ROLES OF PROSTAGLANDIN $F_{2\alpha}$ AND CORTISOL IN REGULATING BOVINE UTERINE AND OVARIAN FUNCTION

March 2013

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PREFACE

The experiments described in this dissertation were carried out at the Graduate School of Natural Science and Technology (Doctor's Course), Okayama University, Japan in collaboration with the Polish Academy of Sciences from April 2010 to March 2013, under the supervision of Dr. Tomas J. ACOSTA, Associate Professor, Dr. Kiyoshi OKUDA, Professor and Dr. Yasuhiro KONDO, Professor of the Graduate School of Natural Science and Technology, Okayama University, Japan.

This dissertation has not been previously submitted wholly or in part to a council, a University or any other professional institution for degree, diploma or other professional qualification.

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ABSTRACT

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The objective of this study was to determine the roles of prostaglandin $F_{2\alpha}$ (PGF) and cortisol in the regulation of bovine uterine and ovarian functions *in vivo.* In the first experiment, injection of a PGF analogue induced more than a twofold increase in the level of PGF in uterine venous (UV) plasma between 0.25 and 1 h after injection, but it did not affect the level of PGF in ovarian venous (OV) plasma. Injection of PGF significantly increased ($P<0.05$) the concentrations of cortisol in OV, UV and jugular venous (JV) plasma between 0.5 and 1 h after injection. The cortisol levels in OV, UV and JV plasma were similar. The PGF levels in UV plasma decreased after cortisol reached its highest levels. In a second series of experiments, intravaginal application of cortisone increased plasma concentrations of cortisol between 0.5 and 1.5 h after application in UV, at 0.5 h in *vena cava caudalis* (VCC), at 1 h in JV and at 1.5 h in *aorta abdominalis* (AA). The plasma concentrations of PGF in UV and the plasma concentration of a PGF metabolite in JV increased between 0.5 and 1 h, and then decreased to near the levels observed before cortisone treatment at 2 h. The levels of PGF in UV blood plasma decreased after cortisol reached its highest levels. In a third series of experiments, plasma concentrations of progesterone (P4) were lower in cortisol-treated heifers than in control heifers (application of gel only) on Days 17 and 18 of the estrous cycle. However, inter-estrus intervals were not different between control and cortisol-treated animals ($P<0.05$). Moreover, treatment with metyrapone, an inhibitor of cortisol biosynthesis, increased P4 and prolonged the luteal phase ($P<0.05$). Interestingly, in inseminated heifers, pregnancy rate was greater ($P<0.05$) in cortisol-treated animals than in control animals (9/12, 75% vs. 7/12, 58%, respectively), whereas pregnancy rate was lower ($P<0.05$) in metyrapone-treated animals than in control animals (2/12, 16.7% vs. 7/12, 58 %, respectively).

The overall results of the present study indicate that 1) the uterus rather than the ovary increases PGF production in response to PGF injection, 2) the reproductive tract (uterus and/or vagina) has the capacity to convert cortisone to cortisol and that cortisol

may act to reduce the excessive uterine PGF secretion in non-pregnant cows *in vivo* and 3) depending on the physiological status (pregnant vs. nonpregnant), cortisol modulates bovine CL function by influencing P4 secretion. Thus, cortisol may have a positive influence on CL function during early pregnancy, which would promote embryo implantation and thus result in higher rates of pregnancy in heifers.

CHAPTER 1: ACUTE CHANGES IN THE CONCENTRATIONS OF PROSTAGLANDIN $F_{2\alpha}$ (PGF) AND CORTISOL IN UTERINE AND OVARIAN VENOUS BLOOD DURING PGF-INDUCED LUTEOLYSIS IN COWS

INTRODUCTION

In ruminants, prostaglandin $F_{2\alpha}$ (PGF) is a hormone synthesised and secreted from the uterus [1-3]. This hormone is involved in the control of the oestrous cycle, ovulation, and regression of the corpus luteum (CL) [4, 5]. In non-pregnant cows, the uterus increases PGF production on Day 17 post-ovulation [6]. Uterine PGF is transported to the ovary by a countercurrent transfer mechanism [7], which is facilitated by a prostaglandin transporter-mediated mechanism [8] to induce regression of the CL [7, 9].

Cortisol (Cr) is a steroid hormone produced by the adrenal cortex. Cr has been shown to be involved in the regulation of endometrial production of PGF in cattle [10]. Interestingly, a recent study using cultured bovine endometrial stromal cells demonstrated that PGF increase the expression and enzymatic activity of 11β-hydroxysteroid dehydrogenase 1 (HSD11B1), which converts inactive cortisone to active cortisol [11]. On the other hand, Cr suppresses PGF production in non-pregnant bovine endometrial stromal cells [10]. These findings suggest that Cr has a role in regulating uterine PGF production and that a dynamic interrelationship between uterine Cr and PGF exist in bovine endometrium. However, the temporal interrelationship between PGF and Cr in the ovarian and uterine circulations of non-pregnant bovine *in vivo* around the time of luteolysis remains unknown.

PGF treatment has been shown to increase luteal production of PGF in ruminants *in vitro* [12]. More recent studies have suggested that endometrial/extraluteal PGF triggers intraluteal production of PGF, which induces PGF production in luteal cells by an auto/paracrine action in the regressing CL [12, 13]. Based on changes in the circulating levels of PGF metabolite, Kotwica *et al.* (1999) suggested that injection of PGF analogue stimulates the secretion of endogenous PGF from the uterus in cattle [14]. Moreover, PGF could regulate cyclooxygenase 2 (COX-2) expression in an autocrine/paracrine manner to establish a positive feedback system for regulating

endometrial tumorigenesis [15]. However, it remains unclear whether exogenous PGF mainly affects uterine and/or ovarian PGF production during the time of luteolysis in cow.

After PGF is released into the circulation, it is rapidly metabolised in the lung, liver and kidney to 13, 14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM) [16, 17] by the enzyme 15-hydroxy prostaglandin dehydrogenase [18]. In cattle, peaks of major pulses of PGF and PGFM during luteolysis occurred concomitantly, based on sampling at 4-h intervals. Therefore, PGFM is often measured to examine the circulating systemic concentrations of PGF [19]. However, there is no *in vivo* information available so far on the real-time changes in the concentrations of PGF in the blood plasma collected directly from the uterine vein (UV) and ovarian vein (OV) during PGF-induced luteolysis in cattle.

This study was carried out to test whether exogenous PGF increases ovarian and/or uterine PGF production, and to determine the temporal relationship between PGF and Cr in ovarian and uterine circulations during PGF-induced luteolysis in cows.

MATERIALS AND METHODS

All procedures were approved by the Local Animal Care and Use Committee in Olsztyn, Poland (Agreement No. 31/2006/N and 06/2007/N). The experimental animals were conducted at the Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland.

Animals and surgical procedures

Healthy, normally cycling Polish Holstein Black and White cows were used for the present studies. The animals were culled by their owners (Spolka Rolna "Wroblik", Lidzbark Warminski, and Gospoka Rolne "Farmer", Zalesie, Szczytno, Poland) from dairy cows herds because of low milk production. Oestrus was synchronised in the cows using implants of a progesterone (P4) analogue (Crestar: Intervet, International B.V. Boxmeer, Holland) with additional intramuscular (i.m.) injection of an analogue of PGF

(Cloprostenol; Bioestrophan, Biowet, Gorzow Wielkopolski, Poland), as recommended by the manufacturer for oestrous synchronisation of multiparous cows and described previously [20]. Oestrus was determined by observing external signs (i.e., vaginal mucus, standing behavior). Before surgery, the ovaries were examined daily by ultrasonography to determine the day and side of ovulation and CL development. The presence of a pre-ovulatory follicle, ovulation and normal CL development were confirmed by a veterinarian using a sectorial rectal probe connected to an ultrasound (Dranminski Animal Profi Scanner, Draminski Electronics in Agriculture, Olsztyn, Poland). The Day of ovulation was defined as Day 0 of the oestrous cycle.

The cows were premedicated with an i.m. injection of xylazine at a dose of 25-30 mg per cow (Sedanzin; Biolwet, Pulawy, Poland). Local anaesthesia was induced by s.c. and i.m. injections of 2% procaine hydrochloride (Polocainum Hydrochloricum; Biowet, Drwelew, Poland) in the paralumbar fossa of the side of the CL. On Day 9, catheters (Medicut Catheter Kit; Argyle, Japan Sherwood, Tokyo, Japan) were inserted into the ovarian vein (OV), uterine vein (UV) and jugular vein (JV) in 10 cows for frequent blood collection. A lateral laparotomy was performed for cannulation of the ovarian and uterine vein. At surgery, 18-gauge catheters were inserted into the ovarian and the uterine vein ipsilateral to the functional CL and fixed to the surrounding connective tissue [21]. After surgery, the cows were moved to a barn, where they were fed with grass hay twice daily and were given free access to water. On Day 10, the cows were divided randomly into two groups (n=5 cows/group). The animals in the first group received an i.m. injection of a luteolytic dose of 500 µg of cloprostenol (Estrumate; Mallinckrodt Burgwedel, Germany), a PGF analogue to induce luteolysis, whereas the second group received an i.m. injection of 5 ml of normal saline solution. Blood samples were simultaneously collected from OV, UV and JV at -0.25, 0, 0.25, 0.5, 1 and 2 h and then at 2-h intervals until 12 h after PGF injection. The time of PGF or saline injection on Day 10 of the cycle was defined as 0 h.

For P4, PGF and Cr measurement, blood samples were collected into sterile 10-ml tubes containing 200 µl of a stabiliser solution (0.3 M EDTA, 1% acid acetyl salicylic, pH 7.4). All tubes were immediately chilled on ice for 10 min, centrifuged at 2000 x g for 10 min at 4^{o}C, and the obtained plasma was stored at - 30^{o}C until further analysis.

Progesterone determination

The progesterone concentrations in the plasma samples were assayed using a direct enzyme immunoassay (EIA) as described previously [22]. The P4 standard curve ranged from 0.05 to 25 ng/ml, and the median effective dose (ED_{50}) of the assay was 2.56 ng/ml. The average intra- and interassay coefficients of variation (CVs) were 4.7% and 6.5%, respectively.

Prostaglandin $F_{2\alpha}$ determination

The concentrations of PGF in the plasma was determined directly with a double-antibody enzyme immunoassay as described previously [23] by using horseradish peroxidase enzyme-labeled PGF as a tracer (1:75,000 final dilutions) and PGF antibody (kindly donated by Dr. Seiji Ito of Kansai Medical University, Osaka, Japan; 1:100,000 final dilutions). The cross-reactivity of PGF first antibody with cloprostenol injected to induce luteolysis and with PGFM at 50% binding were 0.95% and 0.1%, respectively. The samples (uterine and ovarian venous blood plasma) for the PGF assay were diluted 10 times with EIA assay buffer. The standard curve ranged from 15.6 to 4,000 pg/ml, and the ED_{50} of the assay was 400 pg/ml. The intra- and inter-assay CVs were 7.34% and 13.16%, respectively.

Cortisol determination

The concentrations of Cr in the plasma were determined in duplicate after diethyl ether extraction by second antibody EIA as described previously [24] by using horseradish peroxidase enzyme-labeled Cr as a tracer (1:400,000 final dilutions) and Cr antibody (raised in a rabbit against cortisol-3-CMO; Cosmo Bio Co., Tokyo, Japan; 1:80,000 final dilutions). Each plasma sample (200 μl) was extracted by diethyl ether as described previously [21]. The residue was evaporated and then dissolved in 200 μl assay buffer (40 mM PBS 0.1% BSA, pH 7.2). To estimate the recovery rate, Cr were added to plasma (1 ng/ml), and the obtained values were on average 75% (n=5). The standard curve ranged from 0.4 to 400 ng/ml, and the ED_{50} of the assay was 1.6 ng/ml. The intra- and inter-assay CVs were on average 5.4% and 6%, respectively.

Statistical analysis

Experimental data are shown as the mean ± SEM of values obtained from five PGF-treated and five saline-treated cows. The concentrations of PGF and Cr in the blood collected at -0.25 and 0 h were used to calculate the individual baseline. The statistical significance of differences of P4, PGF and Cr in OV, UV and JV blood plasma between pre- and post-PGF injection period, and between OV, UV and JV were assessed by analysis of variance (ANOVA) using GraphPAD Prism Version 5.00, San Diego, CA, USA; followed by protected least significant difference (PLSD) as a multiple comparison test. Differences were considered significant when the probability was less than 5% ($P<0.05$).

RESULTS

Effect of an injection of a prostaglandin $F_{2\alpha}$ analogue on the plasma concentrations of progesterone in ovarian venous blood

An injection of a luteolytic dose of PGF induced a significant ($P<0.05$) decrease in the plasma concentrations of P4 in OV plasma at 2 h, indicating functional luteolysis, as expected (Fig. 1).

Effect of an injection of a prostaglandin $F_{2\alpha}$ analogue on the plasma concentrations of PGF in the ovarian and uterine venous blood

On Day 10 of the oestrous cycle, the basal concentrations of PGF in OV plasma were not significantly different from those in UV plasma (Fig. 2). An injection of a luteolytic dose of PGF induced a transient increase of PGF concentrations ($P<0.05$) in UV blood plasma between 0.25 and 1 h, but not in OV blood plasma (Fig. 2).

Effect of an injection of a prostaglandin $F_{2\alpha}$ analogue on the plasma concentrations of cortisol in the ovarian, uterine and jugular venous blood

On Day 10 of the oestrous cycle, the basal concentrations of Cr in OV, UV and JV blood plasma were similar (Fig. 3). An injection of a PGF analogue induced an acute increase ($P<0.05$) in the concentrations of Cr in ovarian, uterine and jugular venous blood plasma between 0.5 and 1 h (Fig. 3). The plasma concentrations of Cr in OV, UV and JV were not significantly different.

The increases in concentrations of Cr in OV, UV and JV blood plasma occurred after the increase in the levels of PGF in UV blood plasma. In addition, the levels of PGF in UV blood plasma decreased after Cr reached its highest levels (Table 1).

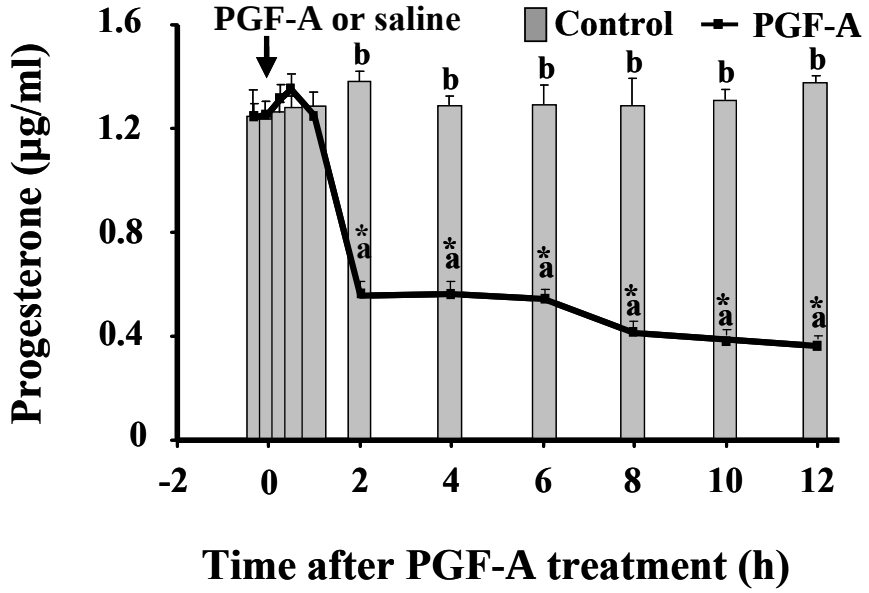

Fig. 1: Concentrations of progesterone (P4) in blood plasma collected from the ovarian vein. Cows were treated intramuscularly with cloprostenol, a prostaglandin $F_{2\alpha}$ analogue (PGF-A, n=5) or saline solution (Control, n=5) on Day 10 of the oestrous cycle. Asterisks indicate significant differences ($P<0.05$) compared with the baseline (pretreatment period). Different superscript letters indicate significant differences ($P<0.05$) between the cow treated with PGF-A and control groups as assessed by ANOVA followed by protected least significant difference test (PLSD).

