

## Hormones, IgG and lactose changes around parturition in plasma, and colostrum or saliva of multiparous sows

Nicolas DEVILLERS<sup>a</sup>, Chantal FARMER<sup>b</sup>, Anne-Marie MOUNIER<sup>a</sup>,  
Jean LE DIVIDICH<sup>a</sup>, Armelle PRUNIER<sup>a,\*</sup>

<sup>a</sup> Institut National de la Recherche Agronomique, Unité Mixte de Recherche sur le Veau et le Porc, 35590 Saint-Gilles, France

<sup>b</sup> Agriculture and Agri-Food Canada, Dairy and Swine R & D Center, PO Box 90, Lennoxville, Québec, Canada J1M 1Z3

(Received 25 January 2004; accepted 3 May 2004)

**Abstract** – Blood, colostrum and saliva samples were serially taken from 6 multiparous sows from day 109 of gestation until day 3 postpartum. Plasma was assayed for oestradiol-17 $\beta$  (E2), progesterone (P4), prolactin (PRL), cortisol, immunoglobulin G (IgG) and lactose. Colostrum was assayed for E2, P4, IgG and lactose. Lactoserum, obtained after ultra centrifugation of colostrum, was assayed for PRL. Saliva was assayed for cortisol. Time-related variations in hormone, IgG and lactose concentrations measured in plasma were parallel to those measured in colostrum, lactoserum or saliva. However, the concentrations were higher in colostrum or lactoserum and lower in saliva than in plasma. Ratios of concentrations of cortisol in saliva and PRL in lactoserum over those in plasma did not vary with time and averaged 0.2 and 1.6, respectively. Conversely, the ratios of concentrations of E2 and P4 in colostrum over those in plasma varied with time ( $P < 0.05$ ) but were quite constant before the end of parturition, averaging 2.7 and 3.6, respectively. The ratios of concentrations of IgG and lactose in colostrum over those in plasma also varied with time ( $P < 0.05$ ). The concentrations of hormones in plasma on the one hand and in colostrum, lactoserum or saliva on the other hand were significantly correlated but correlations varied with time (PRL across periods:  $r = 0.31$ ; cortisol across periods:  $r = 0.60$ ; E2 during parturition:  $r = 0.83$ ; P4 before parturition:  $r = 0.82$ ; P4 during parturition:  $r = 0.67$ ). The present results indicate that around parturition, assays of hormones in colostrum or saliva can be used to study the hormonal status of sows. Furthermore, variations in colostrum and plasma concentrations of IgG and lactose are good indicators of the transition from colostrum to milk synthesis.

sow / parturition / steroid / prolactin / lactose / immunoglobulins

### 1. INTRODUCTION

Hormonal variations around parturition are implicated in the onset of lactation. The roles of hormones such as progesterone (P4), oestradiol-17 $\beta$  (E2), cortisol and prolactin (PRL) on the initiation of lactation

and colostrum production have been demonstrated [1, 2]. The withdrawal of P4 before parturition acts as a trigger for a succession of hormonal changes leading to farrowing and to colostrum and milk production [3–5]. This fall in P4 allows PRL secretion to increase while E2 and cortisol amplify the

---

\* Corresponding author: Armelle.Prunier@rennes.inra.fr

stimulatory action of PRL on the mammary glands [2]. Indeed, the PRL surge at parturition is necessary for the onset of lactation [6, 7].

Colostrum synthesis in the mammary glands begins before parturition [5, 8]. During the last month of gestation, tight junctions between secretory cells of the mammary glands are open, thereby enabling the massive transfer of immunoglobulins (Ig) into the colostrum and the transfer of lactose from colostrum to plasma. Closure of tight junctions is one of the main events which indicates the transition from colostrum to milk production [5, 9]. It can be depicted from variations of concentrations of Ig and lactose in colostrum and plasma. This closure occurs between 24 and 36 h after the onset of parturition and is under the control of P4, cortisol and PRL [10, 11].

Hormones implicated in the control of gestation, parturition and lactation are present in colostrum and milk [12]. Assays of hormones in fluids such as urine, saliva or milk are an alternative to invasive blood collection and could be used for measuring hormone variations around parturition. Indeed, E2 and PRL have been assayed in sow milk [13, 14], and milk P4 is currently used for gestation diagnosis in ruminants [15, 16]. The aim of the present study was to determine patterns of variation of P4, PRL, E2, cortisol, lactose and IgG in plasma and to compare them to variations in colostrum/lactoserum or saliva around parturition in sows.

## 2. MATERIALS AND METHODS

### 2.1. Animals

Eight Large White  $\times$  Landrace multiparous sows were housed in individual farrowing crates (2  $\times$  2.5 m) with straw bedding. During pregnancy, the sows were fed daily 1.1 kg  $\cdot$  100 kg<sup>-1</sup> live weight of a diet containing 13.3% crude proteins, 3090 kcal of digestible energy (DE) per kg and 0.49%

lysine. Throughout lactation, they were fed a diet providing 17.3% crude proteins, 3190 kcal of DE per kg and 0.8% lysine. On the day of farrowing, the sows were offered 2.0 kg of the lactation diet. Thereafter, this amount was increased by 500 g daily until ad libitum feeding. Water was supplied ad libitum throughout the experiment. Water intake and rectal temperature were recorded daily from day 109 of gestation until day 3 postpartum.

On day 108 of gestation, an indwelling Silastic<sup>®</sup> catheter was surgically inserted into the right jugular vein of all sows under general anaesthesia [17]. Parturition was not induced and only one sow (No. 56) received 5 mg serotonin (Sergotonine<sup>®</sup>, Merial, Lyon, France) after the birth of her 11th piglet because of farrowing difficulties.

Time and weight at birth were recorded for all piglets. The start of parturition (T0) was defined as the moment when the first piglet was born. At one and two weeks of age, the piglets were again weighed. Litter size was recorded at farrowing, 24 h after the birth of the first piglet (T24) and two weeks later (Tab. I). Four piglets were removed only from one sow (No. 55) on day 4 postpartum since the number of pigs was higher than the number of functional teats.

Colostrum production during the first 24 h following parturition was estimated according to a previously described method which predicts piglets' colostrum intake from their birth weight and their weight gain [18]. Two sows were excluded from the analyses since they farrowed on day 111 of gestation and numerous samples were missing.

### 2.2. Sampling

Sampling volumes were 10 mL for blood, 1–2 mL for saliva, and 10–30 mL for colostrum. In order to establish the collection schedule, a "pre-farrowing" phase was defined. It started when colostrum could be easily expressed manually from the teats (this was

**Table I.** Reproductive performance of sows and piglets growth.

Sow	51	52	55	56	57	58
Parity	4	2	4	5	2	6
Body weight on day 107 of gestation (kg)	292	263	268	274	261	292
Farrowing duration (h:min) <sup>1</sup>	5:05	12:15	5:21	17:20	7:50	3:20
Number of piglets born	15	15	17	12	17	9
Number of stillbirth piglets	1	3	0	1	5	1
Litter size at T24	14	12	17	10	11	8
Litter size at day 15 post-partum	13	9	11	10	9	8
Colostrum production during the first 24 h (kg)	2.86	4.13	2.85	3.77	2.61	3.36
Piglet birth weight (kg) <sup>2</sup>	1.16 ± 0.06	1.24 ± 0.07	1.12 ± 0.05	1.25 ± 0.11	1.43 ± 0.07	1.71 ± 0.08
Piglet body weight at T24 (kg) <sup>2</sup>	1.27 ± 0.06	1.47 ± 0.07	1.17 ± 0.05	1.50 ± 0.09	1.52 ± 0.09	1.83 ± 0.10
Piglet body weight one week after birth (kg) <sup>2</sup>	1.94 ± 0.07	2.48 ± 0.12	2.44 ± 0.12	2.39 ± 0.17	2.38 ± 0.14	2.84 ± 0.13

<sup>1</sup> Calculated as the time elapsed between birth of the first and last piglet.

