Influence of litter size on insulin sensitivity in multiparous sows¹

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ABSTRACT: The objectives were to examine effects of litter size on insulin sensitivity in multiparous sows at the end of pregnancy. Twelve sows were allocated in two treatments after weaning: control (CTR) or ligature of the left oviduct (LIG). At 68 d of the subsequent pregnancy, catheters were implanted in a jugular vein, in a carotid artery, and in the main vein draining one uterine horn. A blood flow probe was fitted around the artery irrigating the same uterine horn. A meal test, a tolerance test, and an euglycemic hyperinsulinemic clamp test were performed at 108 ± 3 d of pregnancy. Serial blood samples were drawn simultaneously from the uterine vein and the carotid artery before and during the tests. The number of fetuses in the studied uterine horn was lower (3.7 vs. 8.0 , $P < 0.001$), and piglets at birth were heavier (1.71 vs. 1.31 kg, $P = 0.04$) in the LIG sows than in the CTR sows. Treatment did not affect uterine blood flow (UBF), but UBF/

fetus in the uterine horn was greater for the LIG treatment (0.67 vs. 0.34 L/min, $P = 0.002$). During meal test, glycemia, glucose uptake in the uterine horn and glucose uterine uptake/fetus were similar in both groups of sows, while insulin levels were higher in the LIG sows ($P = 0.04$). The decrease of NEFA concentrations was similar across treatments. Glucose half-life did not differ between treatments (13.4 min as a mean; $P = 0.63$) during tolerance test, but area under the insulin curve was greater in the LIG sows ($P = 0.02$). The glucose infusion rate during euglycemic hyperinsulinemic clamps was lower in the LIG sows than in CTR sows (6.1 ± 0.2 vs. 7.8 ± 0.1 mg glucose kg⁻¹ min⁻¹; $P = 0.01$). The LIG sows are less sensitive to insulin than the CTR sows without adjustment of maternal glycemia and glucose tolerance. Insulin sensitivity adaptation to litter size in late pregnancy of sows would rather be connected to growth rate than to number of fetuses.

Key words: insulin sensitivity, litter size, pregnancy, sows

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INTRODUCTION

The increased nutritional requirements of breeding females resulting from fetal development require physiological and metabolic adaptations to cope with them. Uterine blood flow (UBF) has been reported to increase throughout pregnancy in most species such as ewes (Caton et al., 1983), cows (Reynolds and Ferrell, 1987), goats

(Huckabee et al., 1961), guinea pigs (Peeters et al., 1982), rabbits (Gilbert et al., 1984), and sows (Père and Etienne, 2000). A physiological, progressive, and reversible insulin resistance develops in the female during the last one-third of pregnancy to fulfill the increasing glucose requirements of the gravid uterus. This was shown in many species including rats, ewes, humans and rabbits (Leturque et al., 1980; Pethick et al., 1983; Ryan et al., 1985; Catalano et al., 1991; Gilbert et al., 1993). This adaptation allows sparing glucose, the main fetal source of energy, for the gravid uterus at the expense of the maternal tissues that utilize other energetic substrates such as NEFA (Knopp et al., 1973). This adaptation was also demonstrated in

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sows, but it seems to be more limited than in the species studied previously (Père et al., 2000).

Otherwise, litter size of sows has greatly increased during the past decades, which may contribute to increased uterine requirements. One may then wonder whether litter size affects physiological and metabolic adaptations to pregnancy. It was shown that UBF increases with the number of fetuses, at least up to five fetuses per uterine horn (Père and Etienne, 2000). This study aimed to investigate whether, in addition to UBF, litter size affects insulin sensitivity in sows. It was hypothesized that insulin resistance would be greater in sows bearing an important number of fetuses. Three complementary methods: a meal test, a glucose tolerance test, and an euglycemic hyperinsulinemic clamp were carried out during the last week of pregnancy in two groups of multiparous sows that differed by litter size.

MATERIALS AND METHODS

All animal procedures were performed according to the French legislation on experimental animal care (authorization to experiment on living animals No. 04740 delivered by the French Ministry of Agriculture to M.-C. Père).

Animals and Feeding

The study was performed on 17 multiparous crossbred Landrace $\times$ Large White sows that had already weaned 2.0 ± 0.3 litters (mean $\pm$ SEM). Sows were inseminated with semen from Pietrain boars at 605 ± 53 d of age after estrous synchronization (Regumate, Roussel-Uclaf, Paris, France). Sows were housed individually during the whole gestation, and kept on a flat concrete surface with straw bedding during the first 60 d, and on flat decks in the experimental building thereafter.

