Jurnal Veteriner Maret 2008 Vol. 9 No. 1 : 7-12
ISSN : 1411 - 8327

Correlation of Progesterone and Cortisol Plasma Levels between Ovulated and Non-ovulated Ettawa Crossbreds Does

(KORELASI KADAR PROGESTERON DAN KORTISOL DALAM PLASMA KAMBING PERANAKAN ETTAWA ANTARA YANG TIDAK DAN YANG BEROVULASI)

Pudji Astuti[1] Diah Tri Widayati[2] Sunendar[2] Kresno Suharto[3] Asmarani.Kusumawati[3] Aris Junaidi[3]

[1] Department of Physiology, [3] Department Reproductive.
Faculty of Veterinary Medicine
[2] Department of Reproduction, Faculty of Animal Husbandry.
Gadjah Mada University
Jl. Olah Raga, Karang Malang, Yogyakarta.
Email: pastuti2001@yahoo.com, *Phone: 08156851163*

ABSTRACT

This experiment was conducted to determine the correlation of progesterone and cortisol levels in plasma between ovulated and non-ovulated Crossbreds Ettawa does. Eight does were used in this experiment and they were divided equally into 2 groups, i.e. group I consisting of 4 normal ovulated does with the average body weight of 37.5 ± 3.109 kg and group II consisting of 4 non-ovulated does with the average body weight of 28 ± 2.160 kg as group II. The estrus cycles of all does were synchronized using intravaginal device of controlled internal drug release (CIDR) accompanied by intra-muscular injection of prostaglandin F2 (PGF2) alpha. Immediately after the onset of estrus, blood samples were collected from jugular every 3 and 6 hours for 72 hours after onset of estrus. The concentration of cortisol and progesterone in plasma was assayed by enzyme linked immunosorbent assay (ELISA). The results showed that the average concentrations of cortisol in ovulated does (90.89±26.22 ng/mL) was higher than in non-ovulated does (42.70±37.18 ng/mL). Similarly, the concentrations of progesterone in ovulated does (0.098±0.0423 ng/mL) was higher than in non-ovulated does (0.093±0.056 ng/mL). It was evident that the change in progesterone level was closely associated with the change in cortisol level in plasma.

Key words: progesterone, cortisol, ovulated, non-ovulated, does

ABSTRAK

Penelitian ini bertujuan untuk mengetahui korelasi kadar progesteron dan kortisol dalam plasma kambing Peranakan Ettawa (PE) antara yang bersiklus estrus normal (mengalami ovulasi) dan yang tidak mengalami ovulasi. Dalam penelitian ini digunakan 8 kambing PE betina dewasa yang dibagi menjadi 2 grup, yaitu grup I yang terdiri atas 4 kambing betina bersiklus ovulasi normal (mengalami ovulasi) dengan rataan bobot badan 37.5 ± 3.109 kg, dan grup II yang terdiri atas 4 kambing betina yang tidak mengalami ovulasi dengan rataan bobot badan : 28 ± 2.160 kg. Sinkronisasi estrus dilakukan dengan *controlled internal drug release* (CIDR) secara intravaginal dan disertai dengan injeksi prostaglandin F2 (PGF2) alfa secara intramuskuler. Segera setelah estrus, darah diambil dari vena jugularis setiap 3 dan 6 jam selama 72 jam pascaestrus. Kadar hormon dalam plasma ditentukan dengan *enzyme linked immunosorbent assay* (ELISA). Selanjutnya, data tentang kadar hormon dalam plasma diolah dengan uji t. Hasil penelitian menunjukkan bahwa konsentrasi kortisol pada kambing PE yang mengalami ovulasi (90.89±26.22 ng/ml) lebih tinggi jika dibandingkan dengan yang tidak mengalami ovulasi (42.70±37.18 ng/ml). Kadar progesterone pada kambing yang mengalami ovulasi (0.098±0.0423 ng/ml) juga lebih tinggi daripada yang tidak mengalami ovulasi (0.093±0.056 ng/ml). Hasil ini menunjukkan bahwa bahwa perubahan kadar progesteron sangat berkaitan dengan perubahan kadar kortisol dalam plasma.

Kata kunci: progesteron, kortisol, ovulasi, non-ovulasi, kambing PE

INTRODUCTION

Progesterone is generally used to manufacture three different types of estrogen i.e. testosterone, cortisol, and aldosterone (Stimmer *et al.* 2003). Although, progesterone is generally known as a gonadally- released reproductive hormone, it is also produced in the brain and by the adrenal gland, where progesterone is an indirect precursor to cortisol (Baulieu *et al.*, 2001). One of the effective ways to block the luteinizing hormone (LH) surge is by administering intermediate concentration of progesterone (Kinder *et al.* 1996).