<sup>2</sup> Litter mean ± SEM.

further calculated to be 8 h before the onset of parturition on average). From day 109 of gestation, 2 blood samples were collected daily at 9.30 and 21.30. Starting on day 113 of gestation, 2 additional blood samples were collected at 3.30 and 15.30. From the start of the “pre-farrowing” phase until 12 h after the beginning of parturition (T12), blood samples were collected at hourly intervals. Between T12 and T60, blood was again collected at 3.30, 9.30, 15.30 and 21.30. The time schedule for colostrum collection was similar to that for blood except that samples were collected every 2 h (instead of hourly) from the start of the “pre-farrowing” phase until T12. Saliva was collected at 3.30, 9.30, 15.30 and 21.30 from day 113 of gestation until T48.

Blood samples were collected in heparinized tubes, placed immediately on ice and centrifuged for 10 min at 3000 g and 4 °C. Saliva was collected on a cotton pad which was thereafter inserted in a tube (Salivette, Sarstedt 51588 Nümbrecht, Germany) that

was centrifuged for 5 min at 3000 g and 4 °C. Colostrum was collected from all teats without the use of oxytocin, filtered on a gauze tissue and stored at 4 °C. The trial of colostrum collection was limited to 15 min. After T12, collection of colostrum became more difficult and numerous samples were missing. Within 24 h of collection, a 5-mL colostrum sample was warmed at 38 °C, homogenized and ultra-centrifuged twice at 50 000 g and 0 °C over 1 h before lactoserum collection. All samples were stored at –20 °C.

### 2.3. Sample analyses

Prolactin concentrations in plasma and lactoserum were determined by a validated homologous double-antibody radioimmunoassay [19]. Assay sensitivity, estimated as 90% of total binding, was 1.8 ng·mL<sup>-1</sup>. The intra- and interassay coefficients of variation were 3.5 and 2.9%, respectively, for a plasma containing 50 ng·mL<sup>-1</sup> and

were 6.9 and 6.4%, respectively, for a lactoserum containing 24 ng·mL<sup>-1</sup>.

Progesterone, E2 and cortisol concentrations were determined with commercial kits (P4, E2 and cortisol in plasma and colostrum: Immunotech RIA kit, 13276 Marseille, France, Ref. 2465, 2464 and 2466, respectively; cortisol in saliva: Diasorin RIA kit, Minnesota, USA, Ref. CA-1529). One milliliter of colostrum was previously homogenized with ultrasound for 5 to 15 s. For P4 measurement in colostrum, average recovery after the addition of known amounts of the hormone (1.8, 4.4, 8.8 and 22 ng·mL<sup>-1</sup>) was 107% and assay sensitivity was 0.6 ng·mL<sup>-1</sup>. For E2 measurement in colostrum, average recovery after the addition of known amounts of the hormone (44, 88, 200, 440, 1100 pg·mL<sup>-1</sup>) was 104 % and assay sensitivity was 26 pg·mL<sup>-1</sup>. The intra- and inter-assay coefficients of variation for P4 were 4.9 and 14.3%, respectively, for a colostrum containing 9.9 ng·mL<sup>-1</sup>. The intra- and inter-assay coefficients of variation for E2 were 1.7 and 4.6%, respectively, for a colostrum containing 366 pg·mL<sup>-1</sup>.

For the cortisol measurement in saliva, average recovery after the addition of known amounts of the hormone (0.5, 1.5, 5, 12.5, 30 and 90 ng·mL<sup>-1</sup>) was 101% and the intra-assay coefficient of variation was 8.1% at 12 ng·mL<sup>-1</sup>. All saliva samples were measured in a single assay.

For measurements in plasma, assay sensitivities were 1.4 ng·mL<sup>-1</sup>, 16 pg·mL<sup>-1</sup> and 1.9 ng·mL<sup>-1</sup> for P4, E2 and cortisol, respectively. The intra-assay coefficient of variation was 7.8% at 12 ng P4·mL<sup>-1</sup>, 3.0% at 473 pg E2·mL<sup>-1</sup> and 5.5% at 69 ng cortisol·mL<sup>-1</sup>. Measurements were realized in single assays.

Immunoglobulins G were assayed by ELISA in whole colostrum using a pig IgG ELISA Quantitation Kit (Bethyl, Texas, USA, Ref. E100-104). The plates were coated overnight at 4 °C with 100 µL of goat anti-pig IgG-Fc fragment diluted at 1% in 0.05 M sodium carbonate solution (pH 9.6, Sigma, St-Louis, USA, Ref. S2127). Thereafter, the

plates were saturated for 1 h at 20 °C with TBS (Sigma, Ref. T6664, St-Louis, USA) containing 1% BSA (Sigma, Ref. A7906). Colostrum samples were diluted at 1/10<sup>6</sup> in TBS with 0.05% Tween 20 (Sigma, Ref. P1379) and 1% BSA, added in duplicates to the plates (200 µL·well<sup>-1</sup>) and incubated for 2 h at 20 °C. The plates were subsequently incubated for 2 h at 20 °C with peroxidase-labeled anti-pig IgG-Fc fragment and 100 µL of substrate (3,3',5,5'-tetramethylbenzidine, Sigma, Ref. T4444). Between each step, plates were washed at least three times with TBS containing 0.05% Tween 20. The colored reaction was stopped with 100 µL of a 3 M H<sub>2</sub>SO<sub>4</sub> solution and the absorbancy at 450 nm was recorded using an ELISA plate reader (ARGUS 300, Packard, Meriden, USA). Average recoveries were 111% and 103% for plasma and colostrum, respectively. Assay sensitivity was 23 ng·mL<sup>-1</sup>. Dilution curves of plasma (1/25 000 to 1/200 000) or colostrum (1/150 000 to 1/2 400 000) and standard curves were parallel. The intra- and inter-assay coefficients of variation were 5.3% and 15.7%, respectively, for a plasma containing 11 mg·mL<sup>-1</sup> and were 2.6% and 5.8%, respectively, for a colostrum containing 67 mg·mL<sup>-1</sup>.

Nitrogen (N) was determined according to the method of Dumas (AOAC 7 024, 1831) based on sample pyrolysis and direct determination of N<sub>2</sub> using an automatic device (Leco FP-428, LECO Corporation, St-Joseph, USA). Crude proteins were estimated to be N × 6.38 [20]. Total lipids were measured according to the Gerber method (AOAC 1990 [21]).

Lactose in colostrum was assayed using an enzymatic method (Lactose/D-galactose test combination, R-Biopharm – Roche, Darmstadt, Germany, Ref. 0176303). Lactose in plasma was assayed with the same commercial kit after deproteinization with perchloric acid and neutralization with potassium hydroxide according to the method described by the manufacturer. The sensitivity of the assay was 4 µg lactose·mL<sup>-1</sup>.

The intraassay coefficient of variation was 3% for a plasma containing  $96 \mu\text{g}\cdot\text{mL}^{-1}$ .