Sows were fed 2.8 kg/d of a standard gestation diet containing 9.53 MJ NE/kg, 13.5% CP, and 0.65% lysine, and had free access to water. Diets were pelleted and fed as two equal meals at 0800 and 1600 hours.

Experimental Treatments

Insulin sensitivity was studied in two groups of sows at the end of gestation: a control group with a normal litter size (CTR), and a second group (LIG) having about half the number of piglets compared with the control group. After weaning of the previous litter, 17 sows were allocated to the CTR or LIG treatments according to litter origin, live weight, and parity number.

A surgery was performed on nine sows after weaning to ligate and sever the left oviduct (LIG treatment) as detailed earlier (Père et al., 1997). These sows returned normally into estrus thereafter, were inseminated at the second estrus postsurgery, i.e., at 33.5 ± 1.1 d after surgery, and constituted the LIG group. Breeding management of sows was the same in the two groups. Whatever treatment, sows were inseminated at 46.3 ± 0.3 d after weaning their previous litter.

All the sows, including the nine LIG sows, underwent surgery at 68.3 ± 0.7 d of pregnancy to implant catheters and blood flow probe. Fetuses were counted by palpation of uterine horns. All sows (CTR sows, $n = 8$; LIG sows, $n = 9$) were fitted a blood flow probe and vascular catheters allowing measurements of uterine glucose uptake. An ultrasonic transit time flow probe (8-mm probe, R-series, Transonic System Inc., Ithaca, NY) was implanted around the middle artery irrigating the selected uterine horn (six to eight fetuses in the CTR group; two to four fetuses in the LIG group). Details concerning the chronic implantations of flow probes have been described previously by Père and Etienne (2000). Three catheters were implanted. The first one (Tygon Tubing, Cole Parmer Inst. Co., Vernon Hills, IL; 1.78 mm o.d., 1.02 mm i.d.) was implanted in a small collateral uterine vein and advanced until its end laid in the main vein draining the whole uterine horn studied. The catheter was fixed in the collateral vein by tying the vessel with 2-0 plaited polyester (Ethicon SAS, Issy-les-Moulineaux, France) on each side of silicone rings that were previously attached to the catheter. The abdominal cavity was closed by suturing separately the peritoneum and the muscle layer. Oxytetracyclin (5 mg/kg BW, Pfizer, Orsay, France) was injected in the abdominal cavity. The second one (Silastic, Dow Corning Corporation, Midland; 2.16 mm o.d., 1.02 mm i.d.) was implanted through a collateral vein in the right external jugular vein, and the third one (Tygon catheter; 2.29 mm o.d., 1.27 mm i.d.) was inserted in the carotid artery for a distance of 35 cm. Composition of the blood collected via this catheter was assumed to be the same as that of blood supplying the uterus. Surgery required ~ 2 h.

Before surgeries, sows were fasted for 24 h. Sows were premedicated with atropine (20 μ g/kg BW given i.v.), and anesthesia was then induced with sodium thiopental (10 mg/kg BW given i.v.). Anesthesia was maintained with 2% to 5% halothane (Fluothane, Pitman-Moore, Meaux, France) in oxygen (2 to 3 L/min). Postsurgical antibiotic therapy consisted of i.m. injections of Ampicillin (10 mg/kg BW $^{-1}$ d $^{-1}$) for 3 d as a prophylactic measure and Flunixin (2 mg/kg

$\text{BW}^{-1} \cdot \text{d}^{-1}$) for postoperative analgesia. Rectal temperature was recorded daily during the first 5 d after surgery. Sows resumed normal activity and feed intake within 1 to 2 d after surgery. Catheters were flushed three times a week with 10 mL normal saline solution (154 mM NaCl) containing 200 IU of heparin/mL.

Measurements

Sows were weighed at 102 ± 1 d of pregnancy. After an overnight fast, a meal test, a glucose tolerance test, and an euglycemic hyperinsulinemic clamp were applied in the morning during three successive days, on average at 108 ± 3 d of pregnancy.