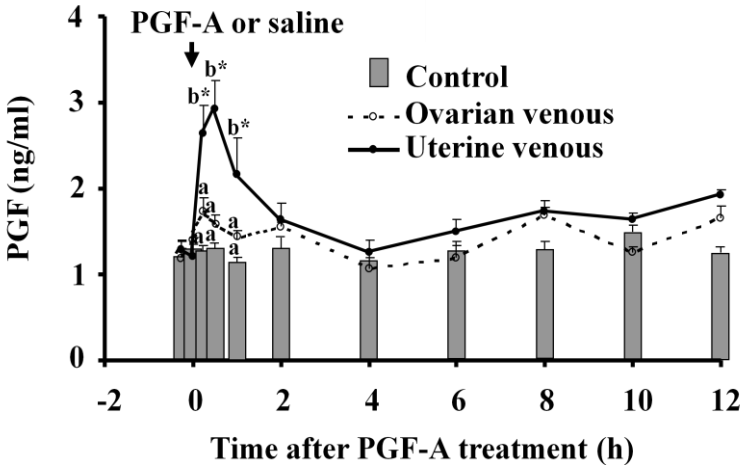

Fig. 2: Concentrations of prostaglandin $F_{2\alpha}$ (PGF) in uterine venous blood plasma of saline-treated group (Control), in ovarian and uterine venous blood plasma of cow treated with cloprostenol, a prostaglandin $F_{2\alpha}$ analogue (PGF-A) group. Data are the mean ± SEM for 5 samples/time-point. Asterisks indicate significant difference ($P<0.05$) compared with the baseline (before PGF-A or saline injection). Different superscript letters indicate significant differences ($P<0.05$) between uterine and ovarian venous blood plasma of cow treated with PGF-A group, or between uterine venous blood plasma of cow treated with PGF-A and control groups. Bars show PGF concentration in uterine venous blood of the saline-treated group (n=5), as determined by ANOVA followed by protected least significant difference test (PLSD).

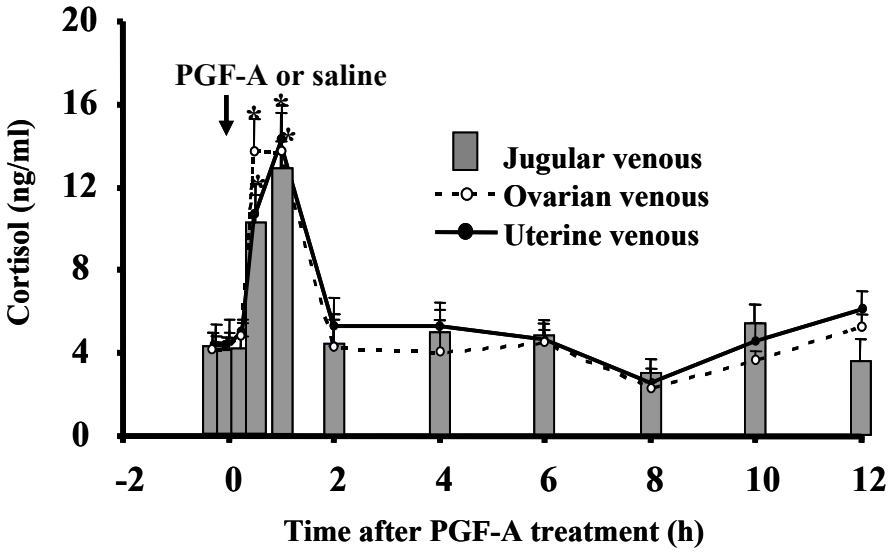

Fig. 3: Concentrations of cortisol (Cr) in jugular, ovarian and uterine venous blood plasma of cows treated with cloprostenol, a prostaglandin $F_{2\alpha}$ analogue (PGF-A). Data are the mean ± SEM for 5 samples/time-point. Asterisks indicate significant difference ($P<0.05$) in Cr concentrations compared with the baseline (before PGF-A or saline injection), as determined by ANOVA followed by protected least significant difference test (PLSD).

Table 1: Acute changes in the concentrations of prostaglandin $F_{2\alpha}$ (PGF) and cortisol (Cr) in uterine venous (UV) and ovarian venous (OV) blood plasma during prostaglandin $F_{2\alpha}$-induced luteolysis in cows.

		Time after prostaglandin $F_{2\alpha}$ injection				
		0 h	**0.25 h**	**0.5 h**	**1 h**	**2 h**
PGF	**OV**	1.4 ± 0.2	1.7 ± 0.2	1.6 ± 0.1	1.4 ± 0.1	1.6 ± 0.3
(ng/ml)	**UV**	1.2 ± 0.2^{a}	2.6 ± 0.6^{b}	2.9 ± 0.9^{b}	2.2 ± 0.4^{b}	1.6 ± 0.1^{a}
Cr	**OV**	4.2 ± 1.4^{a}	5.0 ± 0.5^{a}	13.7 ± 2.6^{b}	13.6 ± 2.0^{b}	4.2 ± 1.4^{a}
(ng/ml)	**UV**	4.4 ± 0.6^{a}	5.1 ± 0.6^{a}	10.6 ± 2.1^{b}	14.1 ± 3.0^{b}	5.3 ± 1.4^{a}

Data are the mean ± SEM for 5 samples/time-point. Different letters indicate significantly different value ($P<0.05$) in Cr and PGF concentrations among time-points related to prostaglandin $F_{2\alpha}$ injection, as determined by ANOVA followed by protected least significant difference test (PLSD).

DISCUSSION

In cattle, injection of a luteolytic dose of PGF analogue induces luteolysis which is characterized by a decrease in the circulating levels of P4 and a concomitant increase in the levels of PGFM in the jugular venous plasma [14, 19]. It is well known that the bovine uterus and ovary both have the capacity to produce PGF [25, 26]. However, it had been unclear whether exogenous PGF affects uterine and/or ovarian PGF production. In the present study, injection of a PGF analogue induced a significant increase in the levels of PGF in UV plasma within 1 h, whereas the increase in the levels of PGF in OV plasma was not statistically significant. These results suggest that there is an increase in the luteal production of PGF, but the response to an intramuscular injection of PGF analogue is not as great as the observed in UV blood plasma. A recent *in vitro* study also demonstrated that bovine endometrium increases PGF production in response to PGF treatment and that the strongest stimulatory effect of PGF was observed between days 15 and 17 of the oestrous cycle [27]. These results imply that exogenous PGF increases uterine PGF production and that the endometrium becomes more responsive to PGF at the time of luteolysis.

A study using a micro dialysis system demonstrated that administration of a PGF analogue induces an acute increase in intraluteal PGF secretion during the first 4 h post-treatment [13]. The same study demonstrated that injection of a PGF analogue (Cloprostenol) did not induce a significant increase of PGF levels in OV plasma up to 24 h after treatment, in agreement with the present results. Furthermore, PGF has the ability to activate PGF production within the CL of ewes and cows [25, 26]. These results indicate that the acute increase in the intraluteal production of PGF is not reflected in the profiles of PGF in OV plasma.

Previous *in vitro* studies showed that PGF increases the levels of Cr in cultured bovine adrenocortical cells [28] and the conversion of cortisone to cortisol by stimulating HSD11B1 in non-pregnant bovine endometrium [11]. *In vivo* studies demonstrated that a luteolytic dose of PGF analogue induced an increase in the levels of Cr in the JV blood plasma [29, 30]. These findings suggest that PGF has the capacity to stimulate Cr *in vitro* and *in vivo*. Moreover, it has been shown that bovine endometrium [10] and corpus luteum tissues [31] have the capacity to convert cortisone to cortisol. It

is of interest to know whether the increase in the levels of Cr in blood plasma collected from the JV, reported by Baishya et al. (1994) [29] and Shrestha et al. (2010) [30], is Cr secreted from the adrenal cortex or converted from cortisone by the ovary or uterus. In the present study, injection of a PGF analogue increased the levels of Cr in ovarian, uterine and jugular venous blood circulation. However, we did not find any significant difference in the levels of Cr among OV, UV and JV blood plasma. These results suggest that PGF stimulates Cr release from the adrenal cortex and that the amount of Cr converted from cortisone by the ovary and uterus is not enough to affect circulating levels of Cr. A recent study demonstrated that PGF stimulates cortisol conversion from cortisone by increasing HSD11B1 activity in endometrial tissue and cultured stromal cells. It is also possible that PGF increase the capacity to convert cortisone to cortisol in other tissues including endothelial cells, making this effect systemic.

It has been demonstrated that PGF is secreted from the uterus in pulses during luteolysis in cattle [32]. The sequential PGF pulses are required to induce natural luteolysis in cattle [19]. Although PGF increases Cr levels in JV blood plasma, it is not known how PGF and Cr in ovarian and uterine circulation change with time *in vivo*. Our previous *in vitro* results showed that Cr has the capacity to reduce basal and tumor necrosis factor α-stimulated PGF production in stromal cells of non-pregnant bovine endometrium [10]. It has been shown that glucocorticoids inhibit PG synthesis by inhibiting the expression of cytosolic phospholipase A2 that convert phospholipids to arachidonic acid (AA) [33], the primary precursor of PGF as well as COX-2, the enzyme that convert AA to PGH2 [34, 35] in most tissues of the body. In the present study, the levels of Cr in OV, UV and JV blood plasma increased immediately after the rise of PGF in UV blood plasma. Interestingly, the present results also showed that the levels of PGF in UV blood plasma decreased after Cr reached its highest levels. These results suggest that Cr inhibits uterine PGF production within a short time period. Thus, Cr may act in reducing the high levels of uterine PGF and may be one of the factors responsible for the generation of PGF pulses in cattle.

In conclusion, exogenous PGF increases uterine PGF production rather than ovarian PGF production. Based on the temporal changes of PGF and Cr in ovarian and uterine circulations, Cr may act to reduce uterine PGF production in non-pregnant cows *in vivo*.

SUMMARY

Prostaglandin $F_{2\alpha}$ (PGF) is considered to be the main luteolysin in cattle. We have previously demonstrated that cortisol (Cr) suppresses PGF production in non-pregnant bovine endometrium. The present study was performed to test whether exogenous PGF increases ovarian and/or uterine PGF production and to determine the temporal relationship between PGF and Cr in ovarian and uterine circulations during PGF-induced luteolysis in cows. Catheters were inserted into the ovarian vein (OV), uterine vein (UV) and jugular vein (JV) of 10 cows on Day 9 of the oestrous cycle (Ovulation = Day 0) for frequent blood collection. On Day 10, the cows were divided randomly into two groups and treated with a luteolytic dose of a PGF analogue (cloprostenol) or saline solution. Blood samples were collected at -0.25, 0, 0.25, 0.5, 1 and 2 h and then at 2-h intervals until 12 h after treatment (0 h). The basal concentrations of PGF and Cr in OV and UV plasma were not significantly different. Injection of a PGF analogue induced more than two-fold increases in the levels of PGF between 0.25 and 1 h in UV plasma, but not in OV plasma. PGF increased ($P<0.05$) the concentrations of Cr in OV, UV and JV plasma between 0.5 and 1 h. The Cr levels in OV, UV and JV plasma were similar. The PGF levels in UV plasma decreased after Cr reached its highest levels. The overall results suggest that the uterus rather than the ovary increases PGF production in response to PGF injection. Based on the temporal changes of PGF and Cr in the ovarian and uterine circulations, Cr may act to reduce uterine PGF production in non-pregnant cows *in vivo*.

CHAPTER 2: CONVERSION OF CORTISONE TO CORTISOL AND PROSTAGLANDIN $F_{2\alpha}$ PRODUCTION BY THE REPRODUCTIVE TRACT OF COWS AT THE LATE LUTEAL STAGE *IN VIVO*

INTRODUCTION

Glucocorticoids (GCs), synthesised from cholesterol in the adrenal cortex, are involved in the regulation of a variety of physiological processes, including metabolism [36], immunological response [37] and female reproductive function [38, 39]. Cortisol (Cr), an active GC, is an anti-inflammatory agent that acts to modulate the production and action of cytokines and prostaglandins required for ovulation, luteolysis, embryo implantation, fetal growth and placenta development as well as parturition [38, 40, 41]. The effects of GCs on target tissues are modulated by 11β-hydroxysteroid dehydrogenases (HSD11Bs) [10, 42]. Two isoforms of the enzyme have been identified. The type 1 enzyme (HSD11B1) mainly converts cortisone to Cr (the active form), while the type 2 isoform (HSD11B2) inactivates Cr by metabolising it to cortisone [43]. We recently demonstrated that HSD11B1 mRNA expression in the bovine endometrium changes throughout the oestrous cycle, and that endometrial tissue has the capacity to convert inactive cortisone to biologically active Cr *in vitro* [10]. However, it remains unknown whether the bovine reproductive tract has the capacity to convert cortisone to Cr *in vivo.*

Prostaglandin $F_{2\alpha}$ (PGF), a hormone synthesised and secreted from the uterus [1-3], has the capacity to stimulate conversion of cortisone to Cr by increasing the expression and enzymatic activity of HSD11B1 in bovine endometrial stromal cells [11]. We recently showed that injection of PGF *in vivo* induced acute increases in the levels of circulating Cr [44]. On the other hand, Cr can modulate tumour necrosis factor α-regulated PGF production and may reduce basal PGF production in non-pregnant bovine endometrium stromal cells *in vitro* [10]. These findings indicate that Cr has a role in regulating uterine PGF production. However, it is unclear whether exogenous cortisone, an inactive GC, can be converted into active Cr and consequently affects uterine PGF production at the late luteal phase in cows. Moreover, the temporal

interrelationship between PGF and Cr in the uterine blood plasma of cows at the late luteal stage remains unknown.

This study was carried out to test the hypothesis that bovine reproductive tract has the capacity to convert cortisone to Cr *in vivo* and to evaluate the effects of intravaginal application of exogenous cortisone on uterine PGF secretion during the late luteal stage. The temporal relationships between PGF and Cr levels in uterine blood plasma were also determined.

MATERIALS AND METHODS

All animal procedures were approved by the Local Animal Care and Use Committee in Olsztyn, Poland (Agreement No. 06/2007/N).

Animals and surgical procedures

Healthy, normally cycling Polish Holstein Black and White cows (n = 18) were used for the present studies. The animals were culled by their owners (the Farm of Polish Academy of Sciences in Baranowo, and a private agriculture farm in Cieszymowo, Poland) from dairy cows herds because of low milk production. The oestrus was synchronised using two injections of an analogue of PGF (dinoprost, Dinolytic; Upjohn - Pharmacia N.V.S.A., Belgium) with an 11-day interval, as described and recommended in our previous study [45]. Oestrus was determined by observing external signs (i.e. vaginal mucus, standing behaviour). Before surgery, the ovaries were examined daily by transrectal ultrasonography (USG) using a Draminski Animalprofi Scanner (Draminski Electronics in Agriculture, Olsztyn, Poland) to determine the day and side of ovulation and corpus luteum (CL) development. The day of ovulation was defined as Day 0 of the oestrous cycle.