#### 2.4. Data processing and statistics

Individual profiles of plasma E2, PRL and cortisol, and salivary cortisol were analyzed according to the method described by Merriam and Wachter [22]. The baseline was evaluated at each point using a weighted regression calculated on the 72 h period surrounding the considered point. A spike was identified when the measured concentrations differed from the baseline at the 1, 0.5 and 0.05% statistical levels for PRL, E2 and cortisol, respectively. Plasma profiles of P4 and IgG did not present spikes but only falls of concentrations whereas plasma profiles of lactose mostly presented rises. To determine the time, amplitude and duration of the falls or rises, a pre-farrowing baseline level was calculated from the concentrations measured at least 72 h before parturition. A post-farrowing level was also calculated from the concentrations measured after T24. Root mean square error (MSE) was calculated for those baseline levels and used to detect concentrations differing at the 5% statistical level. The duration of colostrum sampling was not sufficient to analyze the characteristics of the profiles.

The Pearson Correlation procedure of SAS [23] was used to determine the correlations between the concentrations in plasma, colostrum, lactoserum or saliva. Variations over time were studied by analyses of variance with the GLM procedure of SAS followed by the Dunnett test.

### 3. RESULTS

#### 3.1. Sow performances

Parturition occurred between days 112 and 114 of gestation. Litter size at birth ranged from 9 to 17 with 0 to 5 stillbirth piglets (Tab. I). Farrowing duration ranged from 3.3 h for sow 58 to 17.3 h for sow 56, whose 11 first piglets were extracted manually in 9 h and the 12th piglet was born naturally

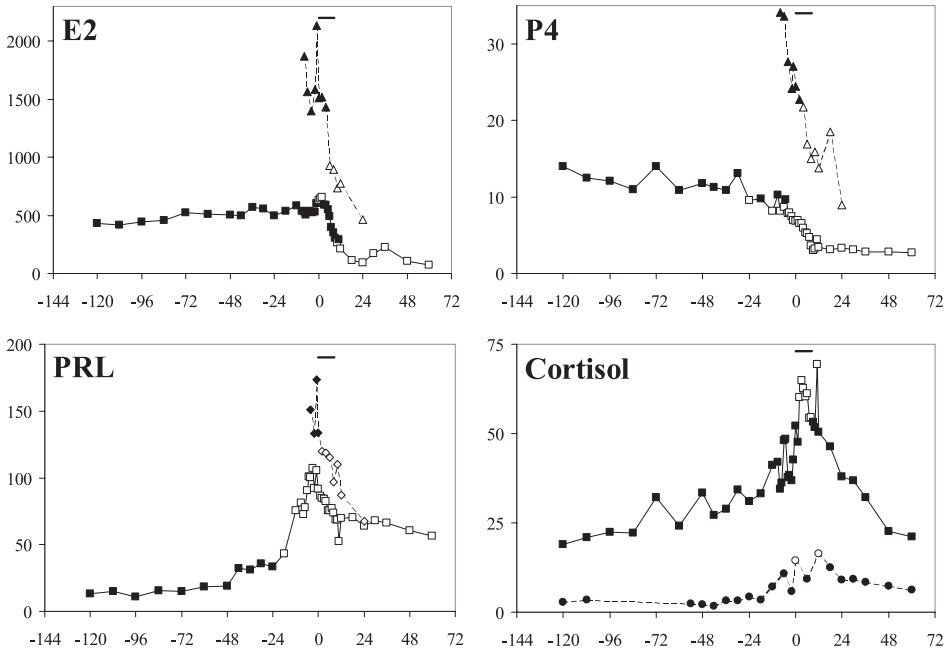
8.3 h later. Significant variations with time were depicted for water intake ( $P < 0.01$ ) and rectal temperature ( $P < 0.001$ ). Water intake was higher on day -1 ( $20.3 \pm 3.7$  L) than before (average daily intake for days -5 to -3:  $11.9 \pm 0.9$  L,  $P < 0.05$ ). Rectal temperature was higher on day 1 ( $38.5 \pm 0.10$  °C) and day 2 ( $38.9 \pm 0.35$  °C) than before (days -5 to -3:  $37.5$  °C  $\pm 0.18$  °C,  $P < 0.05$ ).

#### 3.2. Hormones: oestradiol, progesterone, prolactin and cortisol

There were significant variations with time for all hormones measured either in plasma, colostrum, lactoserum or saliva ( $P < 0.05$  for cortisol in saliva,  $P < 0.0001$  for all other hormonal measurements). Plasma concentrations of E2 were already high at T-120 and continued to increase achieving peak values just before the birth of the first piglet (Fig. 1). They started to decline after parturition and decreased until T24. The values from T10 to T60 were lower than the values measured prior to T-72 ( $P < 0.05$ , Fig. 1). Individual plasma patterns differed between sows, with the number of spikes ranging from 1 (sows 52, 55, 57) to 3 (sow 58). Concentrations of E2 in the colostrum varied in parallel with plasma values even though the decrease after parturition occurred slightly earlier and was more pronounced.

Plasma progesterone decreased slowly between T-120 and T-19 and more rapidly until T12 (Fig. 1). From T-3 until the end of the experiment, concentrations were continuously lower than those measured prior to T-72 ( $P < 0.05$ ). Progesterone concentrations in colostrum also showed a significant drop around parturition.

Plasma PRL concentrations were low until T-48 and increased slowly thereafter. Concentrations from T-18 until the end of the experiment were higher than those measured prior to T-72 ( $P < 0.05$ , Fig. 1). Peak values were reached shortly after the start of parturition (T6 in average). Numerous spikes ( $3.3 \pm 0.2$  per sow) were identified



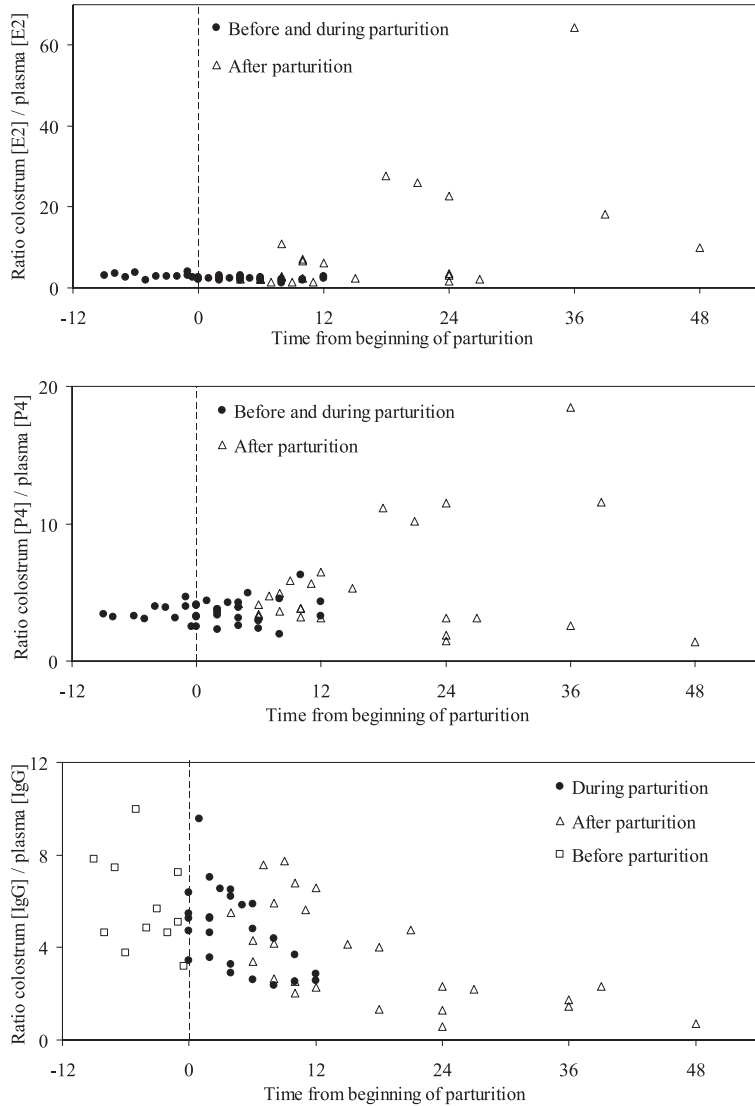
**Figure 1.** Mean concentrations of oestradiol-17 $\beta$  (E2, pg·mL<sup>-1</sup>), progesterone (P4, ng·mL<sup>-1</sup>), prolactin (PRL, ng·mL<sup>-1</sup>) and cortisol (ng·mL<sup>-1</sup>) in plasma (square symbols: ■ or □), colostrum (triangle symbols: ▲ or △), lactoserum (diamond symbol: ◆ or ◇) and saliva (round symbol: ● or ○) around parturition (h). Mean farrowing duration is represented by the black horizontal bars. Empty symbols (□, △, ◇ or ○) represent values that differ ( $P < 0.05$ ) from mean concentrations calculated with values prior to T-72 for plasma and saliva or prior to T0 for colostrum and lactoserum. Standard errors: E2, plasma: 112 pg·mL<sup>-1</sup>; colostrum: 374 pg·mL<sup>-1</sup>. P4, plasma: 1.5 ng·mL<sup>-1</sup>; colostrum: 3.9 ng·mL<sup>-1</sup>. PRL, plasma: 14.7 ng·mL<sup>-1</sup>; lactoserum: 18.4 ng·mL<sup>-1</sup>. Cortisol, plasma: 21.1 ng·mL<sup>-1</sup>; saliva: 4.8 ng·mL<sup>-1</sup>.