Follicular cysts occur when estradiol is unable to induce luteinizing hormone surge at the appropriate time during follicular maturation. Follicular cysts secreting high concentrations of estradiol (Kittock *et al.* 1974; Hamilton *et al.* 1995) has been observed in cows when they fail to release a preovulatory surge of luteinizing hormone (LH). Cystic cows also fail to release LH in response to an exogenously administered estradiol challenge (Silvia *et al.* 2005). Such failure appears to occur at the level of the hypothalamus as Gonadotropin releasing hormone (GnRH) has been shown to be equally effective in stimulating pituitary secretion of LH in both cystic and normal cows (Zaied *et al.* 1980). In addition, Silvia *et al.* (2005) reported that, in cows, no differences in adrenal function was detected between animals with normal cycling and ovarian follicular cysts.

As the progesterone is also produced by adrenal gland besides of that produced by the ovary, it is necessary to predict the profile of cortisol and progesterone in both ovulated and non-ovulated animal, since progesterone produced in adrenal gland is the precursor of cortisol. In addition, it is also important to determine to which extent the progesterone (as cortisol precursor) produced in adrenal gland can substitute ovary in producing progesterone when level of this hormone is very low. This phenomenon was assessed by comparing the concentrations of cortisol and progesterone levels in plasma of normal ovulated and non-ovulated does.

METHODS

Experimental Animals and Synchronization of Estrus

Eight adult (1.5-2 years old) does with body weight of 25-40 kg were used in this study. All of animals were housed in the Farm Unit of Cross breeds does in Animal Husbandry Breeding and Feeding Training Center (BPT-HMT), Toyomerto, Singosari, Malang. They were fed with a standard feed containing 5 kg/ day/animal of Napier grass (*Pennisettum purpurreum*), 500-800 g/day/animal, and *ad libitum* of water. Before the blood was collected, all animals were examined for parasitic infection.

The does were divided into 2 groups i.e. group I consisting of 4 non-ovulated does with average body weight of 28 ± 2.160 kg and group II consisting of 4 ovulated does with average body weight of 37.5 ± 3.109 kg. Ovulated animals were assessed from the peak concentration of estradiol and LH as described by Suharto (2007) and Sunendar (2007). The estrus cycles of all does were synchronized using intravaginal device of controlled internal drug release (CIDR; 0.3 g progesterone, Pharmacia & Upjohn Pty Limited, NSW) for 10 days and intramuscular injection of PGF2alpha at day 8 following the CIDR insertion. The onset of estrus occurred 26.6-36.6 hours after removal of CIDR, and the peak of LH occurred 45-51 after removal of CIDR. The blood was collected from each does every 3 and 6 hours the occurrence of estrus. The blood was collected using 3-mL syringes and then was transferred into 5-mL glass tubes containing heparin and centrifuged at 500 g for 15 minutes.. Plasma was collected and stored at –20° C.

Determination of Hormone Concentration in Plasma

The concentration of cortisol and progesterone in plasma was determined a solid phase competitive enzyme–linked immunosorbent assay (ELISA, DRG, Germany). Each well of ELISA microtitration plate was coated with monoclonal antibody against cortisol/ progesterone. Plasma samples from each doe were added to each well followed by cortisol/ progesterone-horseradish peroxidase (HRP). Following an incubation for 1 hour for cortisol and 2 hours for progesterone at room temperature (± 25° C), the bound cortisol/progesterone-HRP was determined by adding Tetramethylbenzidine (TMB) substrate. Each plasma sample was tested in duplicate and the average absorbance reading of each sample was read using multiscan spectrophotometer. The concentration of cortisol/progesterone plasma sample was determined by simple interpolation of the absorbance reading of each sample to standard curve of standard hormone, the concentration of which has been determined by manufacturer. The concentration of bound cortisol/progesterone HRP is inversely proportional to the concentration of hormone in plasma sample.

RESULTS AND DISCUSSIONS

The average cortisol concentrations immediately after the removal of CIDR were 42.70±37.18 ng/ml for non-ovulated does and 92.27±43.94 ng/ml for ovulated (Table 1 and Figure 1). The average concentration of progesterone was (0.098±0.0423 ng/ml) for ovulated does and (0.093±0.056 ng/ml) for non-ovulated does (Table 2 and Figure 2). The concentrations of cortisol is normally used as an index for basal adrenal activity. The concentration of cortisol and progesterone in ovulated was higher than in non-ovulated does (Figure 1 and 2). This clearly indicates that in non-ovulated, although progesterone was still produced by the adrenal gland, it can not compensate the ovary to produce progesterone and therefore the level of both hormones decreased significantly. Like cortisol, progesterone is released in response to adrenocorticotropin hormone (ACTH) (Genazzani *et al*., 1998; Burt *et al*. 2006). Normal value of cortisol in doe is 75–80 ng/ml (Wagenmaker *et al*. 2004 *in* Kellie *et al*. 2005).