To determine the effective dose of cortisone on Cr and PGF output, a polyvinyl catheter was inserted into the jugular vein of 12 cows on Day 15 of the cycle for collection of the blood samples as described previously [46].

In the main experiment, the cows (n = 6) were premedicated with an intramuscular (i.m.) injection of xylazine at a dose of 25-30 mg per cow (Sedanzin; Biolwet, Pulawy, Poland). Local anaesthesia was induced by s.c. and i.m. injections of 2% procaine hydrochloride (Polocainum Hydrochloricum; Biowet, Drwelew, Poland) in the paralumbar fossa on the side of ovary with CL. On Day 15, catheters (Medicut Catheter Kit; Argyle, Japan Sherwood, Tokyo, Japan) were inserted into jugular vein (JV) uterine vein (UV), *vena cava caudalis* (VCC) and *aorta abdominalis* (AA) for frequent blood collection. A lateral laparotomy was performed for cannulation of the uterine vein. At surgery, 18-gauge catheters were inserted into the UV 3-5 cm from uterine horn before joining the utero-ovarian vein ipsilateral to the functional CL [44, 47], the VCC and AA and fixed to the surrounding connective tissue [48]. After surgery, the cows were moved to a barn, where they were fed with grass hay twice daily and were given free access to water.

Determination of cortisone doses

Twelve cows were used to choose the effective doses of cortisone. All doses were applied to the vagina via a catheter near the cervix of the uterus on Day 16 of the oestrous cycle. The cows were divided randomly into four groups (n = 3 cows/group) and then infused with vaseline gel (10 ml; control group) or three different doses of cortisone (1 mg, 10 mg, 100 mg; cortisone groups; Sigma – Aldrich, Chemie Gmbh, Munich; Germany; No. C2755) dissolved in vaseline gel. The blood samples were collected from JV at -2, -1, -0.5, 0, 0.5, 1, 1.5 and 2 h and then at 1-h intervals until 6 h after vaseline or cortisone infusions. The time of cortisone or vaseline infusion was defined as 0 hour. Plasma concentrations of Cr and 13, 14-dihydro, 15-keto-PGF (PGFM) in plasma samples were measured. For further examination of cortisone action at the late luteal stage in cows, a dose of 100 mg cortisone was used.

Effects of cortisone applications on prostaglandin $F_{2\alpha}$ and Cr concentrations

To examine the possible influence of cortisone on PGF and Cr release from the reproductive tract, cows were divided randomly into two groups (n = 3 cows/group) and infused intravaginally with vaseline gel (control) or 100 mg of cortisone dissolved in

vaseline gel (cortisone) on Day 16 of the oestrous cycle. Blood samples were collected from the JV, UV, VCC, and AA at -2, -1, -0.5, 0, 0.5, 1, 1.5 and 2 h and then 1-h intervals until 6 h after treatments. The time of vaseline gel or cortisone infusion was defined as 0 hour. For Cr, PGF and PGFM determination, blood samples were collected into sterile 10-ml tubes containing 200 µl of stabiliser solution (0.3 M EDTA, 1% acetyl salicylic acid, pH 7.4). All tubes were immediately chilled on ice for 10 min, centrifuged at 2000 X ***g*** for 10 min at 4°C, and the obtained plasma was stored at -30°C until further analysis.

13, 14-dihydro, 15-keto-prostaglandin $F_{2\alpha}$ (PGFM) determination

The concentrations of PGFM in the plasma samples were determined with direct EIAs following the method described previously [46] by using horseradish peroxidase enzyme-labelled PGFM as a tracer (1:40,000 final dilutions) and PGFM antibody (1:10,000 final dilutions). The anti-PGFM serum (WS4468-5) was kindly donated Dr. W.J. Silvia, University of Kentucky, Lexington, USA and characterised before [46]. The cross-reactivity of the PGFM first antibody with PGF at 50% binding was 2.8%. The sensitivity of PGFM assays was 50 pg/ml. The PGFM standard curve ranged from 32.5 to 8,000 pg/ml and the median effective dose (ED_{50}) of the assay was 315 pg/ml. The intra- and inter-assay coefficients of variation (CVs) were on average 7.6% and 10.4%, respectively.

Prostaglandin $F_{2\alpha}$ determination

The concentrations of PGF in the plasma was determined directly with a double-antibody enzyme immunoassay as described previously [1] by using horseradish peroxidase enzyme-labelled PGF as a tracer (1:75,000 final dilutions) and PGF antibody (kindly donated by Dr. Seiji Ito of Kansai Medical University, Osaka, Japan; 1:100,000 final dilutions). The cross-reactivity of PGF first antibody with PGFM at 50% binding was 0.1%. The sensitivity of PGF assays was 30 pg/ml. The standard curve ranged from 15.6 to 4,000 pg/ml, and the ED_{50} of the assay was 400 pg/ml. The intra- and inter-assay CVs were 7.3% and 13.2%, respectively.

Cortisol determination

The concentrations of Cr in the plasma were determined in duplicate after diethyl ether extraction by second antibody EIA as described previously [24] by using horseradish peroxidase enzyme-labelled Cr as a tracer (1:80,000 final dilutions) and Cr antibody (raised in a rabbit against Cr-3-CMO; Cosmo Bio Co., Tokyo, Japan; 1:400,000 final dilutions). The cross-reactivity of Cr first antibody with cortisone treated at 50% binding was 0.6%. The sensitivity of Cr assays was 0.5 ng/ml. Each plasma sample (200 μl) was extracted by diethyl ether as described previously [21]. The residue was evaporated and then dissolved in 200 μl assay buffer (40 mM PBS 0.1% BSA, pH 7.2). To estimate the recovery rate, Cr was added to plasma (1 ng/ml), and the obtained values were on average 75% (n=5). The standard curve ranged from 0.4 to 400 ng/ml, and the ED_{50} of the assay was 1.6 ng/ml. The intra- and inter-assay CVs were on average 5.4% and 6%, respectively.

Statistical analysis

Experimental data are shown as the mean ± SEM (n = 3). The concentrations of PGF, PGFM and Cr in the blood collected at -0.5 and 0 h were used to calculate the individual baseline. The data were not normally distributed (no Gaussian distributions). The analyses of Cr, PGF and PGFM in plasma samples were performed using a repeated measures design approach with treatments and time of sample collection (hours) being fixed effects with all interactions included, as described before [48, 49]. The non-parametric Freidman and Kruskal-Wallis tests with post-hoc test (repeated measurement test - multiple comparisons of mean ranks) has been used (GraphPAD Prism Version 5.00, San Diego, CA; USA; $P<0.05$ was considered significant). Least adjusted means and standard errors, as well as median and quartiles were determined. The correlation between PGF and Cr concentrations after cortisone application in different blood vessels was additionally measured using linear regression and Pearson correlation (GraphPAD Prism).

RESULTS

Determination of cortisone dose

The concentrations of Cr and PGFM in JV plasma in fours groups are shown in Fig. 4. For the PGF and PGFM concentrations in JV plasma, the main effects of hour and group and the group x hour interaction were significant ($P<0.05$). The Cr concentration increased ($P<0.05$) between hours 0 and 2, decreased ($P<0.05$) between hours 2 and 4, and did not change thereafter in the 100 mg cortisone treated animals. There was not temporal change in Cr levels in control and animals infused with lower doses of cortisone (1 and 10 mg; Fig. 4a). Furthermore, the plasma concentration of PGFM in JV first increased between hours 0 and 1, decreased between hours 1 and 2, and again increased between hours 2 and 3, then decreased until hour 6 in the 100 mg cortisone treated animals. However, the Cr level did not change in the control and animals treated with lower doses of cortisone (1 and 10 mg; Fig. 4b). The Cr and PGFM levels were greater in the 100 mg cortisone treated animals than in the control and animals treated with lower doses of cortisone (1 and 10 mg cortisone; $P<0.05$) at 1 and 3 h post treatment.

Based on these results, a cortisone dose of 100 mg was used for further studies to investigate the local effect of cortisone on release of Cr and PGF during the late luteal phase in cows.

Effects of cortisone application on Cr concentrations during the late luteal phase

For the Cr concentrations, the hour effect was significant ($P<0.05$). Intravaginal application of cortisone on Day 16 of oestrous cycle induced a significant ($P<0.05$) increase in the plasma concentrations of Cr between 0.5 and 1.5 h in UV, at 0.5 h in VCC, at 1 h in JV and at 1.5 h in AA (Fig. 5). Furthermore, plasma Cr levels were highest in UV.

Effects of cortisone application on prostaglandin $F_{2\alpha}$ and PGFM concentrations during the late luteal phase

For the concentrations of PGF in UV and PGFM in JV plasma, the main effect of hour and groups and the group x hour interaction were significant ($P<0.05$). Concentrations of PGF in UV and PGFM in JV (Fig. 6) increased between hours 0 and 1, decreased between hour 1 and 1.5 and did not change thereafter. The levels of PGF in UV and PGFM in JV plasma were greater ($P<0.05$) at 0.5 and 1 h in cortisone-treated animals than in control animals on Day 16 of the oestrous cycle.

The increase in the concentration of Cr (Fig. 5) and PGF (Fig. 6) in UV plasma occurred at the same time and a high correlation between both measurements was found (Pearson $r = 0.76$; $P<0.001$). However, the levels of UV PGF decreased after Cr reached its highest levels.

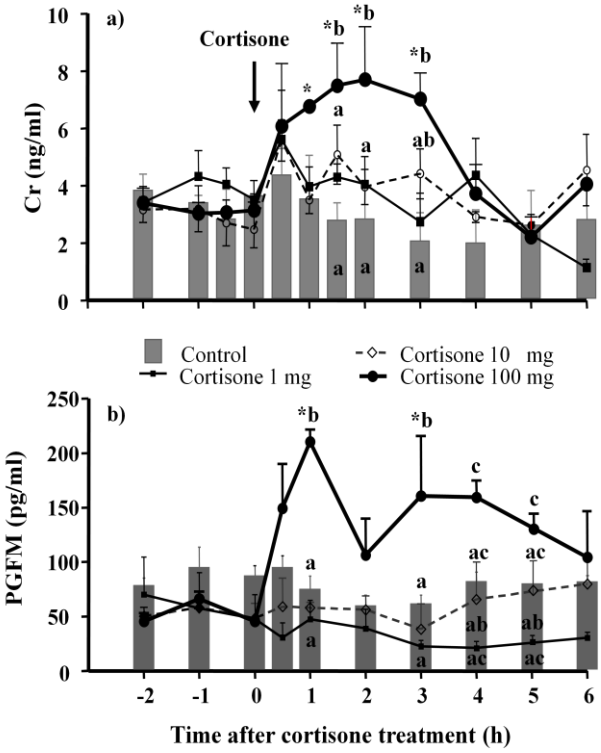

Fig. 4. Plasma concentration of cortisol (Cr; upper panel, a) and prostaglandin $F_{2\alpha}$ metabolite (PGFM; lower panel, b) in jugular vein blood after intravaginal infusion of vaseline gel (Control; n=3) or cortisone at doses of 1 mg, 10 mg, 100 mg dissolved in vaseline gel (n=3/dose) on Day 16 of the oestrous cycle. Data are the mean ± SEM for three samples/time-point. Asterisks indicate significant differences ($P<0.05$) compared with baseline (before cortisone or vaseline gel treatment). Different superscript letters indicate significant differences ($P<0.05$) among groups at the same time point. Bars show Cr concentration in jugular venous plasma of the cows infused intravaginally with vaseline gel (Control, n=3).

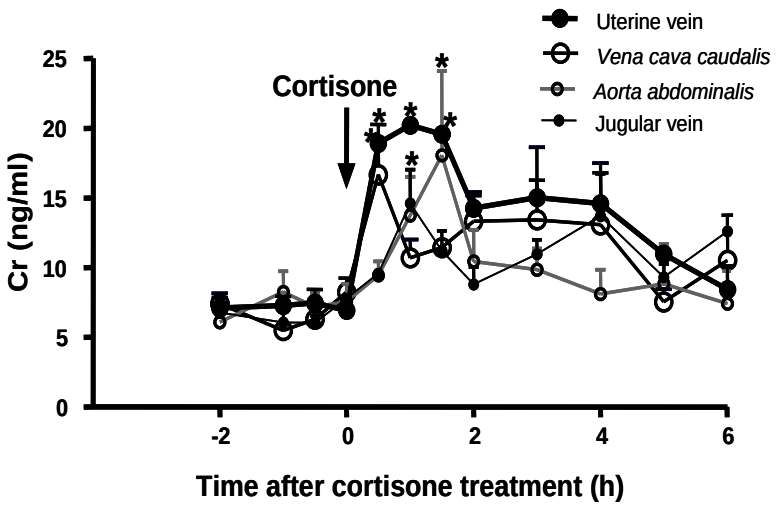

Fig. 5. Plasma concentrations of cortisol (Cr) in uterine vein, *vena cava caudalis*, *aorta abdominalis* and jugular venous blood in cortisone-treated group (n=3) on Day 16 of the oestrous cycle. Data are the mean ± SEM for three samples/time-point. Asterisks indicate significant differences ($P<0.05$) in Cr concentrations compared with the baseline (before cortisone application).

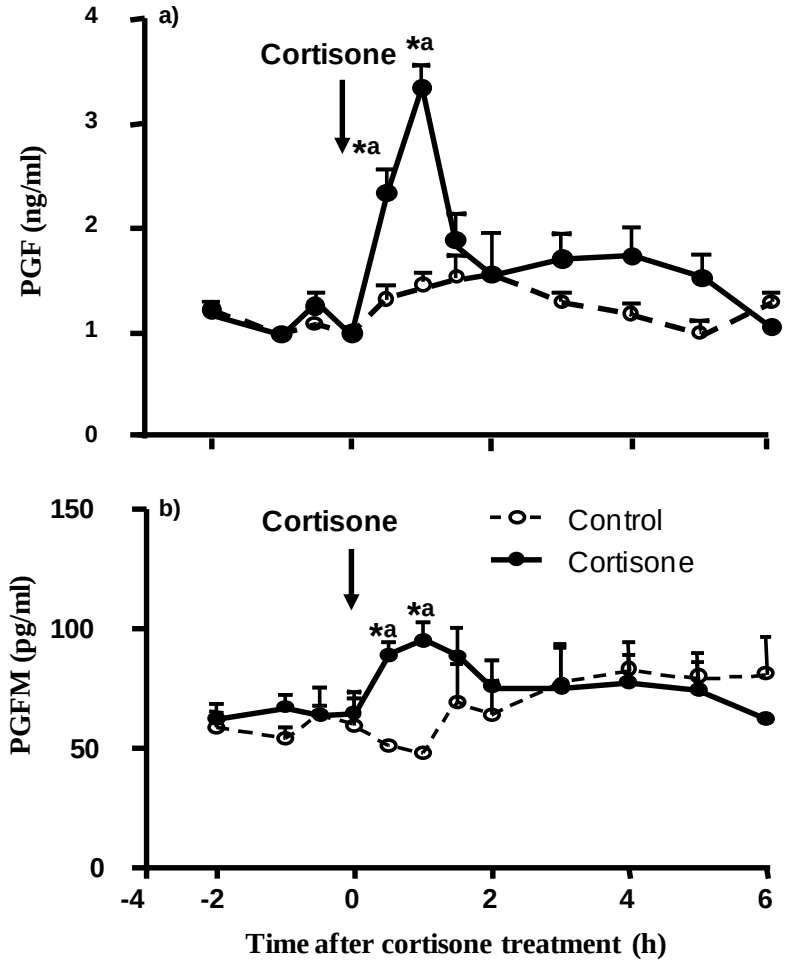

Fig. 6. Plasma concentrations of prostaglandin $F_{2\alpha}$ in uterine venous blood (PGF, upper panel, a) and prostaglandin $F_{2\alpha}$ metabolite (PGFM, lower panel, b) in jugular vein of cortisone-treated group (cortisone, n=3) or vaseline-treated group (control, n=3) on Day 16 of the oestrous cycle. Data are the mean ± SEM for three samples/time-point. Asterisks indicate significant differences ($P<0.05$) compared with baseline (before cortisone or vaseline gel treatment). Superscript letters indicate significant differences ($P<0.05$) between cortisone and control groups at the same time point.