between T-69 and T48 and their number varied from 2 (sow 56) to 5 (sow 51). Concentrations of PRL in lactoserum were also the highest just before parturition and declined rapidly thereafter in parallel to those measured in plasma (Fig. 1).

Plasma concentrations of cortisol increased slowly from T-120 to T-24 and rapidly thereafter, reaching peak values during parturition (Fig. 1). Numerous spikes ( $4.7 \pm 0.3$  per sow) were present between T-56 and T48 and their number varied from 2 (sow 56) to 7 (sows 55, 58). Variations in concentrations of cortisol in saliva paralleled those in plasma, with peak values being seen during parturition.

Across sows, plasma concentrations of P4 and E2 were negatively correlated before parturition ( $r = -0.44$ ,  $P < 0.001$ ,  $n = 90$ ). Similarly, plasma P4 and PRL were negatively linked before parturition ( $r = -0.56$ ,  $P < 0.001$ ,  $n = 90$ ). Progesterone and oestradiol concentrations in colostrum were not significantly correlated with the colostrum lipid level ( $P > 0.1$ ).

Mean ratios of concentrations measured in colostrum/lactoserum (or saliva) and plasma did not vary with time ( $P > 0.1$ ) for PRL ( $1.6 \pm 0.6$ ) or cortisol ( $0.2 \pm 0.2$ ), but varied with time for E2 ( $P < 0.0001$ ) and P4 ( $P < 0.01$ , Fig. 2). This ratio was lower and less variable before and during parturition



**Figure 2.** Ratios of concentrations of oestradiol (E2), progesterone (P4) and immunoglobulin G (IgG) in colostrum on those in plasma upon time (h) around parturition. Vertical dashed lines indicate the beginning of parturition.

(E2:  $2.5 \pm 0.1$ , P4:  $3.6 \pm 0.1$ ) than after parturition (E2:  $9.6 \pm 2.9$ , P4:  $5.5 \pm 0.8$ ).

Correlations between concentrations measured in plasma vs. colostrum, lactoserum, or saliva were examined across sows (Tab. II). For cortisol and PRL, the correlations were

significant across periods (i.e. over the course of the experiment from T-120 to T54). For E2, the concentrations were closely correlated only during parturition ( $P < 0.001$ ) whereas for P4, they were closely correlated also before parturition ( $P < 0.001$ ).

**Table II.** Correlations between concentrations of oestradiol-17 $\beta$ , progesterone, prolactin, cortisol, immunoglobulin G and lactose in plasma on the one hand, and in colostrum, lactoserum or saliva on the other hand for samples collected before, during or after parturition or over the course of the experiment ( $r$  = coefficient of correlation,  $n$  = number of pairs; \*  $P \leq 0.05$ ; \*\*  $P \leq 0.01$ ; \*\*\*  $P \leq 0.001$ ).

		Before parturition	During parturition	After parturition	Across periods
Oestradiol-17 $\beta$ <sup>1,2</sup>	$r$	0.52	<b>0.83</b> ***	-0.16	
	$n$	11	<b>26</b>	24	
Progesterone <sup>1,2</sup>	$r$	<b>0.82</b> **	<b>0.67</b> ***	-0.15	
	$n$	<b>10</b>	<b>24</b>	26	
Prolactin <sup>3,4</sup>	$r$				<b>0.31</b> *
	$n$				<b>44</b>
Cortisol <sup>5,4</sup>	$r$				<b>0.60</b> ***
	$n$				<b>97</b>
Immunoglobulin G <sup>1,2</sup>	$r$	-0.51	0.18	-0.08	
	$n$	11	26	26	
Lactose <sup>1,2</sup>	$r$	0.39	<b>0.40</b> *	0.20	
	$n$	10	<b>25</b>	26	

<sup>1</sup> Correlation between concentrations in plasma and colostrum.

<sup>2</sup> Correlation across periods was not calculated since the ratio of concentrations in colostrum over those in plasma varied significantly with time.

<sup>3</sup> Correlation between concentrations in plasma and lactoserum.

<sup>4</sup> Correlations for each period were not calculated since the ratio of concentrations in lactoserum or saliva over those in plasma did not vary significantly with time.

<sup>5</sup> Correlation between concentrations in plasma and saliva.

### 3.3. Immunoglobulin G and lactose

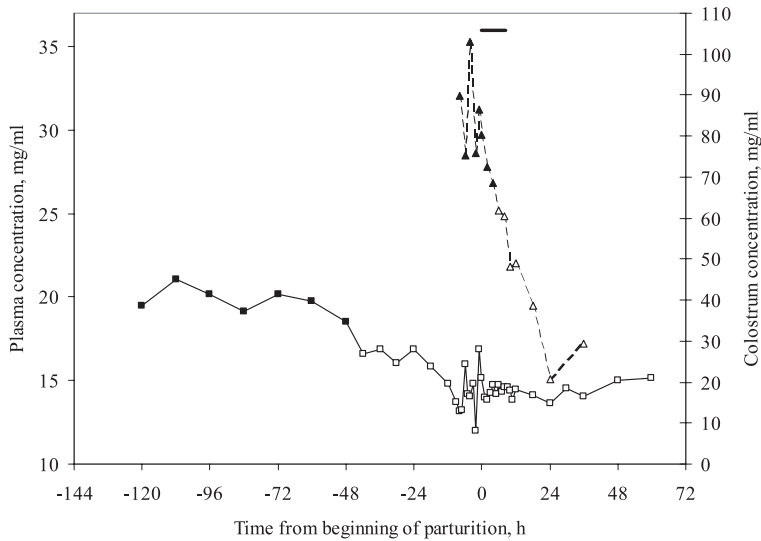
Plasma concentrations of IgG decreased from T-108 until parturition and were lower between T-42 and T60 than prior to T-72 ( $P < 0.05$ , Fig. 3). On the contrary, concentrations of IgG in colostrum were very high just before parturition and declined very rapidly from the start of farrowing until T24. They were lower between T6 and T60 than prior to parturition ( $P < 0.05$ ). Across sows and over the course of the experiment, concentrations of IgG in colostrum and in plasma were not correlated (Tab. II). Ratios of concentrations in colostrum over those in plasma varied with time ( $P < 0.0001$ , Fig. 2) and ratios measured later than 10 h postpartum were lower than the mean ratios obtained before parturition ( $P < 0.05$ ).

