both P_4 ($r^2=0.164^*$, $P<0.05$) and cortisol ($r^2=0.154^*$, $P<0.05$). However, in the non-resumption group, cholesterol had a significant regression with P_4 ($r^2=0.084^*$, $P<0.05$) and showed no correlation with cortisol. Also, the linear regression between P_4 and cortisol was significant in the ovarian resumption group than non-resumption group throughout the postpartum period ($r^2=0.1082^*$ vs. 0.0083, respectively).

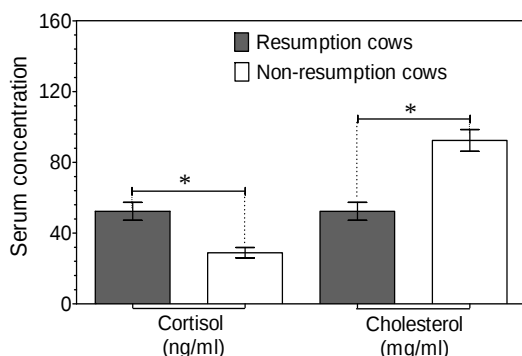


Figure 4 Mean serum cortisol and cholesterol concentrations during the P.P. transition period in ovarian resumption and non-resumption cows

* $P<0.05$ compared with non-resumption cows

Each value represents the mean $\pm$ SEM for 11 cows

Stress was believed to have a negative effect on the secretion of pituitary gonadotropins through the stimulated secretions of adrenal glucocorticoids (Bearden *et al.* 1992; Breen and Karsch, 2004).

The environmental condition during the period of our study, from late March to early June, is considered the most favourable condition for animal health and biological activities according to the values of THI which showed no significant correlation with P_4 , cortisol / or cholesterol either. This could exclude the effect of heat stress on the studied parameters and cortisol mainly. In general, glucocorticoids were suggested to enhance the secretion of P_4 (Kawate *et al.* 1993) which was evidenced by the expression of glucocorticoid receptors by luteal cells (Sugino *et al.* 1997). Several papers have investigated the effect of cortisol on the luteal function. It was thought to be an antiapoptotic factor in the corpus luteum (Rueda *et al.* 2000) and play a luteotropic role in the regulation and remodeling of the luteal cells (Tetsuka *et al.* 2003; Myers *et al.* 2007). However, the ovarian biosynthesis of cortisol is still unclear. Andersen, (2002) stated that ovaries lack the necessary enzymes for cortisol synthesis while others have suggested that the corpus luteum has the potential to respond to a locally generated cortisol (Michael *et al.* 2003). In our study, the concentrations of P_4 and cortisol were lowest on days 1 and 6 after parturition, which agreed to a

previous study stated that plasma corticoid levels decreased following parturition and plasma P_4 remained at a low level from parturition until first estrus (Echternkamp and Hansel, 1973). Furthermore, significantly higher concentrations of cortisol were observed in cows in which ovarian activity was resumed during the postpartum period.

In which, a significant correlation was observed between cortisol and cholesterol concentrations while P_4 concentrations tended to correlate with cortisol concentrations in the ovarian resumption group throughout the postpartum period. These findings suggest that there is a strong relationship between cortisol and luteal function during the postpartum period in cows. Since the formation and function of the corpus luteum was suggested to benefit from a high local concentration of free cortisol (Andersen, 2002). Although it was reported that high concentrations of cortisol in blood during stress suppressed the release of pulsatile GnRH (Breen and Karsch, 2004) or inhibited its effect on the ovary, according to our findings we suggest that cortisol may play an important role in the regulation of the oestrous cycle and maintenance of the corpus luteum. However, additional research should be performed to examine the mechanism of luteolysis under a stress-induced cortisol condition.

Table 1 Linear regression among temperature humidity index (THI), progesterone (P_4), cortisol and cholesterol

Index	Ovarian resumption cows			Non-resumption cows		
	P_4	Cortisol	Cholesterol	P_4	Cortisol	Cholesterol
THI	0.025	0.005	0.017	0.008	0.0002	0.0002
P_4	-	0.108*	0.164*	-	0.008	0.083*
Cortisol	-	-	0.154*	-	-	0.022

* ($P<0.05$).

The cholesterol profile in serum was investigated because cholesterol is a precursor of all steroid hormones like glucocorticoids (Gwazdauskas *et al.* 1972) and P_4 (Spicer and Echternkamp, 1995). The plasma cholesterol concentration was found to steadily increase after parturition (Spicer *et al.* 1993) and undergo cyclic changes during oestrus in miniature pigs (Lussier-cacun *et al.* 1977). The pattern was characterized by a marked decline in plasma cholesterol concentrations during the luteal phase and the total cholesterol level decreases during pregnancy when the plasma P_4 concentration is very high (Wolf *et al.* 1967).