DISCUSSION

The present study clearly demonstrated that the bovine reproductive tract (uterus or/and vagina) has the capacity to convert cortisone to Cr at the late luteal stage *in vivo*. Moreover, intravaginal application of exogenous cortisone (converted to endogenous Cr) on Day 16 of oestrous cycle significantly increased uterine PGF output in cows. These findings support that Cr plays a role in regulating uterine PGF secretion at the late luteal phase in cows.

Cr synthesised in the adrenal cortex affects many organs. The circulating peripheral levels of Cr are relatively constant throughout the oestrous cycle in cattle [50-52]. However, local Cr concentrations have been shown to be modulated by HSD11B1 and HSD11B2 in different organs of several species [43]. HSD11B1 acts predominantly as a NADP(H)-dependent reductase to generate active Cr from inactive cortisone. Our previous *in vitro* study demonstrated that bovine endometrial explants, as well as cultured endometrial cells have the capacity to convert cortisone to Cr [10]. It has been shown that the HSD11B1 is expressed in liver and adipose tissue, with the highest activity normally observed in the liver [53, 54]. In addition to liver, HSD11B1 was previously found to be expressed in the ovine and bovine uterus during the oestrous cycle [10, 55, 56]. It was of interest to know whether the bovine reproductive tract has the ability to convert cortisone to Cr *in vivo* at the late luteal stage. In the present study, the concentrations of Cr increased in UV earlier than in VCC, JV and AA; and plasma Cr levels were highest in UV. These findings indicated that the increase in the levels of Cr detected in the UV plasma is mainly due to the conversion of cortisone to Cr by the bovine reproductive tract at the late luteal stage, and that Cr converted by the reproductive tract enters into the uterine vein and then is diluted in the systemic circulation. Therefore, more time is required to detect a significant increase of Cr concentrations in AA.

Furthermore, intravaginal application of cortisone increased the concentrations of PGF and its metabolite-PGFM within 3 h after treatment. Presently, intravaginal administration is commonly used to administer antimicrobials, labour-inducing agents, prostaglandins and steroids [57, 58]. The vaginal mucosa has good absorption potential

[59]. Many vaginal formulations are applied in the form of suppositories [60, 61], gelatin capsules [62] and recently as bio-adhesive gels [63]. Intra-vaginal administration of progesterone avoids liver first-pass metabolism, and has no systemic side-effects [64]. Recently, vaginal drug delivery has gained further interest due to investigations showing the existence of uterine first-pass effect [65]. The findings indicate that drugs administered through the vaginal route are transported into the uterus achieving higher tissue concentrations than if administered orally or intramuscularly [66]. Thus, in our experiment we have used the intravaginal route to introduce the cortisone dissolved in vaseline gel into the cows to study its local biological effects.

PGF was found to stimulate HSD11B1 in human placenta [67] and increased the local conversion of cortisone to Cr by increasing HSD11B1 in the cow [11]. GCs stimulated PG synthesis in fetal membranes of human [68, 69] and sheep [70]. These findings imply the presence of a positive feedback loop between local PGF and Cr synthesis during late pregnancy and labor [67, 71]. In cattle, our previous *in vitro* study showed that Cr reduces basal PGF production in non-pregnant bovine endometrial stromal cells, whereas it did not affect epithelial cells PGF production [10]. However, stromal cells are the main source of PGE_2 production [72] while epithelial cells are the main source of PGF synthesis [1, 3]. Until now, it has been unclear whether exogenous cortisone, which is converted to endogenous Cr, affects uterine PGF secretion in cow at the late luteal stage *in vivo*. The increased levels of HSD11B1 mRNA and bioactivity were temporally coincident with the increase in the basal release of PGF during the oestrous cycle [10, 72, 73]. Furthermore, treatment of metyrapone, an inhibitor of HSD11B1, to reduce the local availability of Cr converted from cortisone in the bovine reproductive tract at the late luteal stage prolonged the luteal phase in cows [74]. Thus, Cr may play a role in regulating PGF secretion *in vivo*. A recent *in vitro* study using HSD11B1 inhibitor to block the conversion of cortisone to Cr showed that cortisone does not affect directly PGF production in nonpregnant endometrial tissue at late luteal stage (Duong HT, Okuda K & Acosta TJ, unpublished data). In the present study, we found that cortisone infusion increased PGF and PGFM concentrations in the UV and JV blood plasma, respectively and that the increase in uterine levels of PGF and Cr occurred synchronously. These findings suggest that Cr converted from exogenous cortisone can stimulate PGF secretion from the bovine uterus. In fact, PGF treatment

has been shown to increase the levels of Cr *in vitro* [11] and *in vivo* [30, 44]. The above results suggest that a positive feedback loop between local Cr and PGF could play a role during luteolysis in the bovine uterus. However, the exact role of Cr, as well the mechanisms of it action on PGF output from the bovine uterus are not clarified yet.

Regulation of the pulsatile PGF release could be a possible role of Cr during luteolysis. The pulsatile release of PGF from the uterus in the late luteal phase induces luteolysis in many species including cattle [4]. Many local, uterine factors may serve as signals or triggers of PGF output from uterus [73, 75]. Recent *in vivo* studies [30, 44] have shown that Cr plays a role in regulating PGF secretion in the bovine uterus during PGF-induced luteolysis. In addition, it has been demonstrated the temporal association between a PGFM pulse and a Cr pulse during spontaneous luteolysis in mare [76]. Thus, Cr may be also one of the factors responsible for the generation of PGF pulses during luteolysis [44]. However, it is unknown whether Cr directly regulates PGF secretion at the late luteal stage. Lee et al. (2007) showed that HSD11B1 mRNA expression and activity are highest during luteolysis and the follicular phase. The frequent-pulsatile release of PGF with high amplitude was also observed during spontaneous luteolysis and later (Days 17-20 of the cycle) [77-79]. Furthermore, a concomitant elevation of uterine PGF and Cr concentrations was observed after an analogue of PGF injection, and then concentration of PGF decreased after Cr reached its highest levels [44]. Interestingly, in the present study, the levels of PGF in UV blood plasma also decreased after Cr reached its highest levels. Thus, Cr may prevent excessive uterine PGF secretion within a short time period. The above findings support our previous hypothesis [44] that Cr is one of the factors responsible for the generation of PGF pulses in cattle. However, future studies are needed to clarify how Cr induces an acute elevation of PGF concentration and prevents long-lasting-excessive PGF secretion in the bovine uterus resulting in successive PGF pulses.

In conclusion, the bovine reproductive tract has the capacity to convert inactive cortisone to bioactive Cr *in vivo*. Based on the temporal changes of PGF and Cr in the uterine circulation, a biphasic response in PGF secretion was found to be associated to the Cr increase induced by the cortisone treatment at the late luteal stage in nonpregnant cows.

SUMMARY

Previous *in vitro* studies demonstrated that bovine endometrium has the capacity to convert inactive cortisone to biologically active cortisol (Cr) and that Cr inhibits cytokine-stimulated prostaglandin $F_{2\alpha}$ (PGF) production. This study was carried out to test the hypothesis that bovine reproductive tract has the capacity to convert cortisone to Cr *in vivo* and to evaluate the effects of intravaginal application of exogenous cortisone on uterine PGF secretion during the late luteal stage. The temporal relationships between PGF and Cr levels in uterine plasma were also determined. Catheters were inserted into jugular vein (JV), uterine vein (UV), *vena cava caudalis* (VCC) and *aorta abdominalis* (AA) of six cows on Day 15 of the oestrous cycle (ovulation=Day 0) for frequent blood collection. On Day 16, the cows were divided randomly into two groups and infused intravaginally with vaseline gel (10 ml; control; n = 3) or cortisone dissolved in vaseline gel (100 mg; n = 3). Blood samples were collected at -2, -1, -0.5, 0, 0.5, 1, 1.5, 2, 3, 4, 5 and 6h after treatments (0 h). Intravaginal application of cortisone increased plasma concentrations of Cr between 0.5 and 1.5 h in UV, at 0.5 h in VCC, at 1 h in JV and at 1.5 h in AA. The plasma concentrations of PGF in UV and of PGF metabolite in JV were greater at 0.5 and 1 h in the cortisone-treated animals than in control animals. The levels of PGF in UV blood plasma decreased after Cr reached its highest levels. The overall findings suggest that the female reproductive tract has the capacity to convert cortisone to Cr *in vivo*. Based on the temporal changes of PGF and Cr levels in the uterine plasma, a biphasic response in PGF secretion was found to be associated to the Cr increase induced by the cortisone treatment at the late luteal stage in nonpregnant cows.

CHAPTER 3: EFFECTS OF CORTISOL ON PREGNANCY RATE AND CORPUS LUTEUM FUNCTION IN HEIFERS: AN *IN VIVO* STUDY

INTRODUCTION

Glucocorticoids (GCs) are involved in many physiological processes [36, 37], including female reproductive functions [38] in rabbits [80], ewes [81] and humans [82]. Cortisol-Cr, an active GC, is an anti-inflammatory agent that acts to modulate the production and action of cytokines and prostaglandins required for ovulation, luteolysis, embryo implantation, fetal growth and placental development [38, 40]. It is synthesized from cholesterol in the adrenal cortex and is locally regulated by 11β-hydroxysteroid dehydrogenases (HSD11Bs) [10]. The biological action of GCs is mediated through the activation of intracellular GR receptors (GC-R). Two isoforms of GC-R, GC-Rα and GC-Rβ, have been identified [83, 84]. Access of GCs to GC receptors in target tissues is regulated by two HSD11Bs, bidirectional HSD11B type 1 (HSD11B1) that mainly converts cortisone to active cortisol (Cr) [85] and HSD11B type 2 (HSD11B2) that inactivates cortisol to cortisone [86]. Although both HSD11Bs and GC receptors are expressed in the bovine corpus luteum (CL) [31, 87] and endometrium [10] throughout the estrous cycle and early pregnancy [88], the role of GC in regulating CL function is still controversial.

Our previous *in vitro* studies have suggested that cortisol suppresses tumor necrosis factor α (TNFα)-stimulated prostaglandin $F_{2\alpha}$ (PGF) production in endometrial stromal cells [10] and inhibits apoptosis of cultured luteal cells induced by TNFα and interferon-γ (IFNG) [31]. Based on the results of *in vitro* studies, it seems that cortisol plays a role in preventing excessive uterine PGF production and protecting the CL against apoptosis in nonpregnant cattle [11, 31]. In addition, a previous *in vivo* study in cattle has shown that repeated administrations of exogenous GCs during the luteal phase prolong luteal life span and the length of the estrous cycle [89]. Dexamethasone treatment in cattle extended luteal function due to delayed or impaired preovulatory follicular development [90]. Moreover, dexamethasone injection increased CL size but reduced systemic progesterone (P4) concentrations [91]. However, whether and how endogenous Cr may affect bovine CL functions during the estrous cycle and early stages of pregnancy *in vivo* is poorly understood. Metyrapone, an inhibitor of Cr biosynthesis, influences peripheral GC metabolism by regulating the reductase and dehydrogenase activity of HSD11B1 [92]. Previous studies have indicated that metyrapone not

only blocks systemic Cr production, but also decreases the local interconversion of cortisone to Cr by directly inhibiting HSD11B in adrenal cells and hepatocytes [93-97]. Furthermore, the bovine endometrium [10, 98] and corpus luteum [31] have the capacity to convert cortisone to Cr. Thus, metyrapone may be used to reduce the local availability of Cr in the lumen of the reproductive tract and in the CL and to examine the effects of reduced levels of Cr on the pregnancy rate and CL function in cattle.

Intravaginal administration is a widespread method of drug administration for antimicrobials, labor-inducing agents, prostaglandins and steroids [57, 58]. The vaginal mucosa has good absorption potential, and drugs administered via the vaginal route are easily, safely and effectively absorbed and distributed throughout the blood vessels of reproductive organs for a long period of time [59]. Recently, vaginal drug delivery has gained further interest due to investigations showing the existence of a uterine first-pass effect [65]. The above findings suggest that drugs administered through the vaginal route are transported to the uterus and achieve higher tissue concentrations than if administered orally or via intramuscular injection [66]. Thus, in our experiment in heifers, the intravaginal route was used to administer Cr and metyrapone (HSD11B1 inhibitor) dissolved in Vaseline gel to study their biological effects in this species.

To determine whether glucocorticoids affect pregnancy rate and corpus luteum function, we examined the effects of intravaginal applications of exogenous Cr or reduced endogenous Cr by intravaginal applications of HSD11B1 inhibitor (metyrapone) on pregnancy rate and on the secretion of P4 during the estrous cycle and early stage of pregnancy in heifers.

MATERIALS AND METHODS

All animal procedures were approved by the Local Animal Care and Use Committee in Olsztyn, Poland (Agreement No. 06/2007/N).

Animals and surgical procedures

A total of 87 healthy Polish Holstein-Friesian heifers were used for experiments. The animals were made available for this study by their owners (Experimental Animal Farm of the Polish Academy of Sciences in Baranowo, and a private farm in Cieszymowo, Poland). After the study was finished, the heifers were returned to the owners as fully productive animals. Estrus in the heifers was synchronized using two injections of an analogue of PGF (dinoprost, 5

mg, Dinolytic; Upjohn - Pharmacia N.V.S.A., Belgium) with an 11-day interval, as described and recommended in our previous study [45]. The development of ovarian follicles, changes in the size of the CL and uterine structure during the estrous cycle and early pregnancy were monitored daily by a veterinarian via per rectum ultrasonography examination (USG) and confirmed by observing the signs of estrus (i.e., vaginal mucus, standing behavior). The onset of estrus was taken as Day 0 of the estrous cycle. Only heifers with signs of estrus were chosen for the studies.

Determination of cortisol and metyrapone doses

Thirty-three heifers were used to choose the effective doses of Cr and metyrapone. All applications were performed intravaginally on Day 15 of the estrous cycle via a catheter placed into the vagina lumen down to the *orificium uteri externum*, as described previously [47]. The heifers were infused with Vaseline gel (10 ml; control group; n=6), TNFα (n=3), four different doses of Cr (Hydrocortisone, Sigma-Aldrich Chemie GmbH, Munich; Germany; No. H4001) dissolved in Vaseline gel (0.1 mg, 0.5 mg, 1 mg, 10 mg; Cr group; n=12) followed by an intrauterine infusion of 10 ng of TNFα (recombinant human TNFα: rhTNF HF-13; kindly donated by Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan) as previously shown [49] or four different doses of metyrapone dissolved in Vaseline gel (Metopirone^{TM}, HSD11B1 inhibitor; 2-Methyl-1,2-di-3-pyridyl-1-propanone, Sigma-Aldrich Chemie GmbH, Munich, Germany; No. 856525; 1 mg, 10 mg, 100 mg, 500 mg; metyrapone group; n=12). The dose of TNFα and days of experiment were based on our previous data [49]. It has been previously shown *in vivo* that infusion of a low dose (10 ng) of TNFα into the uterus on Day 15 of the estrous cycle increased luteolytic PGF output and induced luteolysis in cattle. Moreover, as shown by *in vitro* data, cortisol inhibited TNFα-stimulated PGF secretion by cultured endometrial cells [10]. Thus, a dose of cortisol, which could be able to inhibit the stimulatory effect of 10 ng of TNFα on PGF output *in vivo*, was tested to determine the effective dose of Cr.