Plasma concentrations of lactose were very low between T-120 and T-80, increased slowly until T-24 and more rapidly until

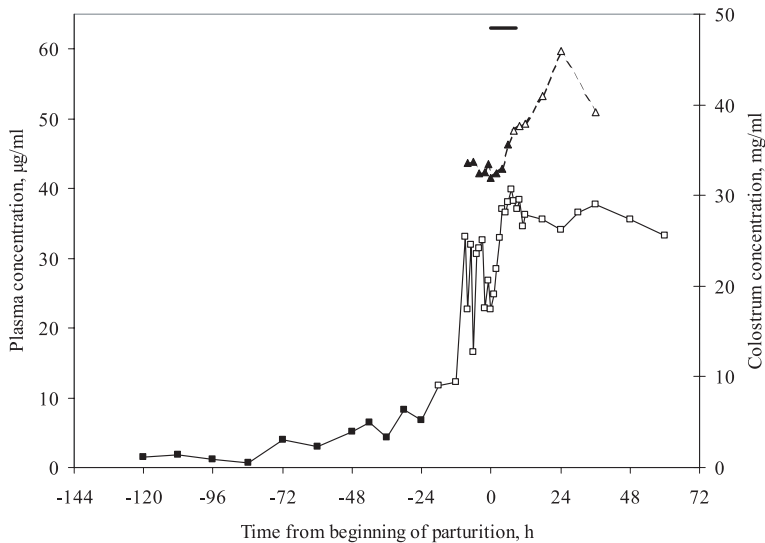
T-12 (Fig. 4). Indeed, plasma concentrations were higher between T-18 and T64 than prior to T-72 ( $P < 0.05$ ). Lactose measurements in colostrum showed a significant increase during parturition and peak values were reached at about T24. The ratios of lactose concentrations in colostrum over those in plasma varied with time ( $P < 0.05$ ). Except for sow 55, this ratio was the lowest during parturition (Fig. 5). Across sows, concentrations of lactose in colostrum and in plasma were closely correlated during parturition ( $P < 0.05$ , Tab. II).

Across sows and periods, plasma P4 concentrations were negatively correlated with the lactose in colostrum ( $r = -0.55$ ,  $P < 0.001$ ,  $n = 61$ ) and positively correlated with IgG in colostrum ( $r = 0.53$ ,  $P < 0.001$ ,  $n = 61$ ). Finally, plasma E2 was positively correlated with the IgG in colostrum ( $r = 0.65$ ,  $P < 0.001$ ,  $n = 64$ ).

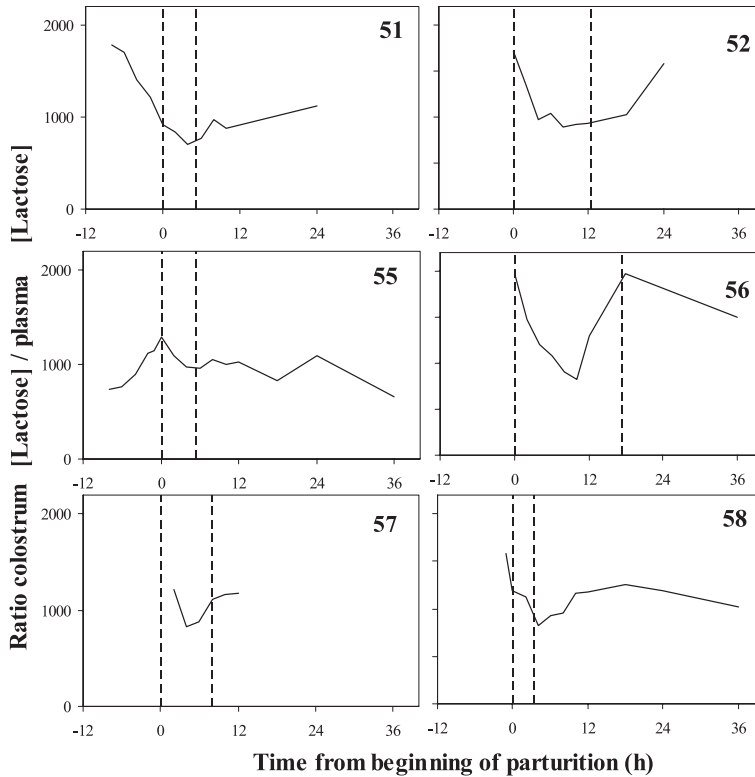




**Figure 3.** Mean concentrations of immunoglobulin G in plasma (square symbols: ■ or □, SE = 1.79 mg·mL<sup>-1</sup>) and colostrum (triangle symbols: ▲ or △, SE = 12.21 mg·mL<sup>-1</sup>) around parturition. Mean farrowing duration is represented by the black horizontal bar. Empty symbols (□ or △) represent values that differ ( $P < 0.05$ ) from mean concentrations calculated with values prior to T-72 for plasma or prior to T0 for colostrum.



**Figure 4.** Mean concentrations of lactose in plasma (square symbols: ■ or □, SE = 5.97 µg·mL<sup>-1</sup>) and colostrum (triangle symbols: ▲ or △, SE = 2.24 mg·mL<sup>-1</sup>) around parturition. Mean farrowing duration is represented by the black horizontal bar. Empty symbols (□ or △) represent values that differ ( $P < 0.05$ ) from mean concentrations calculated with values prior to T-72 for plasma or prior to T0 for colostrum.



**Figure 5.** Variation of the ratio of concentrations of lactose in colostrum on those in plasma upon time (h) around parturition for the 6 sows. Vertical dashed lines delimit parturition.

#### 4. DISCUSSION

To our knowledge, this is the first study comparing the patterns of P4, E2, cortisol and PRL in plasma to those in colostrum (P4 and E2), lactoserum (PRL) or saliva (cortisol) in sows around parturition. It clearly indicates that these hormonal patterns are parallel and that concentrations in plasma and colostrum/lactoserum or saliva are significantly correlated.

##### 4.1. Plasma hormones, lactose and IgG variations around parturition

Time-related variations in plasma concentrations of hormones, lactose and IgG are in good agreement with previous studies

on sow endocrinology around parturition. Plasma P4 decreased slowly during the days preceding parturition and more rapidly just before and during parturition as already described [24–26]. This decrease is due to the regression of the corpora lutea [27, 28]. On the contrary, plasma E2 increased towards the end of gestation, peaked at the beginning of parturition and dropped thereafter. This was in agreement with previous studies [26, 29] and is related to the fact that E2 is produced mainly by the placentas [30, 31]. The very rapid decrease at parturition is therefore the consequence of the removal of the foeto-placental units. Increased plasma concentrations of PRL around parturition were also in accordance with previous data

[25, 32]. The decrease in P4 and the increase in E2 are necessary for lactogenesis to occur in response to the high levels of PRL [3, 33–35]. Despite a decrease following parturition, our data confirm that plasma PRL concentrations remain relatively high thereafter, which is likely due to stimulation by the suckling piglets [36, 37]. The existence of a negative correlation between P4 and E2 and, between P4 and PRL, together with the coincidental fall of P4 on the one hand and rises in E2 and PRL on the other hand, support the proposed role of the P4 decrease as the trigger for other hormonal variations at parturition [3, 4, 35, 38].