Meal test. The meal test consisted of a 30-min control period before and a 240-min sampling period after the morning meal (1.4 kg of feed). Meal consumption lasted about 10 min. Blood samples (2 mL) were drawn simultaneously through the uterine and carotid catheters. Samples were collected at 30 and 10 min before the meal, at 15-min intervals from 15 to 90 min, and at 30-min intervals from 90 to 240 min after meal distribution. Blood samples were collected on ice with heparinized syringes and immediately centrifuged for 2 min at $8,500 \times g$ at 4°C . The supernatant was divided into three subsamples and stored at -20°C until further analysis. Concentrations of plasma glucose and insulin were determined at each sampling time. Plasma NEFA were measured at 30 and 10 min before the meal, and 30, 60, 90, 120, 180, and 240 min after the meal.

Glucose tolerance test. A 1.665 M sterile glucose solution was injected through the jugular catheter to deliver 0.5 g glucose/kg BW. Twenty milliliters of a saline solution (0.9% NaCl) were then injected to rinse the catheter. Total process lasted 7 min on average. The first blood samples of the test were drawn immediately after, at a time considered as time zero. Blood samples were drawn simultaneously from the uterine vein and carotid artery at 30 and 10 min before the test and at 3, 6, 9, 12, 15, 18, 21, 25, 30, 35, 40, 45, 50, and 60 min after time 0. The blood samples (2 to 3 mL) were collected on ice with heparinized syringes and immediately centrifuged for 2 min at $8,500 \times g$ at 4°C . The supernates were stored at -20°C until they were analyzed for glucose and insulin. Insulin was determined on arterial plasma only.

Euglycemic hyperinsulinemic clamp. An euglycemic hyperinsulinemic clamp test was carried out according to procedures described by De Fronzo et al. (1979), Burnol et al. (1983), and Père and

Etienne (2007) with an insulin infusion rate of $55 \text{ ng} \cdot \text{kg BW}^{-1} \cdot \text{min}^{-1}$. Insulin (human insulin, 40 IU/mL, Actrapid, France) was diluted (1/20) with 30 mL of normal saline solution (154 mM NaCl) and 4 mL of the own plasma of the sow sampled 1 h before the start of the clamp. Euglycemia was defined for each sow as the average of blood glucose concentrations measured every 15 min during the 60 min preceding the clamp. During the clamp, a loading dose of insulin ($220 \text{ ng insulin/kg BW}$) was injected through the jugular catheter and followed immediately by the continuous insulin infusion (syringe pump; kdS model 260, KD Scientific Boston, MA) at a constant rate ($180 \pm 2 \mu\text{L/min}$). Duration of the infusion period was 150 min. Arterial concentration of glucose was maintained at the basal preinfusion concentration by infusing a glucose solution (1.665 M sterile glucose) at a variable rate through the jugular catheter with an IPC-04 peristaltic pump (Ismatec SA, Zürich, Switzerland). Glucose infusion began about 5 min after the start of the insulin infusion. The flask containing the glucose solution was kept on a scale. At 5-min intervals during the entire glucose clamp procedure, its weight was registered to measure the glucose infusion rate (**GIR**), and a 1-mL arterial blood sample was sampled. Blood glucose was immediately measured on that sample by the glucose oxidase method using a glucose analyzer (YSI, Yellow Springs Instrument Co, Yellow Springs, OH) to adjust GIR. Every 15 min, 2 to 3 mL of blood were sampled simultaneously from carotid and uterine vein to immediately measure the blood packed-cell volume and for subsequent determination of plasma insulin and glucose concentrations, whereas NEFA, were determined every 30 min.

Blood flow measurements. A 3-m extension cable was fixed at the end of the cable of the probe, and the connector was soldered. It was connected to a dual channel blood flowmeter (model T206, Transonic Systems Inc.) that allows for about 170 measurements/s. The flowmeter was connected to a computer with an application program that integrates measurements during 1-min periods and stores data. UBF was measured during 24-h periods during each test. Times of beginning and end of experiments were recorded.

Chemical Analyses

Glucose and NEFA concentrations in plasma were determined by standard enzymatic colorimetric analysis using commercial kits (bio-Mérieux ref 61269,

Marcy l'Etoile, France, and NEFA C ref 754664, Wako, Dardilly, France, respectively) on an automatic multi-channel analyzer (Cobas Mira, Roche, Switzerland). Plasma insulin concentrations were measured using a porcine commercial RIA kit (CIS Bio International, Gif sur Yvette, France).

Calculations

Basal values of glucose, NEFA, and insulin concentrations were the means of the measurements made before the tests, after an overnight fast. These means were considered as time zero for the meal and clamp tests. Net fluxes of glucose across uterine horn were calculated according to the Fick principle: arteriovenous difference of substrates times UBF. Uterine blood flow used in the calculations was the mean of blood flow during the 5 min around each blood sampling.