A polyvinyl catheter was inserted into the jugular vein on Day 14 of the estrous cycle for collection of blood samples as described previously [99]. The time of TNFα or metyrapone infusion was defined as 0 h. Blood samples were collected at -2, -1, 0, 0.5, 1, 2, 3 and 4 h and then at 2-h intervals until 12 h after infusions in the Cr and metyrapone groups. For further examination of Cr and metyrapone action during the estrous cycle and early pregnancy in heifers, the doses of 10 mg Cr and 500 mg metyrapone were used. Plasma concentrations of P4, Cr and 13,14-dihydro,15-keto-prostaglandin $F_{2\alpha}$ (PGFM) in plasma samples were measured.

Effects of cortisol and metyrapone applications between Days 15 and 18 of the estrous cycle

To demonstrate the possible influence of Cr on the circulating concentration of P4 during the late luteal phase of the cycle, the first group of heifers (n=18) were infused intravaginally once a day with Vaseline gel (10 ml; control group; n=6), Cr dissolved in Vaseline gel (10 mg; n=6) or metyrapone dissolved in Vaseline gel (500 mg; n=6) from Day 15 to 18 of the estrous cycle, as described for the preliminary experiment. Blood samples were collected from the jugular vein on Days 0, 6, 12, 15, 16, 17, 18, 19 and 21. Plasma concentrations of P4 in blood samples were measured.

Effects of cortisol and metyrapone applications between Days 15 and 18 after artificial insemination

To demonstrate the possible influence of Cr on P4 secretion during early pregnancy and on pregnancy rate, the second group of heifers (n=36) were inseminated with semen from the same bull 60 and 72 h after the second PGF injection. From Day 15 to 18 after insemination, heifers were infused intravaginally once a day with Vaseline gel (10 ml; control group; n=12), Cr dissolved in Vaseline gel (10 mg; n=12) or metyrapone dissolved in Vaseline gel (Metopirone^{TM}, 500 mg; n=12), as described for the preliminary experiment. Blood samples were collected on the following days after insemination: Days 0, 6, 12, 15, 16, 17, 18, 19 and 21. Pregnancy was confirmed by USG between Days 28-30. Plasma concentrations of P4 in blood samples were measured.

For P4 and PGFM determination, blood samples were collected into sterile 10-ml tubes containing 200 μl of stabilizer solution (0.3 M EDTA, 1% acid acetyl salicylic, pH 7.4). All tubes were immediately chilled on ice for 10 min and centrifuged at 2000 x g for 10 min at 4C, and the obtained plasma was stored at - 30C until further analysis.

Progesterone determination

The concentrations of P4 in plasma samples were assayed using a direct enzyme immunoassay (EIA) according to the method described previously [46]. The P4 standard curve ranged from 0.39 ng/ml to 25 ng/ml, and the effective dose for 50% inhibition (ED50) of the assay was 2.85 ng/ml. The intra- and interassay coefficients of variation averaged 6.6% and 8.4%, respectively.

13,14-dihydro,15-keto-prostaglandin $F_{2\alpha}$ (PGFM) determination

The concentrations of PGFM in the plasma samples were determined with a direct EIA according to the method described previously [46]. The PGFM standard curve ranged from 32.5 pg/ml to 8000 pg/ml, and the ED50 of the assay was 315 pg/ml. The intra- and interassay coefficients of variation were on average 7.6% and 10.4%, respectively.

Cortisol determination

The concentrations of Cr in the plasma were determined in duplicate after diethyl ether extraction by second antibody EIA using horseradish peroxidase enzyme-labelled Cr as a tracer (1:80,000 final dilutions) and Cr antibody (raised in a rabbit against cortisol-3-CMO; Cosmo Bio Co., Tokyo, Japan; 1:400,000 final dilutions), as described and characterized recently [34]. The standard curve ranged from 0.4 to 400 ng/ml, and the ED_{50} of the assay was 1.68 ng/ml. The intra- and interassay CVs were on average 5.5% and 6.3%, respectively.

Statistical analysis

The analyses of P4, Cr and PGFM in plasma samples collected from the jugular vein during all experiments were performed using a repeated measures design approach with treatments and time of sample collection (hours or days) being fixed effects with all interactions included (two-way ANOVA tests followed by the Bonferroni Multiple Comparison Test; GraphPAD Prism Version 5.00, GraphPAD Prism Software, San Diego, CA, USA). $P<0.05$ was considered significant. Least adjusted means and standard errors were determined. The total amounts of P4 and PGFM released are shown by the area under the curve (relative units; Tables 2 and 3; means ± SEM) and were analyzed using one-way ANOVA followed by the Bonferroni Multiple Comparison Test (GraphPAD Prism) as described previously [47]. The rate of pregnancy was analyzed using Chi-square ($P<0.05$).

RESULTS

Determination of cortisol and metyrapone doses

Intravaginal application of cortisol on Day 15 of the estrous cycle did not significantly affect P4 levels as indicated by the analysis of area under the curve between 0 and 12 h post treatment (Table 2, Fig. 7a). However, Cr at a dose of 10 mg blocked the increase in PGFM induced by intrauterine infusion of TNFα (10 ng/animal; Table 2, Fig. 7b).

Intravaginal application of 500 mg of metyrapone on Day 15 of the estrous cycle induced an acute increase in circulating P4 levels starting 3 h after application (Table 3, Fig. 8a). The

levels of PGFM did not change significantly during the period of 12 h after metyrapone treatment (Table 3, Fig. 8b).

There was no significant effect of intravaginal application of Cr or metyrapone at any doses on the Cr level in peripheral blood compared with that found in the control animals (data not shown).

Based on these results, the most effective doses of Cr (10 mg) and metyrapone (500 mg) were chosen for the further studies to investigate the local effect of Cr on the luteal function in the estrous cycle and early pregnancy.

Effects of cortisol and metyrapone applications between Days 15 and 18 of the estrous cycle on progesterone concentrations

Plasma concentrations of P4 were lower in Cr-treated heifers than in the control heifers (only gel application) on Days 17 and 18 of the estrous cycle. However, the levels of circulating P4 did not decrease to less than 2 ng/ml until Day 19 (Fig. 9). The interestrus intervals were not different between the control and Cr treated groups ($P<0.05$). Moreover, metyrapone prolonged the functional life span of the CL as indicated by significantly greater levels of P4 compared with those in the control animals on Days 19 and 21 ($P<0.05$). In the heifers infused with 500 mg of metyrapone, spontaneous luteolysis was prevented, and the length of the estrous cycle was prolonged compared with that in the control group (over 30 days versus 21.8 ± 0.77 days; $P<0.05$).

Effects of cortisol and metyrapone applications between Days 15 and 18 post-artificial insemination on pregnancy rate

The effects of Cr and metyrapone on P4 secretion in pregnant and nonpregnant heifers is shown in Figure 10. Intravaginal application of metyrapone (500 mg, Fig. 10c) prolonged the functional life span of the CL in nonpregnant heifers as indicated by the levels of P4 higher than 3 ng/ml until Day 21. However, in control (Fig. 4a) and Cr (Fig. 4b) heifers, the levels of P4 were less 1 ng/ml on Day 21. The pregnancy rate of Cr-treated heifers was higher than that of control heifers (75% vs. 58%), whereas the pregnancy rate of metyrapone-treated heifers was lower ($P<0.05$) than that of the control group (16.7% vs. 58%; Table 4).

Table 2. Effects of intravaginal infusion with Vaseline gel (control, n=3), a luteolytic dose of TNFα (10 ng/heifer; n=3) or different doses of cortisol (0.1 mg, 0.5 mg, 1 mg, 10 mg, each dose n=3) followed by intrauterine infusion with TNFα 2 h later on the total amounts of progesterone (P4) and prostaglandin $F_{2\alpha}$ metabolite (PGFM) released during the experiment, as measured by hormones concentrations in jugular venous blood of heifers on Day 15 of the estrous cycle.

Treatment	**Progesterone**	**Prostaglandin $F_{2\alpha}$ metabolite**
Saline/vaseline (control)	81.7 ± 4.7 [a]	952 ± 51.8 [a]
TNFα	67.9 ± 16.5 [a]	2510 ± 248.2 [b]
Cortisol 0.1 mg + TNFα	76.5 ± 3.2 [a]	2299 ± 292.0 [b]
Cortisol 0.5 mg + TNFα	91.1 ± 17.8 [a]	2433 ± 533.7 [b]
Cortisol 1 mg + TNFα	100.1 ± 10.0 [a]	1554 ± 194.3 [a]
Cortisol 10 mg + TNFα	104.8 ± 13.0 [a]	1121 ± 68.5 [a]

Values indicate the area under the curve (relative units, means ± SEM). The area under the curve was analyzed using P4 and PGFM data between 0 and 12 h after intrauterine infusion with a luteolytic dose of TNFα (0 h). [a – b] Different superscript letters within a column indicate significant differences ($P<0.05$) among treated groups.

Table 3. Effects of intravaginal infusion with Vaseline gel (control, n=3) or different doses of metyrapone (1, 10, 100 and 500 mg, each dose n=3) on the total amounts of progesterone (P4) and prostaglandin $F_{2\alpha}$ metabolite (PGFM) released during the experiment, as measured by hormones concentrations in jugular venous blood of heifers on Day 15 of the estrous cycle.

Treatment	Progesterone	Prostaglandin $F_{2\alpha}$ metabolite
Saline (control)	61.8 ± 9.7 [a]	1051 ± 162.0 [a]
Metyrapone 1 mg	86.3 ± 11.2 [ab]	672 ± 47.6 [a]
Metyrapone 10 mg	72.1 ± 19.4 [ab]	743 ± 59.4 [a]
Metyrapone 100 mg	90.8 ± 16.4 [b]	775 ± 60.6 [a]
Metyrapone 500 mg	112.9 ± 10.8 [c]	722 ± 24.2 [a]

Values indicate the area under the curve (relative units, means ± SEM). The area under the curve was analyzed using P4 and PGFM data between 0 and 12 h after intravaginal infusion with metyrapone (0 h). [a-b-c] Different superscript letters within a column indicate significant differences ($P<0.05$) among treated groups.

Table 4: Effects of intravaginal infusion with Vaseline gel (n=12), cortisol (n=12) or metyrapone (n=12) between Days 15 and 18 post artificial insemination on pregnancy rate

Treatment	**Number of pregnant heifers**	**Number of nonpregnant heifers**	**Pregnancy rate (%)**
Vaseline (control)	7	5	58
Cortisol	9	3	75
Metyrapone	2	10	16

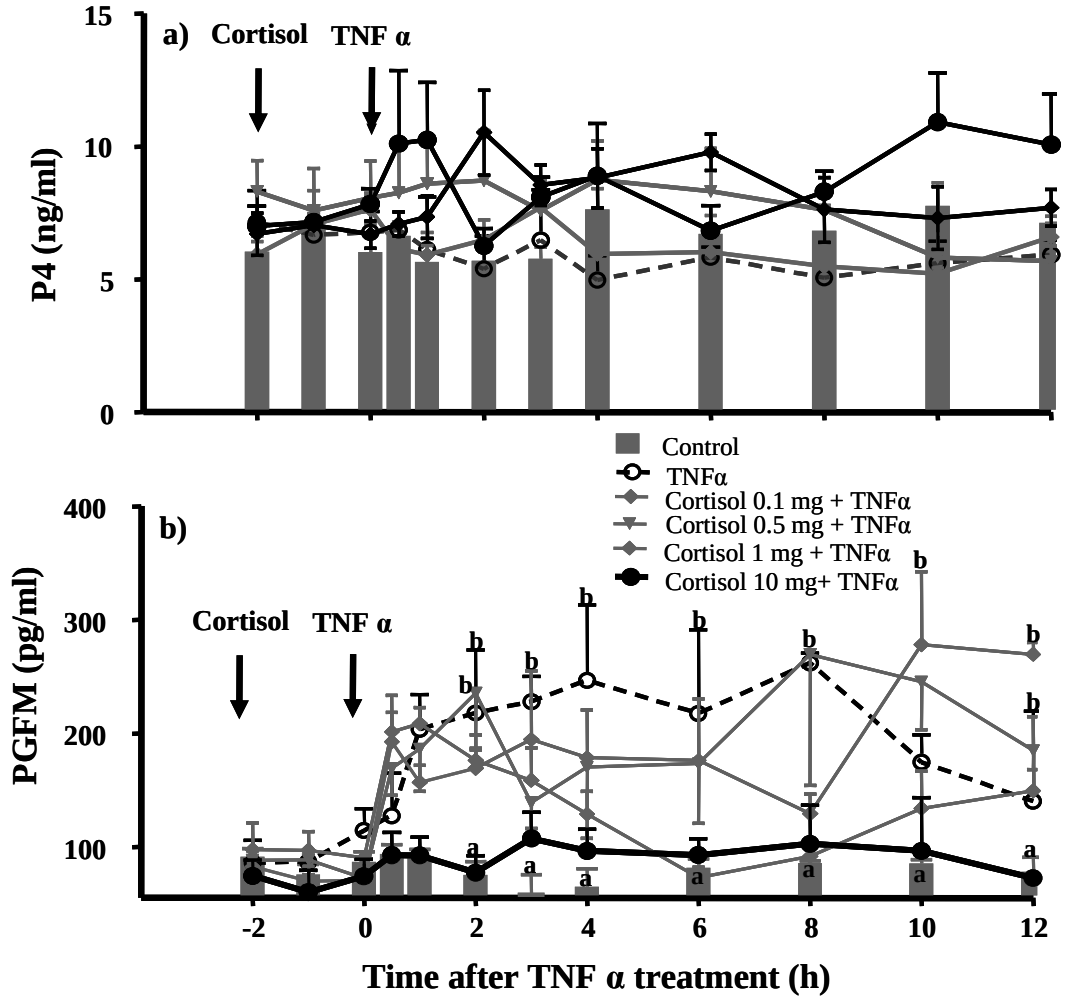

Fig. 7. Concentrations of progesterone (P4, Fig. a) and prostaglandin $F_{2\alpha}$ metabolite (PGFM, Fig.b) in blood plasma from the jugular vein in heifers after intravaginal infusion with Vaseline gel (control; n=3), TNFα at luteolytic dose (TNFα, 10 ng/heifer; n=3) or cortisol at doses of 0.1 mg, 0.5 mg, 1 mg and 10 mg (each dose n=3) dissolved in Vaseline gel followed by an intrauterine infusion of TNFα at a luteolytic dose 2 h later (n=12) on Day 15 of the estrous cycle. Different superscript letters indicate significant differences ($P<0.05$) among treated groups.