As a comparison, salivary progesterone correlated positively with salivary cortisol in men and women taking hormonal contraceptives but not in freely cycling women. This is consistent with the idea that progesterone in men is largely adrenal in origin, whereas in women it is produced by both ovary and the adrenal gland (Wirth and Schultheiss 2006; Wirth *et al*. 2007). Unlike progesterone, Astuti *et al*. (2007) (in press) reported that in ovulated does, estradiol level in serum was positively correlated with serum cortisol. This finding suggests that the circulating progesterone in non-ovulated does comes primarily from the adrenal gland and that in ovulated does comes from the ovary. The release of adrenal progesterone along with cortisol, is likely to be stimulated by ACTH (Genazzani *et al*. 1998). Barbaccia *et al*. (1996) and Konigsson *et al*. (2001) demonstrated that close relationships between progesterone and cortisol is likely to reflect a functional relationship of both hormones. Progesterone is released from the adrenal along with cortisol to

Table 1. Concentrations of Cortisol (ng/ml) in ovulated and non-ovulated does.

Status	Animal 1	Animal 2	Animal 3	Animal 4	Average
Non-ovulated	51.89± 69.44	40.90±15.01	29.19±25.66	48.84±38.62	42.70±37.18[a)]
Ovulated	119.80±44.16	64.33±11.23	115.39±12.92	64.06±36.56	90.89±26.22[b)]

Note: different superscript indicate significant different

Table 2. Concentrations of progesterone (ng/ml) in ovulated and non-ovulated does

Status	Animal 1	Animal 2	Animal 3	Animal 4	Average
Non-ovulated	0.118± 0.059	0.043±0.030	0.125±0.086	0.087±0.050	0.093±0.056[c)]
Ovulated	0.030±0.006	0.026±0.004	0.249±0.104	0.086±0.053	0.098±0.0423[d)]

Note: different superscript indicates significant difference

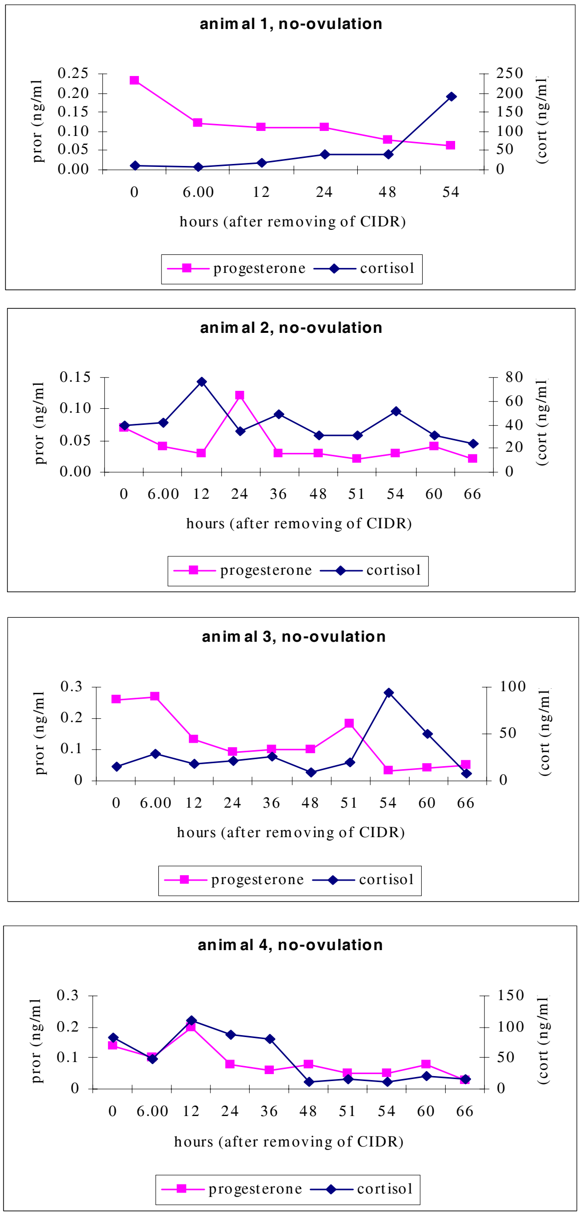

Figure 1. Concentrations of progesterone (ng/ ml) and cortisol (ng/ml) in non-ovulated does