Since the hematocrit did not show significant variations around parturition, the reduction in sow plasma concentrations of IgG noted in the days prior to parturition agreed with the hypothesis that most IgG present in the colostrum are from plasmatic origin [39, 40]. The decrease in the ratio of IgG concentrations in colostrum over those in plasma from 10 h postpartum suggests that closure of mammary epithelium tight junctions is occurring. Progesterone may play an active role in the IgG transfer from plasma to colostrum. Indeed, the decrease in plasma IgG occurred when plasma P4 concentrations were still high. Moreover, P4 was shown to prevent closure of tight junctions between lactocytes in the mouse [10, 11] and, when associated with E2, to enable paracellular transfer of immunoglobulins from plasma to the alveolar lumen of mammary glands in the cow [41]. In sows, Jackson et al. [42] confirmed the positive role of P4 on IgG transfer by showing higher colostrum concentrations of IgG in sows treated with P4 and farrowing at 116 days of gestation instead of 114 days.

We observed that lactose concentrations in plasma were very low at the end of gestation and started to rise from T-72, which was in agreement with previous results [43]. The concentrations in our study were similar to those determined in earlier studies from sows collected by puncture in the ear veins on the day before (around  $10 \mu\text{g}\cdot\text{mL}^{-1}$  in [43]), on the day after (around  $40 \mu\text{g}\cdot\text{mL}^{-1}$

in [44]), and on the following one (around  $30 \mu\text{g}\cdot\text{mL}^{-1}$  in [44]). Peak concentrations in our sows were observed a couple of hours after the beginning of parturition also in agreement with earlier studies [43, 44] but were of lower values (around  $40 \mu\text{g}\cdot\text{mL}^{-1}$  in our study compared to about  $90 \mu\text{g}\cdot\text{mL}^{-1}$  in [43] and  $120 \mu\text{g}\cdot\text{mL}^{-1}$  in [44]). Lactose is specifically produced in the mammary glands and may pass from the alveolar lumen to the blood through paracellular pathways before closure of the tight junctions [5, 46]. The increase in concentrations of lactose in the colostrum occurred under low P4 plasma concentrations but high E2 and PRL concentrations. This was in agreement with the suggested inhibitory action of P4 [4, 47, 48] and stimulatory actions of E2 and PRL [34, 35, 49] on lactose synthesis in the mammary glands. The decrease in the ratio of lactose concentrations in colostrum over those in plasma before and during parturition is in accordance with the passage of lactose from the colostrum to the blood circulation. The following increase in this ratio is probably the consequence of the closure of the tight junctions which begins at parturition [43]. However, it should be noted that plasma concentrations of lactose remained relatively high after T24 in agreement with previous data [44, 46]. This observation strongly suggests that lactose is still transferred from the mammary glands to the peripheral blood after closure of the tight junctions. The mechanisms involved are not known [44].

#### 4.2. Comparison of hormone concentrations in plasma and other fluids

Significant correlations between the concentrations of P4 in plasma and milk have been demonstrated in cows [50, 51], goats [52] and bitches [53]. Several authors reported a strong influence of the lipid content of cows' milk on P4 concentrations in mammary secretions [50, 54–56]. Indeed, after centrifugation of cows' milk, about 80% of P4 is recovered in cream, showing

a high affinity of P4 for milk fat [54, 57]. In the present study, concentrations of P4 were about 4 times higher in colostrum than in plasma until the end of parturition, which was in agreement with data obtained in cows [50].

Oestradiol-17 $\beta$  has already been assayed in sow colostrum, but only after parturition [14]. Our data show a significant correlation between concentrations of E2 in colostrum and those in plasma, especially during parturition. This was also found to be the case in cows [58–60]. Österlundh et al. [14] reported lower concentrations of E2 in colostrum/milk after parturition ( $< 100 \text{ pg}\cdot\text{mL}^{-1}$  vs.  $> 500 \text{ pg}\cdot\text{mL}^{-1}$ ) than in the present study despite similar concentrations in plasma. This was to be expected since these authors measured E2 in skimmed milk mammary secretions instead of in whole colostrum as was the case in the present study. Indeed, oestrogen levels are lower when measured in skimmed [14, 61] than in whole colostrum [62]. Moreover, as was the case for P4, E2 is preferentially bound to the cream fraction in cows' milk [57, 63]. It is noteworthy that the E2 ratio between colostrum and plasma increased with time and was much more variable after parturition as for P4. This phenomenon is probably related to a more rapid decrease of the concentrations in blood than in colostrum. Indeed, the release of P4 and E2 from maternal and placental origins ceases at parturition whereas the metabolic clearance of both these steroids at the sow peripheral level is high. Steroid concentrations in mammary secretions could only decrease as the steroid-rich colostrum is removed by the piglets and replaced by new mammary secretions containing lower concentrations of both steroids.

To our knowledge, this is the first report of parturition-related variations of PRL in sow lactoserum. Our data show that these variations are parallel to those observed in plasma and that concentrations in plasma and lactoserum are significantly correlated. However, the correlation is not very high

( $r = 0.31$ ) and is slightly lower than that previously measured between values in the serum and in whole milk in sows [13]. In this previous study, correlations ranged between 0.29 and 0.85. Concentrations of PRL were greater in lactoserum than in plasma in the present study (ratio = 1.25) whereas concentrations were similar in whole milk and serum in the study from Mulloy and Malven [13]. This difference can be explained by the enrichment of lactoserum in PRL after ultracentrifugation. Indeed, PRL is a peptide hormone with a molecular weight of 24 kD [64] which is probably fully retrieved in the lactoserum after ultracentrifugation whereas the volume of lactoserum represents approximately 80% of the total volume centrifuged.

Salivary cortisol has already been measured in growing pigs and was found to be correlated with plasma cortisol after an acute stress [65]. Cortisol in plasma can be divided into two fractions: the free cortisol, which is biologically active and the cortisol bound to transport proteins like CBG (Corticosteroid Binding Globulin [66]). Only free cortisol can passively diffuse across the secretory cells of salivary glands, and concentrations of cortisol in saliva were found to be close to concentrations of plasmatic free cortisol in man [67]. In our study, total cortisol concentrations in plasma and saliva were highly correlated ( $r = 0.60$ ) and salivary concentrations represented 24% of plasma concentrations which is higher than the values found by Cook et al. [65] in growing pigs, Vincent and Michell [68] in dogs and Vining et al. [67] in humans. This elevated salivary to plasma cortisol ratio around parturition can be explained by a lower binding of cortisol to blood proteins at that time. Indeed, plasmatic concentrations of CBG do not vary around parturition [4], while cortisol concentrations show a 4-fold increase during farrowing. The binding capacity of CBG for cortisol is therefore likely exceeded [69] and the free cortisol fraction increases up to 28% of the whole fraction around parturition instead of 10–13% during gestation [69, 70].

## 5. CONCLUSION

Plasma patterns of the main hormones regulating the parturition process and the initiation of lactation were confirmed. Simultaneously measuring lactose and IgG concentrations in plasma and colostrum may be used to detect when the closure of the tight junctions between mammary secretory cells occurs and hence the transition between colostrum and milk. Further studies should be developed to determine how endogenous- or environmental-related variations in progesterone, oestradiol and cortisol secretion by sows may influence the closure of the tight junctions and hence the amount of colostrum produced by the sows.

Relationships between these hormone concentrations in plasma and in other body fluids, such as colostrum/lactoserum or saliva, were assessed. Measurements of P4, E2 and PRL in colostrum/lactoserum and of cortisol in saliva could be used as alternatives to blood collection in order to determine patterns of variation of these hormones in sows around parturition and how the environment may influence them. However, it should be kept in mind that the correlations were not very high and that the ratio between plasma and colostrum concentrations may vary around parturition. Therefore, particular attention should be given to the moment of sample collection relative to the time of farrowing.