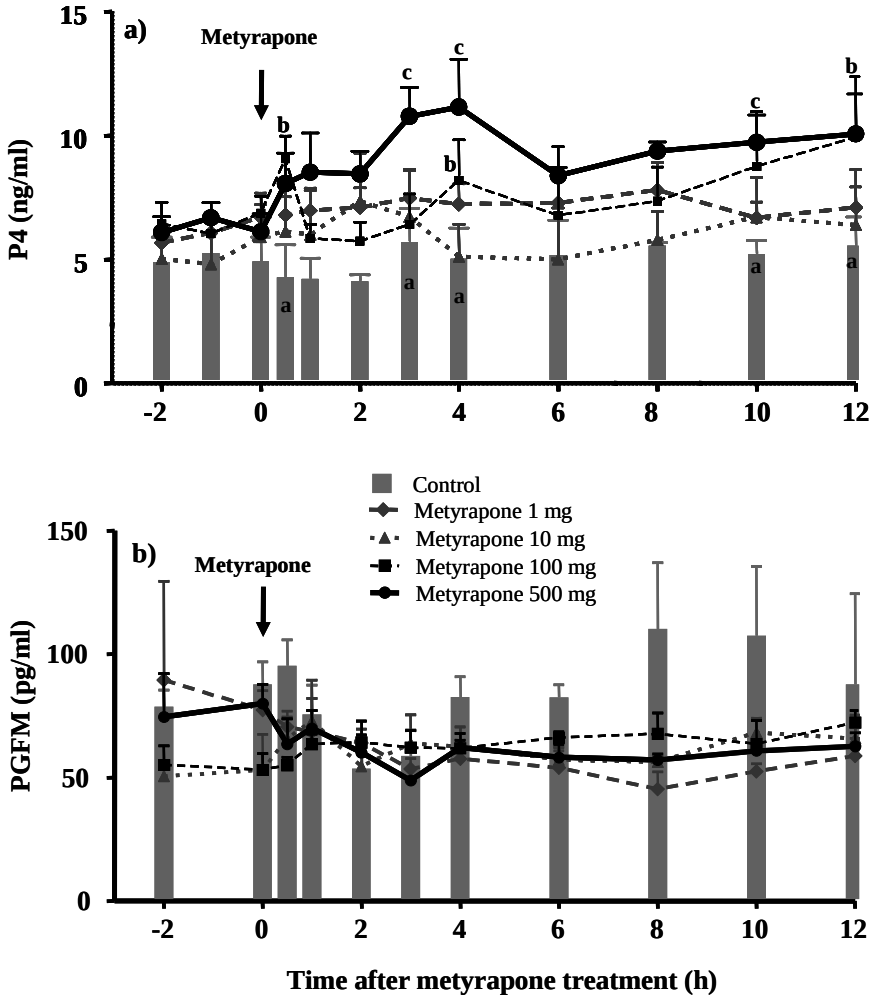

Fig. 8. Concentrations of progesterone (P4, Fig. a) and prostaglandin $F_{2\alpha}$ metabolite (PGFM, Fig.b) in blood plasma from the jugular vein in heifers after intravaginal application with Vaseline gel (control; n=3) or metyrapone at doses of 1 mg, 10 mg, 100 mg and 500 mg dissolved in Vaseline gel (MetopironeTM, HSD11B1 inhibitor; each dose n=3) on Day 15 of the estrous cycle. Different superscript letters indicate significant differences ($P<0.05$) among treated groups.

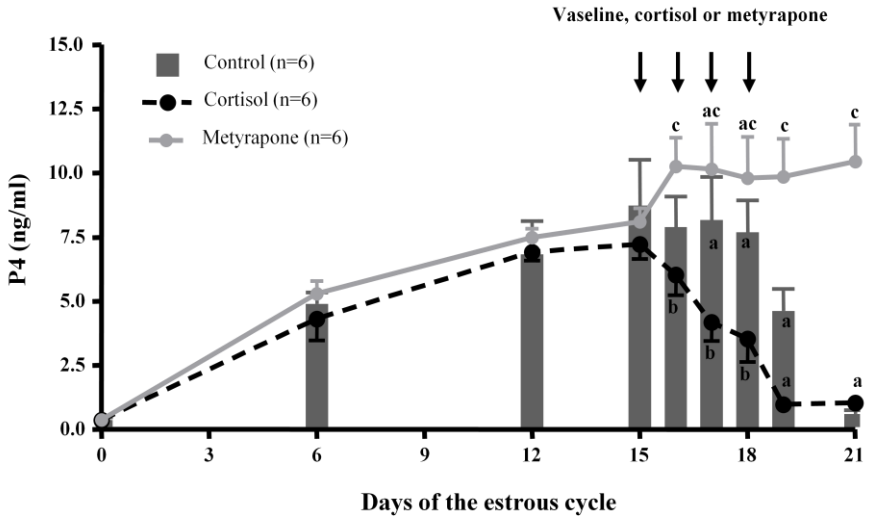

Fig. 9. Changes in plasma concentrations of progesterone (P4) in heifers after intravaginal application with Vaseline gel (control; n=6), cortisol (10 mg; n=6) or metyrapone (MetopironeTM, HSD11B1 inhibitor; 500 mg; n=6) dissolved in 10 ml of Vaseline gel. Intravaginal Vaseline gel or Vaseline gel with cortisol or metyrapone was applied daily from Day 15 to Day 18 of the estrous cycle. Different superscript letters indicate significant differences ($P<0.05$) among treated groups.

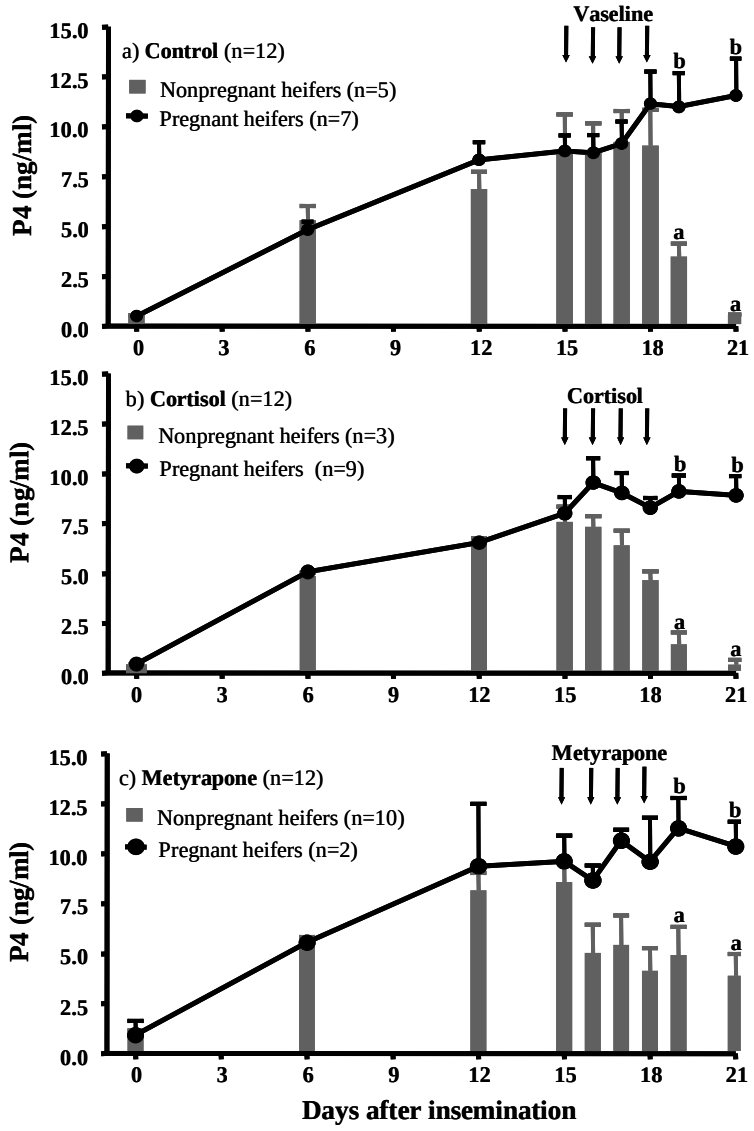

Fig. 10. Change in plasma concentrations of progesterone (P4) in pregnant and nonpregnant heifers. Heifers were inseminated on Day 0 of the estrous cycle. Vaseline gel (control; n=12, Fig. 10a), cortisol (10 mg; n=12, Fig. 10b) or metyrapone (MetopironeTM, HSD11B1 inhibitor; 500 mg; n=12, Fig. 10c) dissolved in 10 ml of Vaseline gel was intravaginally applied once a day from Day 15 to Day 18 after insemination. Different superscript letters indicate significant differences ($P<0.05$) between pregnant and nonpregnant heifers.

DISCUSSION

The results of the present study demonstrated that intravaginal application of exogenous cortisol from Day 15 to 18 post insemination increased the pregnancy rate, whereas inhibition of local cortisol biosynthesis by intravaginal application of metyrapone decreased the pregnancy rate in heifers. The same treatment with cortisol during the estrous cycle (in noninseminated heifers) decreased P4 between Days 17 and 18, but did not affect the time of complete luteolysis. On the other hand, treatment with metyrapone prolonged the life span of the CL, and luteolysis did not occur until Day 30 post ovulation. The above findings suggest that cortisol differently modulates the life span of the CL depending on physiological status of heifers (pregnant vs. nonpregnant).

We have previously shown that cortisol inhibits basal PGF production in nonpregnant bovine endometrial stromal cells, whereas it does not affect PGF production in epithelial cells *in vitro* [10]. This *in vitro* study suggests that cortisol could mainly act as an antiluteolytic factor suppressing PGF production in the bovine endometrium. To examine whether cortisol modulates CL function *in vivo*, heifers were treated with cortisol around the time of the cycle when luteolysis normally begins (Days 15-18 of the estrous cycle). In the present study, the fact that cortisol did not affect the interestrus interval in cycling heifers but significantly increased the pregnancy rate in inseminated heifers suggests that depending on the type of endometrial cells (stromal and epithelial) and physiological status (pregnant versus nonpregnant), cortisol has different roles in regulating CL function. Our previous *in vitro* studies demonstrated that cortisol suppresses PGF production in the endometrium [10] and suppresses apoptosis of luteal cells acting as a survival factor for bovine luteal cells [31] in cattle. Thus, cortisol may act to prevent luteolysis by decreasing luteolytic PGF production and decreasing luteal cell death in cattle.

In ruminants, inhibition of the luteolytic mechanism to maintain the secretion of P4 is essential for the establishment of pregnancy. Interferon-tau (IFNT) has been identified as an embryonic signal responsible for the maternal recognition of pregnancy in ruminants [100]. During maternal recognition of pregnancy, the conceptus synthesizes and secretes IFNT with maximal production on Days 14-16 [101, 102]. It has been shown that the level of cortisol is locally regulated by IFNT in the ovine [55] and bovine [88] endometrium during pregnancy. Therefore, the different effects of exogenous cortisol or the inhibitor of cortisol biosynthesis in pregnant versus nonpregnant heifers observed in the present study may be due to the action of IFNT that is locally present at high concentrations in the uterine lumen of pregnant heifers but

not in nonpregnant heifers. IFNT acts locally within the uterus to inhibit PGF secretion [103], and IFNT inhibits PGF production but stimulates PGE_2 production by the uterus during early pregnancy [104, 105]. PGF is a luteolytic factor [106], whereas PGE_2 acts as a luteotrophic factor by stimulating P4 production [107-109]. In addition, poor embryo development is associated with low IFNT production, failed inhibition of luteolysis and embryo loss [110, 111]. These findings suggest that embryonic loss may occur because the embryos are unable to inhibit endometrial PGF secretion. In the present study, intravaginal application of exogenous cortisol from Days 15 to 18 post insemination increased the pregnancy rate and reduced TNFα-stimulated PGFM levels, whereas the inhibition of local cortisol biosynthesis by intravaginal application of metyrapone decreased the pregnancy rate in heifers. These results agree with those of Boomsma *et al.* (2007), who reported an increase in pregnancy rate as a result of GC administration [112]. The above results suggest that cortisol plays an important role in maintaining CL function by directly inhibiting uterine PGF secretion to support embryo implantation and early embryonic development.

In the present study, intravaginal applications of cortisol reduced P4 production compared with those of control heifers between Days 17 and 18 of the estrous cycle; however, the length of the cycle did not change compared with the control group. In contrast, reduced endogenous cortisol production by metyrapone application extended the length of the estrous cycle and increased P4 production. Glucocorticoids decreased the plasma P4 concentration in cattle [90, 113, 114]. Cortisol has the capacity to suppress luteal P4 secretion indirectly by inhibiting basal and luteinizing hormone-releasing hormone-induced release of luteinizing hormone from bovine pituitary cells [115]. At the end of the estrous cycle, cortisol can be involved in the luteolytic cascade by modulating uterine PGF secretion and its action on the CL. PGF released from the endometrium, especially the intercaruncular region of the surface epithelium of the uterus [116], in a pulsatile manner causes regression of the bovine CL [19, 77]. Thus, for the initiation of bovine luteal regression, the pulsatile character of PGF is much more important and plays a mandatory role rather than its absolute levels [19, 77]. Furthermore, cortisol has been suggested to act in reducing the high levels of uterine PGF and to be one of the factors responsible for the generation of PGF pulses *in vivo* in cattle [44]. Cortisol is well-known as a local regulator/modulator of PG secretion [10]. Therefore, it can be suggested that cortisol is one of the most important components of the intrauterine regulatory system responsible for the autonomous, episodic PGF output during luteolysis in cattle. Blockade of the endogenous cortisol production by metyrapone application may disturb the pulsatile PGF output from the bovine uterus, consequently inhibiting luteolysis and prolonging the life span of the bovine CL.

However, the effects and mechanisms of cortisol action on the autonomous, episodic PGF output and/or on the frequency of PGF pulses need to be determined in the future.

In conclusion, cortisol, depending on the physiological status of the heifers (pregnant vs. nonpregnant), modulates CL function by influencing P4 secretion. Cortisol may have a positive influence on CL function during early pregnancy, leading to support embryo implantation and resulting in higher rates of pregnancy in heifers.

SUMMARY

To determine whether glucocorticoids affect the function of the bovine corpus luteum (CL) during the estrous cycle and early pregnancy, we examined the effects of exogenous cortisol or reduced endogenous cortisol on the secretion of progesterone (P4) and on pregnancy rate. In preliminary experiments, doses of cortisol and metyrapone (an inhibitor of cortisol synthesis) were established (n=33). Cortisol in effective doses of 10 mg blocked tumor necrosis factor-induced prostaglandin $F_{2\alpha}$ secretion as measured by its metabolite (PGFM) concentrations in the blood. Metyrapone in effective doses of 500 mg increased the P4 concentration. Thus, both reagents were then intravaginally applied in the chosen doses daily from Day 15 to 18 after estrus (Day 0) in noninseminated heifers (n=18) or after artificial insemination (n=36). Pregnancy was confirmed by transrectal ultrasonography between Days 28-30 after insemination. Plasma concentrations of P4 were lower in cortisol-treated heifers than in control heifers on Days 17 and 18 of the estrous cycle ($P<0.05$). However, the interestrus intervals were not different between control and cortisol-treated animals ($P>0.05$). Moreover, metyrapone increased P4 and prolonged the CL lifespan in comparison to control animals ($P<0.05$). Interestingly, in inseminated heifers, cortisol increased the pregnancy rate (75%) compared with control animals (58%), whereas metyrapone reduced the pregnancy rate to 16.7% ($P<0.05$). The overall results suggest that cortisol, depending on the physiological status of heifers (pregnant vs. nonpregnant), modulates CL function by influencing P4 secretion. Cortisol may have a positive influence on CL function during early pregnancy, leading to support of embryo implantation and resulting in higher rates of pregnancy in heifers.