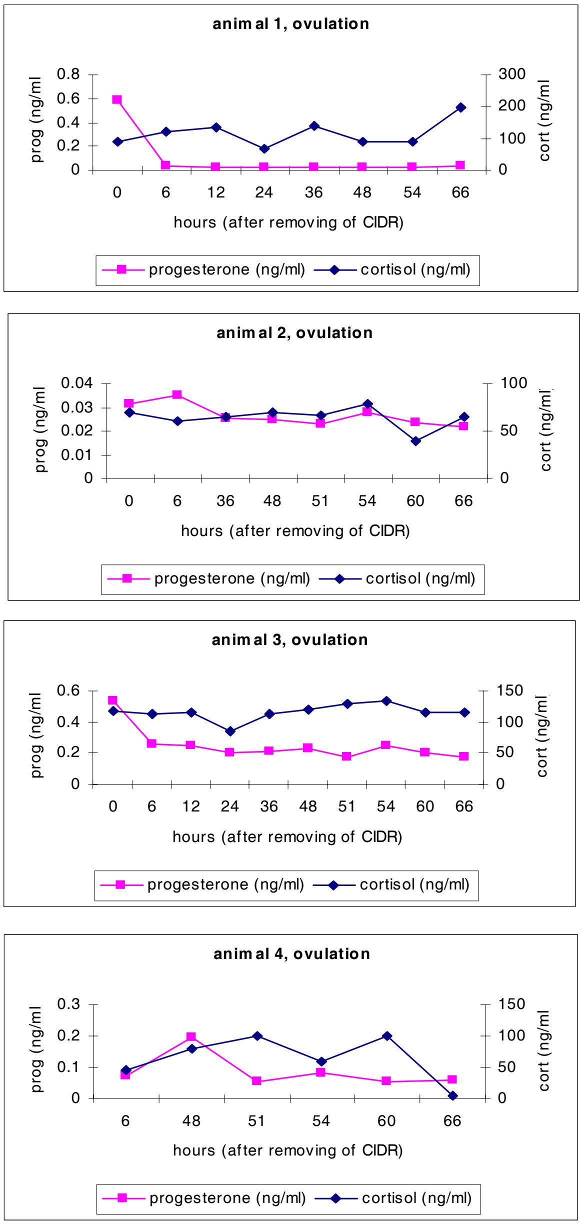

Figure 2. Concentration of progesterone (ng/ml) and cortisol (ng/ml) in ovulated animals

with the finding in this study that the level of cortisol in non-ovulated does was lower than that in ovulated does. Wirth and Schultheiss (2006) and Pirlich *et al* (2002) speculated that progesterone release during stress condition is closely affiliated with the reduction purposes.

In the present study, the level of both progesterone and cortisol in ovulated was significantly lower in non-ovulated that in ovulated does. There are 2 possible explanations down-regulate the Hipothalamo-Pituitary axis, particularly during stress and under basal conditions. Dandrea *et al*. (2001) reported that reduction in maternal during a prolonged period of nutrient restriction is likely to be due to a decrease maternal cortisol secretion. Cortisol (also known as stress hormone) competes with progesterone for receptor sites, leading to a condition of estrogen dominance and less active progesterone. This condition was very similar behind this finding. Firstly, although the adrenal gland remain actively secreting pregnenolone as precursor of cortisol, it could not sufficiently substitute those secreted by the ovary. It means that the adrenal gland only produces the hormone in small quantity. Secondly, in non-ovulated animals, small concentration of progesterone may reflect a small concentration of cortisol , so that there was a positive correlation between the two hormones. It can therefore be concluded that change in progesterone level was positively related with the change in cortisol level and the progesterone produced adrenal gland appears to be unable to compensate the progesterone normally produced by the ovary.

ACKNOWLEDGMENT

The study was funded by Graduate-Competitive grants of Directorate of Higher Education, Department of National Education of Indonesia.

REFFERENCES

Astuti P, Widayati DT, Sunendar, Suharto, Asmarani K, Junaidi. 2007. Cortisol and Estradiol Profile in Crossbreds Ettawa Does: the effects of Body Condition Scoring (BCS). *Jf Ind Biotech* (*in press*).

Barbaccia ML, Roscetti G, Trabucchi M, Mostallino MC. Concas A, Purdy RH. 1996. Time-dependent changes in rat brain neuroactive steroid concentrations and GABA-A receptor function after acute stress. *Neuroendocrinology* 63 : 166–172.