However, our results revealed that the level of cholesterol was directly proportional to the increase in not only P_4 but also cortisol in the post-partum cyclic luteal phase ($P_4>1.0$ ng/mL). On the other hand, it was stated that the plasma cholesterol concentration alone cannot be used to predict the postpartum ovarian activity and luteal function because it is closely correlated with other factors like glucose, milk fat and protein (Francisco *et al.* 2003). The dif-

ference in the serum cholesterol concentrations between the groups may be due to other factors related to lactation and dry matter intake which could explain the significantly lower concentrations of cholesterol in the ovarian resumption group. Thus, utilization of cholesterol appears to be essential and closely related to the ovarian activity during the luteal period and additional research is required to investigate more precisely the cholesterol uptake by the ovarian cells during the luteal phase of the oestrous cycle in relation to other metabolic factors like glucose.

In conclusion, cortisol was stated to regulate the dynamics of ovulatory follicles (Andersen, 2002; Acosta *et al.* 2005) and have a positive influence on the luteal function during oestrus (Duong *et al.* 2012) or pregnancy (Myers *et al.* 2007; Duong *et al.* 2012). Therefore, the present study could support the previous studies for the importance of cortisol in the maintenance and function of the luteal life span, and that cholesterol is relevant indicator of luteal function during the puerperium period in cows which requires more research.

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REFERENCES

- Acosta T.J., Tetsuka M., Matsui M., Shimizu T., Berisha B., Schams D. and Miyamoto A. (2005). *In vivo* evidence that local cortisol production increases in the preovulatory follicle of the cow. *J. Reprod. Dev.* **51**, 483-489.
- Andersen C.Y. (2002). Possible new mechanism of cortisol action in female reproductive organs, physiological implications of the free hormone hypothesis. *Endocrinology*. **173**, 211-217.
- Bearden H.J. and Fuquay J.W. (1992). *Applied Animal Reproduction*. Englewood Cliffs: Prentice Hall.
- Breen K.M. and Karsch F.J. (2004). Does cortisol inhibit pulsatile luteinizing hormone secretion at the hypothalamic or pituitary level? *Endocrinology*. **145**, 692-698.
- Breen K.M. and Karsch F.J. (2006). New insights regarding glucocorticoids, stress and gonadotropin suppression. *Front Neuroendocrinol.* **27**, 233-245.
- Breen K.M., Billings H.J., Wagenmaker E.R., Wessinger E.W. and Karsch F.J. (2005). Endocrine basis for disruptive effects of cortisol on preovulatory events. *Endocrinology*. **146**, 2107-2115.
- Daley C.A., Sakurai H., Adams B.M. and Adams T.E. (1999). Effect of stress-like concentrations of cortisol on gonadotroph function in orchidectomized sheep. *Biol. Reprod.* **60**, 158-163.
- Duong H.T., Piotrowska Tomala K.K., Acosta T.J., Bah M.M., Sinderewicz E., Majewska M., Jankowska K., Okuda K. and Skarzynski D.J. (2012). Effects of cortisol on pregnancy rate and corpus luteum function in heifers: an *in vivo* study. *J. Reprod. Dev.* **58**, 22-230.
- Echternkamp S.E. and Hansel W. (1973). Concurrent changes in bovine plasma hormone level prior to and during the first postpartum oestrous cycle. *J. Anim. Sci.* **37**, 1362-1370.
- Francisco C.C., Spicer L.J. and Payton M.E. (2003). Predicting cholesterol, progesterone, and days to ovulation using postpartum metabolic and endocrine measures. *J. Dairy Sci.* **86**, 2852-2863.
- Gwazdauskas F.C., Thatcher W.W. and Wilcox C.J. (1972). Adrenocorticotropin alteration of bovine peripheral plasma concentrations of cortisol, corticosterone and progesterone. *J. Dairy Sci.* **55**, 1165-1168.
- Hahn G.L. (1969). Predicted vs. measured production differences using summer air conditioning for lactating cows. *J. Dairy Sci.* **52**, 800-801.
- Kawate N., Inaba T. and Mori J. (1993). Effects of cortisol on the amounts of estradiol-17 β and progesterone secreted and the number of luteinizing hormone receptors in cultured bovine granulosa cells. *Anim. Reprod. Sci.* **32**, 15-25.
- Lewis G.S. (1997). Uterine health and disorders. *J. Dairy Sci.* **80**, 984-994.
- Lucy M.C. (2007). Fertility in high-producing dairy cows: reasons for decline and corrective strategies for sustainable improvement. *Soc. Reprod. Fertil. Suppl.* **64**, 237-254.
- Lussier-cacun S., Bolte E., Bidallier M., Huang Y.S. and Davignon J.J. (1977). Cyclic fluctuations of plasma cholesterol in the female miniature swine and its relationship to progesterone secretion. Pp. 471-474 in *Proc. Soc. Exp. Biol. Med.*
- Malven P.V. (1984). Pathophysiology of the puerperium. definition of the problem. Pp. 1-8 in *Proc. 10th Int. Cong. Anim. Reprod. A.I., Urbana-Champaign, USA.*
- Mc Dowell D., Hooven N. and Cameron K. (1979). Effects of climate on performance of Holsteins in first lactation. *J. Dairy Sci.* **68**, 2418-2435.
- Michael A.E., Thuston L.M. and Rae M.T. (2003). Glucocorticoid metabolism and reproduction: a tale of two enzymes. *Reproduction*. **126**, 425-441.
- Myers M.M., Lamont M.C., Driesche S.V., Mary N.K., Thong J., Hillier S.G. and Duncan W.C. (2007). Role of luteal glucocorticoid metabolism during maternal recognition of pregnancy in woman. *Endocrinology*. **148**, 5769-5779.
- Oakley A.E., Breen K.M., Clarke I.J., Karsch F.J., Wagenmaker E.R. and Tilbrook A.J. (2009). Cortisol reduces gonadotropin-releasing hormone pulse frequency in follicular phase ewes: influence of ovarian steroids. *Endocrinology*. **150**, 341-349.
- Opsomer G., Mijten P., Coryn M. and De Kruif A. (1996). Postpartum anoestrus in dairy cows: a review. *Vet. Q.* **18**, 68-75.
- Peter A.T., Vos P.L.A.M. and Ambrose D.J. (2009). Postpartum anoestrus in dairy cattle. *Theriogenology*. **71**, 1333-1342.
- Roche J.F., Dackey D. and Diskin M.D. (2000). Reproductive management of postpartum cows. *Anim. Reprod. Sci.* **61**, 703-712.
- Rueda B.R., Hendry I.R., Hendry I.W., Stormshak F., Slayden O.D. and Davis J.S. (2000). Decreased progesterone levels and progesterone receptor antagonists promote apoptotic cell death in bovine luteal cells. *Biol. Reprod.* **62**, 269-276.