## ACKNOWLEDGEMENTS

The authors wish to acknowledge L. Thibault and S. Horth from Lennoxville R&D centre, F. Thomas, M. Lefebvre, S. Lechaud, M. Massard, H. Renoult and Y. Lebreton from INRA-UMRVP, for expert technical assistance. N. Devillers's Ph.D. studentship was funded by ITP (Paris, France) and INRA (Paris, France).

## REFERENCES

- [1] Delouis C. Physiology of colostrum production. *Ann Rech Vét* 1978, 9: 193–203.
- [2] Martinet J, Houdebine LM. Glande mammaire, mammogénèse, facteurs de croissance, lactogénèse. In: Martinet J, Houdebine LM (Eds), *Biologie de la lactation*, INRA-INSERM, Paris, 1993, p 3–29.
- [3] Liptrap RM. Prostaglandin F2 alpha and progesterone in experimental hypogalactia in sows. *Res Vet Sci* 1980, 29: 240–247.
- [4] Martin CE, Hartmann PE, Gooneratne A. Progesterone and corticosteroids in the initiation of lactation in the sow. *Aust J Biol Sci* 1978, 31: 517–525.
- [5] Neville MC, Morton J, Umemura S. Lactogenesis. The transition from pregnancy to lactation. *Pediatr Clin North Am* 2001, 48: 35–52.
- [6] Farmer C, Robert S, Rushen J. Bromocriptine given orally to periparturient of lactating sows inhibits milk production. *J Anim Sci* 1998, 76: 750–757.
- [7] Whitacre MD, Threlfall WR. Effects of ergocryptine on plasma prolactin, luteinizing hormone, and progesterone in the periparturient sow. *Am J Vet Res* 1981, 42: 1538–1538.
- [8] Hartmann PE, Holmes MA. Sow lactation. In: Barnett JL, Hennessy DP (Eds), *Manipulating Pig Production II*, Australasian Pig Science Association, Werribee, Australia, 1989, p 72–97.
- [9] Klopfenstein C, Couture Y, Martineau GP, Bouchard E. Physiopathologie comparative de la lactation chez la truie et chez la vache. *Médecin Vétérinaire du Québec* 2002, 32: 52–56.
- [10] Nguyen DA, Neville MC. Tight junction regulation in the mammary gland. *J Mammary Gland Biol Neoplasia* 1998, 3: 233–246.
- [11] Nguyen DA, Parlow AF, Neville MC. Hormonal regulation of tight junction closure in the mouse mammary epithelium during the transition from pregnancy to lactation. *J Endocrinol* 2001, 170: 347–356.
- [12] Grosvenor CE, Picciano MF, Baumrucker CR. Hormones and growth factors in milk. *Endocr Rev* 1993, 14: 710–728.
- [13] Mulloy AL, Malven PV. Relationships between concentrations of porcine prolactin in blood serum and milk of lactating sows. *J Anim Sci* 1979, 48: 876–881.
- [14] Österlundh I, Holst H, Magnusson U. Hormonal and immunological changes in blood and mammary secretion in the sow at parturition. *Theriogenology* 1998, 50: 465–477.

- [15] Heap RB, Gwyn M, Laing JA, Walters DE. Pregnancy diagnosis in cows, changes in milk progesterone concentration during the oestrous cycle and pregnancy measured by a rapid radioimmunoassay. *J Agric Sci Camb* 1973, 81: 151–157.
- [16] Murray RD, Newstead R. Determination of steroid hormones in goats' milk and plasma as an aid to pregnancy diagnosis using an ELISA. *Vet Rec* 1988, 122: 158–161.
- [17] Camous S, Prunier A, Pelletier J. Plasma prolactin, LH, FSH and estrogen excretion patterns in gilts during sexual development. *J Anim Sci* 1985, 60: 1308–1317.
- [18] Devillers N, van Milgen J, Prunier A, Le Dividich J. Estimation of colostrum intake in the neonatal pig. *Anim Sci* 2004, 78: 305–313.
- [19] Robert S, de Passillé AM, St-Pierre N, Dubreuil P, Pelletier G, Petitclerc D, Brazeau P. Effect of the stress of injections on the serum concentration of cortisol, prolactin and growth hormone in gilts and lactating sows. *Can J Anim Sci* 1989, 69: 663–672.
- [20] Gordon GO, Whittier EO. Proteins of milk. In: Webb BH, Johnson AH (Eds), *Fundamentals of dairy chemistry*, Avi Publishing Company, Westport (Connecticut), 1965, p 54–90.
- [21] AOAC Official methods of analysis. 15th ed, Association of Official Analytical Chemists, Washington DC, 1990.
- [22] Merriam GR, Wachter KW. Algorithms for the study of episodic hormone secretion. *Am J Physiol* 1982, 243: E310–E318.
- [23] SAS Software release 8.01. SAS institute Inc, Cary NC, 2000.
- [24] Ash RW, Heap RB. Oestrogen, progesterone and corticosteroid concentrations in peripheral plasma of sows during pregnancy, parturition, lactation and after weaning. *J Endocrinol* 1975, 64: 141–154.
- [25] Meunier-Salaün MC, Gort F, Prunier A, Schouten WPG. Behavioural patterns and progesterone, cortisol and prolactin levels around parturition in European (Large-White) and Chinese (Meishan) sows. *Appl Anim Behav Sci* 1991, 31: 43–59.
- [26] Taverne M, Bevers MM, Bradshaw JMC, Dieleman SJ, Willems AH, Porter DG. Plasma concentrations of prolactin, progesterone, relaxin and oestradiol-17 $\beta$  in sows treated with progesterone, bromocryptine or indomethacin during late pregnancy. *J Reprod Fertil* 1982, 65: 85–96.
- [27] Coggins EG, Van Horn D, First NL. Influence of prostaglandin F2 alpha, dexamethasone, progesterone and induced corpora lutea on porcine parturition. *J Anim Sci* 1977, 45: 754–762.
- [28] Gustafsson B, Einarsson S, Larsson K, Edquist LE. Sequential changes of estrogens and progesterone at prostaglandin-induced parturition in the sow. *Am J Vet Res* 1976, 37: 1017–1020.
- [29] Robertson HA, King GJ. Plasma concentrations of progesterone, oestrone, oestradiol-17beta and of oestrone sulphate in the pig at implantation, during pregnancy and at parturition. *J Reprod Fertil* 1974, 40: 133–141.
- [30] Baldwin DM, Stabenfeldt GH. Endocrine changes in the pig during late pregnancy, parturition and lactation. *Biol Reprod* 1975, 12: 508–515.
- [31] Martal J, Haddad B. Physiologie et endocrinologie placentaires. In: Thibault C, Levasseur MC (Eds), *La reproduction chez les mammifères et l'homme*, INRA Editions – Ellipses, Paris, 2001, p 505–532.
- [32] Dusza L, Krzymowska H. Plasma prolactin levels in sows during pregnancy, parturition and early lactation. *J Reprod Fertil* 1981, 61: 131–134.
- [33] Falconer IR. Aspects of the biochemistry, physiology and endocrinology of lactation. *Aust J Biol Sci* 1980, 33: 71–84.
- [34] Houdebine LM, Djiane J, Dusanter-Fourt I, Martel P, Kelly PA, Devinoy E, Servely JL. Hormonal action controlling mammary activity. *J Dairy Sci* 1985, 68: 489–500.
- [35] Willcox DL, Arthur PG, Hartmann PE, Whitely JL. Perinatal changes in plasma oestradiol-17 beta, cortisol and progesterone and the initiation of lactation in sows. *Aust J Biol Sci* 1983, 36: 173–181.
- [36] Algers B, Madej A, Rojanasthien S, Uvnäs-Moberg K. Quantitative relationships between suckling-induced teat stimulation and the release of prolactin, gastrin, somatostatin, insulin, glucagon and vasoactive intestinal polypeptide in sow. *Vet Res Commun* 1991, 15: 395–407.
- [37] Rushen J, Foxcroft GR, DePassillé AM. Nursing-induced changes in pain sensitivity, prolactin, and somatotropin in the pig. *Physiol Behav* 1993, 53: 265–270.
- [38] Gooneratne A, Hartmann PE, McCauley I, Martin CE. Control of parturition in the sow