GENERAL CONCLUSION

The present study determined the roles of PGF and cortisol in the regulation of bovine uterine and luteal functions *in vivo.* The experiment in chapter 1 demonstrated that the uterus rather than the ovary increases PGF production in response to PGF injection by showing that injection of a PGF analogue induced more than a twofold increase in the levels of PGF in uterine venous (UV) plasma between 0.25 and 1 h after injection, but it did not affect the levels of PGF in ovarian venous (OV) plasma. The series of experiments in chapter 2 demonstrated that the bovine reproductive tract (uterus and/or vagina) has the capacity to convert cortisone to cortisone and that cortisol may act to reduce the excessive uterine PGF secretion in non-pregnant cows *in vivo.* The series of experiments in chapter 3 demonstrated that depending on physiological status (pregnant vs. nonpregnant), cortisol modulates bovine CL function by influencing P4 secretion. Thus, cortisol may have a positive influence on CL function during early pregnancy, which would promote embryo implantation and thus result in higher rates of pregnancy in heifers. The overall results of the present study suggest that PGF and cortisol play the roles in regulating bovine uterine and ovarian functions.

REFERENCES

1. **Skarzynski DJ, Miyamoto Y, Okuda K.** Production of prostaglandin $F_{2\alpha}$ by cultured bovine endometrial cells in response to tumor necrosis factor α: cell type specificity and intracellular mechanisms. *Biol Reprod* 2000; **62**: 1116-1120.
2. **McCracken JA, Custer EE, Lamsa JC.** Luteolysis: A neuroendocrine-mediated event. *Physiol Rev* 1999; **79**: 263-323.
3. **Kim JJ, Fortier MA.** Cell type specificity and protein kinase C dependency on the stimulation of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ production by oxytocin and platelet-activating factor in bovine endometrial cells. *J Reprod Fertil* 1995; **103**: 239-247.
4. **Poyser NL.** The control of prostaglandin production by the endometrium in relation to luteolysis and menstruation. *Prostaglandins Leukot Essent Fatty Acids* 1995; **53**: 147-195.
5. **Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LBA, Lipsky PE.** Cyclooxygenase in biology and disease. *Faseb Journal* 1998; **12**: 1063-1073.
6. **Wolfenson D, Thatcher WW, Drost M, Caton D, Foster DB, Leblanc MM.** Characteristics of prostaglandin-F measurements in the ovarian circulation during the estrous-cycle and early-pregnancy in the cow. *J Reprod Fertil* 1985; **75**: 491-499.
7. **McCracken JA, Carlson JC, Glew ME, Goding JR, Baird DT, Green K, Samuelsson B.** Prostaglandin $F_{2\alpha}$ identified as a luteolytic hormone in sheep. *Natural Science* 1972; **238**: 129-134.
8. **Lee J, McCracken JA, Banu SK, Rodriguez R, Nithy TK, Arosh JA.** Transport of prostaglandin $F_{2\alpha}$ pulses from the uterus to the ovary at the time of luteolysis in ruminants is regulated by prostaglandin transporter-mediated mechanisms. *Endocrinology* 2010; **151**: 3326-3335.
9. **Inskeep EK, Murdoch WJ.** Relation of ovarian functions to uterine and ovarian secretion of prostaglandins during the estrous cycle and early pregnancy in the ewe and cow. *Int Rev Physiol* 1980; **22**: 325-356.

10. **Lee HY, Acosta TJ, Tanikawa M, Sakumoto R, Komiyama J, Tasaki Y, Piskula M, Skarzynski DJ, Tetsuka M, Okuda K.** The role of glucocorticoid in the regulation of prostaglandin biosynthesis in non-pregnant bovine endometrium. *J Endocrinol* 2007; **193**: 127-135.
11. **Lee HY, Acosta TJ, Skarzynski DJ, Okuda K.** Prostaglandin $F_{2\alpha}$ stimulates 11β-hydroxysteroid dehydrogenase 1 enzyme bioactivity and protein expression in bovine endometrial stromal cells. *Biol Reprod* 2009; **80**: 657-664.
12. **Tsai SJ, Wiltbank MC.** Prostaglandin $F_{2\alpha}$ induces expression of prostaglandin G/H synthase-2 in the ovine corpus luteum: a potential positive feedback loop during luteolysis. *Biol Reprod* 1997; **57**: 1016-1022.
13. **Hayashi K, Acosta TJ, Berisha B, Kobayashi S, Ohtani M, Schams D, Miyamoto A.** Changes in prostaglandin secretion by the regressing bovine corpus luteum. *Prostag Oth Lipid M* 2003; **70**: 339-349.
14. **Kotwica J, Skarzynski D, Miszkiel G, Melin P, Okuda K.** Oxytocin modulates the pulsatile secretion of prostaglandin $F_{2\alpha}$ in initiated luteolysis in cattle. *Res Vet Sci* 1999; **66**: 1-5.
15. **Jabbour HN, Sales KJ, Boddy SC, Anderson RA, Williams ARW.** A positive feedback loop that regulates cyclooxygenase-2 expression and prostaglandin $F_{2\alpha}$ synthesis via the F-series-prostanoid receptor and extracellular signal-regulated kinase 1/2 signaling pathway. *Endocrinology* 2005; **146**: 4657-4664.
16. **Granstrom E, Kindahl H.** Species differences in circulating prostaglandin metabolites. Relevance for the assay of prostaglandin release. *Biochim Biophys Acta* 1982; **713**: 555-569.
17. **Goff AK, Basu S, Kindahl H.** Measurement of 11-ketotetranor PGF metabolites: An approach for monitoring prostaglandin $F_{2\alpha}$ release in the mare. *Theriogenology* 1984; **21**: 887-896.
18. **Keirse MJ, Turnbull AC.** Metabolism of prostaglandins within the pregnant uterus. *Br J Obstet Gynaecol* 1975; **82**: 887-893.
19. **Ginther OJ, Araujo RR, Palhao MP, Rodrigues BL, Beg MA.** Necessity of sequential pulses of prostaglandin $F_{2\alpha}$ for complete physiologic luteolysis in cattle. *Biol Reprod* 2009; **80**: 641-648.

20. **Bah MM, Acosta TJ, Pilawski W, Deptula K, Okuda K, Skarzynski DJ.** Role of intraluteal prostaglandin $F_{2\alpha}$, progesterone and oxytocin in basal and pulsatile progesterone release from developing bovine corpus luteum. *Prostag Oth Lipid M* 2006; **79**: 218-229.
21. **Acosta TJ, Ozawa T, Kobayashi S, Hayashi K, Ohtani M, Kraetzl WD, Sato K, Schams D, Miyamoto A.** Periovulatory changes in the local release of vasoactive peptides, prostaglandin $F_{2\alpha}$, and steroid hormones from bovine mature follicles in vivo. *Biol Reprod* 2000; **63**: 1253-1261.
22. **Piotrowska KK, Woclawek-Potocka I, Bah MA, Piskula MK, Pilawski W, Bober A, Skarzynski DJ.** Phytoestrogens and their metabolites inhibit the sensitivity of the bovine corpus luteum to luteotropic factors. *J Reprod Dev* 2006; **52**: 33-41.
23. **Skarzynski DJ, Okuda K.** Different actions of noradrenaline and nitric oxide on the output of prostaglandins and progesterone in cultured bovine luteal cells. *Prostag Oth Lipid M* 2000; **60**: 35-47.
24. **Acosta TJ, Yoshizawa N, Ohtani M, Miyamoto A.** Local changes in blood flow within the early and midcycle corpus luteum after prostaglandin $F_{2\alpha}$ injection in the cow. *Biol Reprod* 2002; **66**: 651-658.
25. **Wiltbank MC, Ottobre JS.** Regulation of intraluteal production of prostaglandins. *Reprod Biol Endocrinol* 2003; **1**: 91.
26. **Arosh JA, Banu SK, Chapdelaine P, Madore E, Sirois J, Fortier MA.** Prostaglandin biosynthesis, transport, and signaling in corpus luteum: a basis for autoregulation of luteal function. *Endocrinology* 2004; **145**: 2551-2560.
27. **Tasaki Y, Lee HY, Cui JP, Acosta TJ, Okuda K.** Local autoamplification system for prostaglandin $F_{2\alpha}$ in bovine endometrium. *Meeting of the Society for the Study of Reproduction* 2010: 93-94.
28. **Wang H, Walker SW, Mason JI, Morley SD, Williams BC.** Role of arachidonic acid metabolism in ACTH-stimulated cortisol secretion by bovine adrenocortical cells. *Endocr Res* 2000; **26**: 705-709.
29. **Baishya N, Cooper MJ, Hart IC, Jackson PS, Furr BJ, Jenkin G, Pope GS.** Effects of luteolytic doses of prostaglandin $F_{2\alpha}$ and cloprostenol on concentrations of progesterone, luteinizing hormone, follicle-stimulating hormone, glucose,

insulin, growth hormone, thyroxine, prolactin and cortisol in jugular plasma of lactating dairy cows. *Br Vet J* 1994; **150**: 569-583.

30. **Shrestha HK, Beg MA, Imam S, Ginther OJ.** Luteal blood flow and concentrations of circulating progesterone and other hormones associated with a simulated pulse of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ in heifers. *Reproduction* 2010; **139**: 673-683.
31. **Komiyama J, Nishimura R, Lee HY, Sakumoto R, Tetsuka M, Acosta TJ, Skarzynski DJ, Okuda K.** Cortisol is a suppressor of apoptosis in bovine corpus luteum. *Biol Reprod* 2008; **78**: 888-895.
32. **Mann GE, Lamming GE.** Timing of prostaglandin $F_{2\alpha}$ release episodes and oxytocin receptor development during luteolysis in the cow. *Anim Reprod Sci* 2006; **93**: 328-336.
33. **Newton R, Kuitert LM, Slater DM, Adcock IM, Barnes PJ.** Cytokine induction of cytosolic phospholipase A2 and cyclooxygenase-2 mRNA is suppressed by glucocorticoids in human epithelial cells. *Life Sci* 1997; **60**: 67-78.
34. **Xue S, Slater DM, Bennett PR, Myatt L.** Induction of both cytosolic phospholipase A2 and prostaglandin H synthase-2 by interleukin-1 [beta] in WISH cells is inhibited by dexamethasone. *Prostaglandins* 1996; **51**: 107-124.
35. **Rhen T, Cidlowski JA.** Antiinflammatory action of glucocorticoids--new mechanisms for old drugs. *N Engl J Med* 2005; **353**: 1711-1723.
36. **Wang M.** The role of glucocorticoid action in the pathophysiology of the metabolic syndrome. *Nutr Metab* 2005; **2**: 1-14.
37. **McKay LI, Cidlowski JA.** Molecular control of immune/inflammatory responses: Interactions between nuclear factor-kappa B and steroid receptor-signaling pathways. *Endocr Rev* 1999; **20**: 435-459.
38. **Andersen CY.** Commentary - Possible new mechanism of cortisol action in female reproductive organs: physiological implications of the free hormone hypothesis. *J Endocrinol* 2002; **173**: 211-217.
39. **Brann DW, Mahesh VB.** Role of corticosteroids in female reproduction. *Faseb J* 1991; **5**: 2691-2698.
40. **Hillier SG, Tetsuka M.** An anti-inflammatory role for glucocorticoids in the ovaries? *J Reprod Immunol* 1998; **39**: 21-27.

41. **Goppelt-Struebe M.** Molecular mechanisms involved in the regulation of prostaglandin biosynthesis by glucocorticoids. *Biochem Pharmacol* 1997; **53**: 1389-1395.
42. **Bush IE, Hunter SA, Meigs RA.** Metabolism of 11-oxygenated steroids. Metabolism in vitro by preparations of liver. *Biochem J* 1968; **107**: 239-258.
43. **Krozowski Z, Li KX, Koyama K, Smith RE, Obeyesekere VR, Stein-Oakley A, Sasano H, Coulter C, Cole T, Sheppard KE.** The type I and type II 11β-hydroxysteroid dehydrogenase enzymes. *J Steroid Biochem Mol Biol* 1999; **69**: 391-401.
44. **Duong HT, Vu HV, Bah MM, Woclawek-Potocka I, Dam TV, Skarzynski DJ, Okuda K, Acosta TJ.** Acute changes in the concentrations of prostaglandin $F_{2\alpha}$ (PGF) and cortisol in uterine and ovarian venous blood during PGF-induced luteolysis in cows. *Reprod Domest Anim* 2012; **47**: 238-243.
45. **Skarzynski DJ, Siemieniuch MJ, Pilawski W, Potocka IW, Bah MM, Majewska M, Jaroszewski JJ.** In vitro assessment of progesterone and prostaglandin E_2 production by the corpus luteum in cattle following pharmacological synchronization of estrus. *J Reprod Dev* 2009; **55**: 170-176.
46. **Skarzynski DJ, Jaroszewski JJ, Bah MM, Deptula KM, Barszczewska B, Gawronska B, Hansel W.** Administration of a nitric oxide synthase inhibitor counteracts prostaglandin-F-induced luteolysis in cattle. *Biol Reprod* 2003; **68**: 1674-1681.
47. **Woclawek-Potocka I, Kowalczyk-Zieba I, Skarzynski DJ.** Lysophosphatidic acid action during early pregnancy in the cow: in vivo and in vitro studies. *J Reprod Dev* 2010; **56**: 411-420.
48. **Skarzynski DJ, Piotrowska KK, Bah MM, Korzekwa A, Woclawek-Potocka I, Sawai K, Okuda K.** Effects of exogenous tumour necrosis factor α on the secretory function of the bovine reproductive tract depend on tumour necrosis factor α concentrations. *Reprod Domest Anim* 2009; **44**: 371-379.
49. **Skarzynski DJ, Woclawek-Potocka I, Korzekwa A, Bah MM, Piotrowska K, Barszczewska B, Okuda K.** Infusion of exogenous tumor necrosis factor dose dependently alters the length of the luteal phase in cattle: Differential responses to

treatment with indomethacin and L-NAME, a nitric oxide synthase inhibitor. *Biol Reprod* 2007; **76**: 619-627.

50. **Garverick HA, Erb RE, Niswender GD, Callahan CJ.** Reproductive steroids in the bovine. 3. Changes during the estrous cycle. *J Anim Sci* 1971; **32**: 946-956.
51. **Lyimo ZC, Nielen M, Ouweltjes W, Kruip TAM, van Eerdenburg F.** Relationship among estradiol, cortisol and intensity of estrous behavior in dairy cattle. *Theriogenology* 2000; **53**: 1783-1795.
52. **Roussel JD, Clement TJ, Aranas TJ, Seybt SH.** Changes in circulating plasma levels of cortisol in lactating and non-lactating dairy cattle during the estrous cycle. *Theriogenology* 1983; **19**: 535-539.
53. **Kushnir MM, Neilson R, Roberts WL, Rockwood AL.** Cortisol and cortisone analysis in serum and plasma by atmospheric pressure photoionization tandem mass spectrometry. *Clin Biochem* 2004; **37**: 357-362.
54. **Yang K, Smith CL, Dales D, Hammond GL, Challis JRG.** Cloning of an ovine 11β-hydroxysteroid dehydrogenase complementary deoxyribonucleic-acid - tissue and temporal distribution of its messenger-ribonucleic-acid during fetal and neonatal development. *Endocrinology* 1992; **131**: 2120-2126.
55. **Simmons RM, Satterfield MC, Welsh TH, Jr., Bazer FW, Spencer TE.** HSD11B1, HSD11B2, PTGS2, and NR3C1 expression in the peri-implantation ovine uterus: effects of pregnancy, progesterone, and interferon tau. *Biol Reprod* 2010; **82**: 35-43.
56. **Yang K, Fraser M, Yu M, Krkosek M, Challis JR, Lamming GE, Campbell LE, Darnel A.** Pattern of 11β-hydroxysteroid dehydrogenase type 1 messenger ribonucleic acid expression in the ovine uterus during the estrous cycle and pregnancy. *Biol Reprod* 1996; **55**: 1231-1236.
57. **Vermani K, Garg S.** The scope and potential of vaginal drug delivery. *Pharm Sci Technolo Today* 2000; **3**: 359-364.
58. **Nyende L, Towobola OA, Mabina MH.** Comparison of vaginal and oral misoprostol, for the induction of labour in women with intra-uterine foetal death. *East Afr Med J* 2004; **81**: 179-182.