Baulieu, E.E., Robel, P., Schumacher, M., 2001. Neurosteroids: beginning of the story. *Int Rev Neurobiol* 46: 1–32.

Burt MG, Gibney J, Ho KK 2006 Characterization of the metabolic phenotypes of Cushing's syndrome and growth hormone deficiency: a study of body composition and energy metabolism. *Clin Endocrinol (Oxf)* 64:436–443

Dandrea J, Wilson V, Gopalakrishnan G, Heasman L, Budge H, Stephenson T, Symonds ME 2001 Maternal nutritional manipulation of placental growth and glucose transporter-1 abundance in sheep. *Reproduction* 122:793–800

Genazzani AR, Petraglia F, Bernardi F, Casarosa E, Salvestroni C, Tonetti A. 1998. Circulating levels of allopregnanolone in humans: gender, age, and endocrine influences. *J. Clin. Endocrinol. Metab*. 83 : 2099–2103.

Groschl, M., Rauh, M., Dorr, H.G., 2003. Circadian rhythm of salivary cortisol,
17alpha-hydroxyprogesterone, and progesterone in healthy children. *Clin Chem* 49: 1688–1691.

Hamilton SA, Garverick HA, Keisler DH, Xu ZZ, Loos K, Youngquist RS, Salfen BE .1995. Characterization of ovarian follicular cysts and associated endocrine profiles in dairy cows. *Biol Reprod* 53: 890-898.

Kellie M. Breen, Heather J. Billings, Elizabeth R. Wagenmaker, Emily W. Wessinger and Fred J. Karsch .2005. Endocrine Basis for Disruptive Effects of Cortisol on Preovulatory Events. *Endocrinology* 146: 2107-2115

Kinder JE, Kojima FN, Bergfeld EMG, Wehrman ME, Fike KE. 1996. Progestin and estrogen regulation of pulsatile LH release and development of persistent ovarian follicles in cattle. *J Anim Sci* 74: 1424-1440.

Kittock RJ, Britt JH, Edgerton LA. 1974. Serum steroids after gonadotropin treatment in cows with ovarian follicular cysts. *Am J Vet Res* 35: 1575-1576.

Königsson K, Kask K, Gustafsson H, Kindahl H and Parvizi N. 2001. 15-Ketodihydro-$PGF_{2\pm}$, Progesterone and Cortisol Profiles in Heifers after Induction of Parturition by Injection of Dexamethasone**.** Acta Vet Scan 42:151-159

.Pirlich M, Biering H, Gerl H, Ventz M, Schmidt B, Ertl S, Lochs H. 2002. Loss of body cell mass in Cushing's syndrome: effect of treatment. *J Clin Endocrinol Metab* 87:1078–1084

Silvia WJ, Angela S, McGinnis T, Ben Hatler. 2005. A comparison of adrenal gland function in lactating dairy cows with or without ovarian follicular cysts. *Reprod Biol* 5: 19-29

Suharto K, A Junaidi, Diah TW, Asmarani K. 2007. Synchronization of oestrus in goat: the relationship between time of occurance of oestrus, preovulatory LH surge and fertility following artificial insemination. *J Anim Prod* (in press).

Steimer Th. 2003. Steroid Hormone Metabolism. http://www.gfmer.ch/books/reproductive health/steroid homorne metabolism

Sunendar, 2007. Profil hormone progesterone dan estrogen kambing peranakan ettawah yang disinkronisasi estrus dengan *implant controlled internal drug release.* Thesis. Sekolah Pascasarjana, UGM

Tataranni PA, Larson DE, Snitker S, Young JB, Flatt JP, Ravussin E 1996 Effects of glucocorticoids on energy metabolism and food intake in humans. *Am J Physiol* 271:E317–E325

Wirth MM, Elizabeth A, Meier, Barbara L. Fredrickson L, Oliver C. 2007. Relationship between salivary cortisol and progesterone levels in humans *Biol Psychol* 74: 104–107

Wirth MM, and Schultheiss OC. 2006. Effects of affiliation arousal (hope of closeness) and affiliation stress (fear of rejection) on progesterone and cortisol. *Hormone and Behave* 50: 786-795

Zaied AA, Garverick HA, Bierschwal CJ, Elmore RG, Youngquist RS, Sharp AJ 1980. Effect of ovarian activity and endogenous reproductive hormones on GnRH-induced ovarian cycles in postpartum dairy cows. *J Anim Sci* 50: 508-513.