- using progesterone and prostaglandin. *Aust J Biol Sci* 1979, 32: 587–595.
- [39] Bourne FJ, Curtis J. The transfer of immunoglobins IgG, IgA and IgM from serum to colostrum and milk in the sow. *Immunology* 1973, 24: 157–162.
- [40] Huang SC, Hu ZL, Hasler-Rapacz J, Rapacz J. Preferential mammary storage and secretion of immunoglobulin gamma (IgG) subclasses in swine. *J Reprod Immunol* 1992, 21: 15–28.
- [41] Smith KL, Muir LA, Ferguson LC, Conrad HR. Selective transport of IgG into the mammary gland: role of estrogen and progesterone. *J Dairy Sci* 1971, 54: 1886–1894.
- [42] Jackson JR, Hurley WL, Easter RA, Jensen AH, Odle J. Effects of induced or delayed parturition and supplemental dietary fat on colostrum and milk composition in sows. *J Anim Sci* 1995, 73: 1906–1913.
- [43] Hartmann PE, Whitely JL, Willcox DL. Lactose in plasma during lactogenesis, established lactation and weaning in sows. *J Physiol.* 1984, 347: 453–463.
- [44] Atwood CS, Hartmann PE. Assessment of mammary gland metabolism in the sow. I. Development of methods for the measurement of cellular metabolites in milk and colostrum. *J Dairy Res* 1995, 62: 189–206.
- [45] Atwood CS, Toussaint JK, Hartmann PE. Assessment of mammary gland metabolism in the sow. II. Cellular metabolites in the mammary secretion and plasma during lactogenesis. *J Dairy Res* 1995, 62: 207–220.
- [46] Meurer KA, Cox NM, Tubbs RC. Absence of insulin affects recruitment and maintenance of preovulatory follicles in cyclic diabetic gilts. *J Anim Sci* 1990, 68 (Suppl. 1): 466.
- [47] Nicholas KR, Hartmann PE. Progesterone control of the initiation of lactose synthesis in the rat. *Aust J Biol Sci* 1981, 34: 435–443.
- [48] Whitely JL, Hartmann PE, Willcox DL, Bryant-Greenwood GD, Greenwood FC. Initiation of parturition and lactation in the sow: effects of delaying parturition with medroxyprogesterone acetate. *J Endocrinol* 1990, 124: 475–484.
- [49] Nicholas KR, Hartmann PE. Progressive changes in plasma progesterone, prolactin and corticosteroid levels during late pregnancy and the initiation of lactose synthesis in the rat. *Aust J Biol Sci* 1981, 34: 445–454.
- [50] Ginther OJ, Nuti LC, Garcia MC, Wentworth BC, Tyler WJ. Factors affecting progesterone concentration in cow's milk and dairy products. *J Anim Sci.* 1976, 42: 155–159.
- [51] Mauer RE, Rippel RH, Hedlund MT, Hutchinson RE. Plasma and milk progesterone, GnRH and LH in cows. *J Anim Sci (Abs.)* 1974, 39: 218.
- [52] Bretzlaff KN, Elmore RG, Nuti LC. Use of an enzyme immunoassay to determine concentrations of progesterone in caprine plasma and milk. *J Am Vet Med Assoc* 1989, 194: 664–668.
- [53] England GC, Verstegen JP. Progesterone concentration and ionic composition of the mammary secretion of periparturient bitches. *J Reprod Fertil (Suppl.)* 1997, 51: 209–214.
- [54] Heap RB, Henville A, Linzell JL. Metabolic clearance rate, production rate, and mammary uptake and metabolism of progesterone in cows. *J Endocrinol* 1975, 66: 239–247.
- [55] Nachreiner RF, Oschmann SJ, Edqvist LE, Richards JJ. Factors affecting skim milk progesterone assay results. *Am J Vet Res* 1992, 53: 1085–1089.
- [56] Pennington JA, Spahr SL, Lodge JR. Influences on progesterone concentration in bovine milk. *J Dairy Sci* 1981, 64: 259–266.
- [57] Schwalm JW, Tucker HA. Glucocorticoids in mammary secretions and blood serum during reproduction and lactation and distributions of glucocorticoids, progesterone, and estrogens in fractions of milk. *J Dairy Sci* 1978, 61: 550–560.
- [58] Erb RE, Chew BP, Keller HF. Relative concentrations of estrogen and progesterone in milk and blood, and excretion of estrogen in urine. *J Anim Sci* 1977, 45: 617–626.
- [59] Kesler DJ, Peterson RC, Erb RE, Callahan CJ. Concentrations of hormones in blood and milk during and after induction of parturition in beef cattle with dexamethasone and estradiol-17beta. *J Anim Sci* 1976, 42: 918–926.
- [60] Monk EL, Erb RE, Mollett TA. Relationships between immunoreactive estrone and estradiol in milk, blood, and urine of dairy cows. *J Dairy Sci* 1975, 58: 34–40.
- [61] Matubsi HY, Bervanakis G, Tritton SM, Campbell RG, Fairclough RJ. Influence of dietary protein of diets given in late gestation and during lactation on protein content and oestrogen concentrations in the colostrum and milk of gilts. *Anim Sci* 1998, 67: 139–145.
- [62] Farmer C, Houtz SK, Hagen DR. Estrone concentration in sow milk during and after parturition. *J Anim Sci* 1987, 64: 1086–1089.

- [63] Wolford ST, Argoudelis CJ. Measurement of estrogens in cow's milk, human milk, and dairy products. *J Dairy Sci* 1979, 62: 1458–1463.
- [64] Li CH. Biochemistry of prolactin. In: Kon SK, Cowie AT (Eds), *Milk, the Mammary Gland and its Secretion*. Vol I, Academic Press, New York and London, 1961, p 205–228.
- [65] Cook NJ, Schaefer AL, Lepage P, Jones SM. Salivary vs. serum cortisol for the assessment of the adrenal activity in swine. *Can J Anim Sci* 1996, 76: 329–335.
- [66] Vining RF, McGinley RA. The measurement of hormones in saliva: possibilities and pitfalls. *J Steroid Biochem* 1987, 27: 81–94.
- [67] Vining RF, McGinley RA, Maksvytis JJ, Ho KY. Salivary cortisol: a better measure of adrenal cortical function than serum cortisol. *Ann Clin Biochem* 1983, 20: 329–335.
- [68] Vincent IC, Michell AR. Comparison of cortisol concentrations in saliva and plasma of dogs. *Res Vet Sci* 1992, 53: 342–345.
- [69] Whitely JL, Willcox DL, Newton JA, Bryant-Greenwood G, Hartmann PE. Total and free plasma concentrations of progesterone, cortisol and oestradiol-17beta during pregnancy, parturition and early lactation in sows. *Aust J Biol Sci* 1984, 37: 267–276.
- [70] Kattesh HG, Baumbach GA, Gillespie BB, Schneider JF, Murai JT. Distribution between protein-bound and free forms of plasma cortisol in the gilt and fetal pig near term. *Biol Neonate* 1997, 72: 192–200.