59. **Baloglu E, Senyigit ZA, Karavana SY, Bernkop-Schnurch A.** Strategies to prolong the intravaginal residence time of drug delivery systems. *J Pharm Pharm Sci* 2009; **12**: 312-336.

60. **Norman TR, Morse CA, Dennerstein L.** Comparative bioavailability of orally and vaginally administered progesterone. *Fertil Steril* 1991; **56**: 1034-1039.

61. **Pasquale SA, Foldesy RG, Levine JP, Bachmann GA, Blackwell RE.** Peripheral progesterone (P) levels and endometrial response to various dosages of vaginally administered P in estrogen-primed women. *Fertil Steril* 1997; **68**: 810-815.

62. **Miles RA, Paulson RJ, Lobo RA, Press MF, Dahmoush L, Sauer MV.** Pharmacokinetics and endometrial tissuel levels of progesterone after administration by intramuscular and vaginal routes - a comparative-study. *Fertil steril* 1994; **62**: 485-490.

63. **Ross D, Cooper AJ, PryseDavies J, Bergeron C, Collins WP, Whitehead MI.** Randomized, double-blind, dose-ranging study of the endometrial effects of a vaginal progesterone gel in estrogen-treated postmenopausal women. *Am J Obstet Gynecol* 1997; **177**: 937-941.

64. **Levy T, Yairi Y, Bar-Hava I, Shalev J, Orvieto R, Ben-Rafael Z.** Pharmacokinetics of the progesterone-containing vaginal tablet and its use in assisted reproduction. *Steroids* 2000; **65**: 645-649.

65. **Bulletti C, deZiegler D, Giacomucci E, Polli V, Rossi S, Alfieri S, Flamigni C.** Vaginal drug delivery: The first uterine pass effect. In: Bulletti C, deZiegler D, Guller S, Levitz M, (eds), Uterus: Endometrium and Myometrium, Ann N Y Acad Sci. 1997: 285-290.

66. **Devroey P, Palermo G, Bourgain C, Van Waesberghe L, Smitz J, Van Steirteghem AC.** Progesterone administration in patients with absent ovaries. *Int J Fertil* 1989; **34**: 188-193.

67. **Alfaidy N, Xiong ZG, Myatt L, Lye SJ, MacDonald JF, Challis JR.** Prostaglandin $F_{2\alpha}$ potentiates cortisol production by stimulating 11β-hydroxysteroid dehydrogenase 1: a novel feedback loop that may contribute to human labor. *J Clin Endocrinol Metab* 2001; **86**: 5585-5592.

68. **Sun K, Ma RL, Cui XL, Campos B, Webster R, Brockman D, Myatt L.** Glucocorticoids induce cytosolic phospholipase A2 and prostaglandin H synthase type 2 but not microsomal prostaglandin E synthase (PGES) and cytosolic PGES expression in cultured primary human amnion cells. *J Clin Endocrinol Metab* 2003; **88**: 5564-5571.

69. **Mirazi N, Alfaidy N, Martin R, Challis JR.** Effects of dexamethasone and sulfasalazine on prostaglandin E_2 output by human placental cells in vitro. *J Soc Gynecol Investig* 2004; **11**: 22-26.

70. **Zhang Q, Collins V, Chakrabarty K, Wolf RF, Unno N, Howe D, Rose JC, Wu WX.** Regulation of membrane-associated prostaglandin E_2 synthase 1 in pregnant sheep intrauterine tissues by glucocorticoid and estradiol. *Endocrinology* 2006; **147**: 3719-3726.

71. **Alfaidy N, Li W, MacIntosh T, Yang K, Challis J.** Late gestation increase in 11β-hydroxysteroid dehydrogenase 1 expression in human fetal membranes: a novel intrauterine source of cortisol. *J Clin Endocrinol Metab* 2003; **88**: 5033-5038.

72. **Murakami S, Miyamoto Y, Skarzynski DJ, Okuda K.** Effects of tumor necrosis factor α on secretion of prostaglandins E_2 and $F_{2\alpha}$ in bovine endometrium throughout the estrous cycle. *Theriogenology* 2001; **55**: 1667-1678.

73. **Miyamoto Y, Skarzynski DJ, Okuda K.** Is tumor necrosis factor α a trigger for the initiation of endometrial prostaglandin $F_{2\alpha}$ release at luteolysis in cattle? *Biol Reprod* 2000; **62**: 1109-1115.

74. **Duong HT, Piotrowska-Tomala KK, Acosta TJ, Bah MM, Sinderewicz E, Majewska M, Jankowska K, Okuda K, Skarzynski DJ.** Effects of cortisol on pregnancy rate and corpus luteum function in heifers: An *in vivo* study. *J Reprod Dev* 2012; **58**: 223-230.

75. **Okuda K, Miyamoto Y, Skarzynski DJ.** Regulation of endometrial prostaglandin $F_{2\alpha}$ synthesis during luteolysis and early pregnancy in cattle. *Domest Anim Endocrinol* 2002; **23**: 255-264.

76. **Ginther OJ, Beg MA.** Concentrations of circulating hormones normalized to pulses of a prostaglandin $F_{2\alpha}$ metabolite during spontaneous luteolysis in mares. *Theriogenology* 2009; **72**: 1111-1119.

77. **Ginther OJ, Shrestha HK, Fuenzalida MJ, Shahiduzzaman AK, Beg MA.** Characteristics of pulses of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ before, during, and after spontaneous luteolysis and temporal intrapulse relationships with progesterone concentrations in cattle. *Biol Reprod* 2010; **82**: 1049-1056.

78. **Kotwica J, Skarzynski D, Jaroszewski J, Williams GL, Bogacki M.** Uterine secretion of prostaglandin $F_{2\alpha}$ stimulated by different doses of oxytocin and released spontaneously during luteolysis in cattle. *Reprod Nutr Dev* 1998; **38**: 217-226.

79. **Parkinson TJ, Lamming GE.** Interrelationships between progesterone, 13,14-dihydro-15-keto $F_{2\alpha}$ (PGFM) and LH in cyclic and early pregnant cows. *J Reprod Fertil* 1990; **90**: 221-233.

80. **Bigsby RM, Everett LM.** Effects of progestin antagonists, glucocorticoids and estrogen on progesterone-induced protein secreted by rabbit endometrial stromal cells in culture. *J Steroid Biochem Mol Biol* 1991; **39**: 27-32.

81. **Gupta S, Gyomorey S, Lye SJ, Gibb W, Challis JR.** Effect of labor on glucocorticoid receptor (GR(Total), GRα , and GRβ) proteins in ovine intrauterine tissues. *J Soc Gynecol Investig* 2003; **10**: 136-144.

82. **Gellersen B, Kempf R, Telgmann R, Dimattia GE.** Nonpituitary human prolactin gene-transcription is independent of pit-1 and differentially controlled in lymphocytes and in endometrial stroma. *Mol Endocrinol* 1994; **8**: 356-373.

83. **Funder JW.** Mineralocorticoids, glucocorticoids, receptors and response elements. *Science* 1993; **259**: 1132-1133.

84. **Giguere V, Hollenberg SM, Rosenfeld MG, Evans RM.** Functional domains of the human glucocorticoid receptor. *Cell* 1986; **46**: 645-652.

85. **Stewart PM, Mason JI.** Cortisol to cortisone: glucocorticoid to mineralocorticoid. *Steroids* 1995; **60**: 143-146.

86. **Albiston AL, Obeyesekere VR, Smith RE, Krozowski ZS.** Cloning and tissue distribution of the human 11β-hydroxysteroid dehydrogenase type 2 enzyme. *Mol Cell Endocrinol* 1994; **105**: R11-17.

87. **Tetsuka M, Yamamoto S, Hayashida N, Hayashi KG, Hayashi M, Acosta TJ, Miyamoto A.** Expression of 11β-hydroxysteroid dehydrogenases in bovine follicle and corpus luteum. *J Endocrinol* 2003; **177**: 445-452.

88. **Majewska M, Lee HY, Tasaki Y, Acosta TJ, Szostek AZ, Siemieniuch M, Okuda K, Skarzynski DJ.** Is cortisol a modulator of interferon tau action in the endometrium during early pregnancy in cattle? *J Reprod Immunol* 2012; **93**: 82-93.
89. **Stoebel DP, Moberg GP.** Effect of adrenocorticotropin and cortisol on luteinizing-hormone surge and estrous behavior of cows. *J Dairy Sci* 1982; **65**: 1016-1024.
90. **Broussard JR, Rocha A, Sirois J, Roussel JD, Thibodeaux JK, Godke RA, Hansel W.** Effects of dexamethasone administration to diestrus cows on systemic progesterone, estrogen and uterine cyclooxygenase production. *Anim Reprod Sci* 1997; **47**: 263-271.
91. **Maciel SM, Chamberlain CS, Wettemann RP, Spicer LJ.** Dexamethasone influences endocrine and ovarian function in dairy cattle. *J Dairy Sci* 2001; **84**: 1998-2009.
92. **Sampath-Kumar R, Yu M, Khalil MW, Yang K.** Metyrapone is a competitive inhibitor of 11β-hydroxysteroid dehydrogenase type 1 reductase. *J Steroid Biochem Mol Biol* 1997; **62**: 195-199.
93. **Lye SJ, Challis JR.** In vivo adrenocorticotropin (1-24)-induced accumulation of cyclic adenosine monophosphate by ovine fetal adrenal cells is inhibited by concomitant infusion of metopirone. *Endocrinology* 1984; **115**: 1584-1587.
94. **Sejian V, Srivastava RS.** Effects of melatonin on adrenal cortical functions of indian goats under thermal stress. *Vet Med Int* 2010: doi: 10.4061/2010/348919.
95. **Young EA, Lopez JF, MurphyWeinberg V, Watson SJ, Akil H.** Normal pituitary response to metyrapone in the morning in depressed patients: Implications for circadian regulation of corticotropin-releasing hormone secretion. *Biol Psychiatry* 1997; **41**: 1149-1155.
96. **Broadley AJM, Korszun A, Abdelaal E, Moskvina V, Jones CJH, Nash GB, Ray C, Deanfield J, Frenneaux MP.** Inhibition of cortisol production with metyrapone prevents mental stress-induced endothelial dysfunction and baroreflex impairment. *J Am Coll Cardiol* 2005; **46**: 344-350.
97. **Thomson LM, Raven PW, Smith KE, Hinson JP.** Effects of metyrapone on hepatic cortisone-cortisol conversion in the rat. *Endocr Res* 1998; **24**: 607-611.

98. **Siemieniuch MI, Majewska M, Takahashi M, Sakatani M, Lukasik K, Okuda K, Skarzynski DJ.** Are glucocorticoids auto- and/or paracrine factors in early bovine embryo development and implantation? *Reprod Biol* 2010; **10**: 249-256.

99. **Skarzynski DJ, Bah MM, Deptula KM, Woclawek-Potocka I, Korzekwa A, Shibaya M, Pilawski W, Okuda K.** Roles of tumor necrosis factor α of the estrous cycle in cattle: an in vivo study. *Biol Reprod* 2003; **69**: 1907-1913.

100. **Bazer FW, Ott TL, Spencer TE.** Endocrinology of the transition from recurring estrous cycles to establishment of pregnancy in subprimate mammals. In: Bazer FW, (ed), Endocrinology of Pregnancy. Totowa: Humana Press; 1998: 1-34.

101. **Godkin JD, Bazer FW, Moffatt J, Sessions F, Roberts RM.** Purification and properties of a major, low molecular weight protein released by the trophoblast of sheep blastocysts at day 13-21. *J Reprod Fertil* 1982; **65**: 141-150.

102. **Spencer TE, Burghardt RC, Johnson GA, Bazer FW.** Conceptus signals for establishment and maintenance of pregnancy. *Anim Reprod Sci* 2004; **82-3**: 537-550.

103. **Farin CE, Imakawa K, Hansen TR, McDonnell JJ, Murphy CN, Farin PW, Roberts RM.** Expression of trophoblastic interferon genes in sheep and cattle. *Biol Reprod* 1990; **43**: 210-218.

104. **Xiao CW, Murphy BD, Sirois J, Goff AK.** Down-regulation of oxytocin-induced cyclooxygenase-2 and prostaglandin F synthase expression by interferon-tau in bovine endometrial cells. *Biol Reprod* 1999; **60**: 656-663.

105. **Parent J, Chapdelaine P, Sirois J, Fortier MA.** Expression of microsomal prostaglandin E synthase in bovine endometrium: Coexpression with cyclooxygenase type 2 and regulation by interferon-tau. *Endocrinology* 2002; **143**: 2936-2943.

106. **McCracken JA, Glew ME, Scaramuzzi RJ.** Corpus luteum regression induced by prostaglandin $F_{2\alpha}$. *J Clin Endocrinol Metab* 1970; **30**: 544-546.

107. **Shelton K, Parkinson TJ, Hunter MG, Kelly RW, Lamming GE.** Prostaglandin E_2 as a potential luteotrophic agent during early-pregnancy in cattle. *J Reprod Fertil* 1990; **90**: 11-17.

108. **Arosh JA, Banu SK, Kimmins S, Chapdelaine P, MacLaren LA, Fortier MA.** Effect of interferon-tau on prostaglandin biosynthesis, transport, and signaling at

the time of maternal recognition of pregnancy in cattle: Evidence of polycrine actions of prostaglandin E_2. *Endocrinology* 2004; **145**: 5280-5293.

109. **Weems YS, Bridges PJ, Sasser RG, Ching L, LeaMaster BR, Vincent DL, Weems CW.** Effect of mifepristone on pregnancy, pregnancy-specific protein B, progesterone, estradiol-17β, prostaglandin $F_{2\alpha}$ and prostaglandin E in ovariectomized 90-day pregnant ewes. *Prostag Oth Lipid M* 2002; **70**: 195-208.
110. **Mann GE, Lamming GE, Fisher PA.** Progesterone control of embryonic interferon-tau production during early pregnancy in the cow *J Reprod Fertil* 1998; **21**: 37
111. **Mann GE, Mann SJ, Lamming GE.** The interrelationship between the maternal hormone environment and the embryo during the early stages of pregnancy *J Reprod Fertil* 1996; **17**: 55-61.
112. **Boomsma CM, Keay SD, Macklon NS.** Peri-implantation glucocorticoid administration for assisted reproductive technology cycles. *Cochrane Database Syst Rev* 2007: CD005996.
113. **Michael AE, Papageorghiou AT.** Potential significance of physiological and pharmacological glucocorticoids in early pregnancy. *Hum Reprod Update* 2008; **14**: 497-517.
114. **Spicer LJ, Chamberlain CS.** Influence of cortisol on insulin- and insulin-like growth factor 1 (IGF-1)-induced steroid production and on IGF-1 receptors in cultured bovine granulosa cells and thecal cells. *Endocrine* 1998; **9**: 153-161.
115. **Padmanabhan V, Keech C, Convey EM.** Cortisol inhibits and adrenocorticotropin has no effect on luteinizing hormone-releasing hormone-induced release of luteinizing hormone from bovine pituitary cells in vitro. *Endocrinology* 1983; **112**: 1782-1787.
116. **Asselin E, Drolet P, Fortier MA.** In vitro response to oxytocin and interferon-tau in bovine endometrial cells from caruncular and inter-caruncular areas. *Biol Reprod* 1998; **59**: 241-247.

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