- Savio J.D., Boland M.P., Hynes N. and Roche J.F. (1990). Resumption of follicular activity in the early postpartum period of dairy cows. *J. Reprod. Fertil.* **88**, 569-579.
- Schopper D., Schemer R. and Claus R. (1989). Analyse der fruchtbarkeitssituation von Miltchk Ühen postpartum in praxisbetrieben anhand von progesterone profilen. *Reprod. Dom. Anim.* **24**, 67-78.
- Spicer L.J. and Echternkamp S.E. (1995). The ovarian insulin and insulin-like growth factor system with an emphasis on domestic animals. *Dom. Anim. Endocrinol.* **12**, 223-245.
- Spicer L.J., Vernon R.K., Tucker W.B., Wettemann R.P., Hogue J.F. and Adams G.D. (1993). Effects of inert fat on energy balance plasma concentrations of hormones and reproduction in dairy cows. *J. Dairy Sci.* **76**, 2664-2673.
- Stevenson J.S. and Britt J.H. (1979). Relationships among LH, estradiol, progesterone, glucocorticoid, milk yield, body weight and postpartum ovarian activity in Holstein cows. *J. Anim. Sci.* **48**, 570-577.
- Sugino N., Teller'a C.M. and Gibori G. (1997). Progesterone inhibits 20 α -hydroxysteroid dehydrogenase expression in the rat corpus luteum through the glucocorticoid receptor. *Endocrinology.* **138**, 4497-4500.
- Tetsuka M., Yamamoto S., Hayashida N., Hayashi K.G., Hayashi M., Acosta T.J. and Miyamoto A. (2003). Expression of 11 β -hydroxysteroid dehydrogenases in bovine follicle and corpus luteum. *J. Endocrinol.* **177**, 445-452.
- Thom E.C. (1959). The discomfort index. *Weath. Wise.* **12**, 57-61.
- Wolf R.C., Tente L. and Meyer R.K. (1967). Plasma cholesterol in pregnant Rhesus monkeys. Pp. 1230-1231 in Proc. Soc. Exp. Biol. Med.