During tolerance test, glucose half-life was estimated from individual regression equations relating the logarithm of glucose concentrations to the time between time 0 and time when the concentration passes through the fasting level on the way down. For meal and tolerance tests, the area under the curve (AUC) for insulin was calculated by linear interpolation of insulin concentrations between the measurements, using the fasting concentration as the base line. For meal test, AUC for glucose was also calculated. These estimations were made between the initiation of the meal and 240 min later for the meal tests and between time 0 and the time when insulinemia returned to the fasting level for the glucose tolerance tests. During tolerance test, time required to reach one-quarter, half, or three-quarters of this area was also determined by interpolation. Glucose infusion rate during the clamps (mg of glucose·kg BW⁻¹·min⁻¹) was calculated during periods 60 to 150 and 105 to 150 min.

Statistical Analyses

Effects of treatment (CTR or LIG) on weight and reproductive performance of sows, areas under the insulin and glucose curves, time required to reach one-quarter, half, or three-quarters of area under insulin curve, were analyzed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC) with treatment as main effect. Glucose, NEFA and insulin arterial levels, UBF, and glucose uterine uptake during meal test, and glucose, insulin arterial levels and GIR during clamp were analyzed by ANOVA using the MIXED procedure for repeated measures of SAS with sow nested within treatment as the experimental unit, considering treatment and sampling time as main effects, and the interaction between treatment and sampling time. Half-life of glucose calculated from arterial and uterine vein blood samplings was analyzed with treatment and blood vessel as main effects, and the treatment × blood vessel interaction. In all analyses, effects were considered significant for $P \leq 0.05$, and $0.05 < P \leq 0.10$ was discussed as a trend.

RESULTS

Catheters and flow probes were implanted in 17 sows. Four sows had flow probe cable destroyed or clogged catheters and one sow aborted shortly after surgery. Our study was finally conducted on six multicatheterized sows per treatment.

Sow Performance

The number of fetuses in the uterine horn studied was one-fold greater ($P < 0.001$) in the CTR than in the LIG treatments (8.0 ± 0.4 vs. 3.7 ± 0.4 , Table 1). Sows were allowed to farrow and duration of pregnancy did not differ between treatments. The number of piglets born was greater ($P < 0.001$) and their

Table 1. Variation of weight, reproductive performance, and uterine blood flow of sows

Treatment group ¹	CTR	LIG	RMSE ²	P-value
Parity number	3.3	2.7	1.0	0.29
Weight of sows at 102 d, kg	278	282	30	0.84
Duration of gestation, d	113.2	114.5	1.7	0.21
Number of fetuses in studied horn	8.0	3.7	1.1	< 0.001
Total number of piglets at birth	13.0	7.5	2.0	< 0.001
Total number of stillborn piglets	0.7	0.2	0.9	0.36
Piglet's mean weight, kg	1.31	1.71	0.30	0.04
Litter weight, kg	17.2	12.6	4.4	0.10
Uterine blood flow at 108 d ³ , L/min	2.69	2.42	0.93	0.63
Uterine blood flow per fetus ³ , L/min	0.34	0.67	0.13	0.002

¹CTR, control; LIG, left oviduct severed.

²Root mean square error.

³Mean uterine blood flow during 24 h at 108 d of pregnancy.

mean weight was lower (1.31 ± 0.08 vs. 1.71 ± 0.15 kg, $P = 0.04$) in the CTR than in the LIG sows. Litter weight did not differ significantly between treatments (17.2 ± 1.9 vs. 12.6 ± 1.7 kg, in the CTR and LIG sows, respectively, $P = 0.10$; Table 1).

Blood Flow

UBF did not differ between the LIG and the CTR sows (2.42 ± 0.28 L/min and 2.69 ± 0.46 L/min, respectively; $P = 0.63$; Table 1). On average, UBF per fetus was one-fold greater in the LIG than in the CTR sows (0.67 ± 0.05 and 0.34 ± 0.06 L/min, respectively; $P = 0.002$; Table 1).

Meal Test

Variations of UBF during the meal test are presented in Figure 1. Irrespective of treatment, UBF was lower 15 min after the meal. It did not differ thereafter from the preprandial values.

Variations of arterial concentrations of glucose, insulin, and NEFA during meal test are presented in Figure 2.

Basal glycemia was similar in the LIG and the CTR sows (4.75 ± 0.09 vs. 4.66 ± 0.12 mM, respectively; $P = 0.53$). Arterial concentration of glucose was affected by time elapsed after meal ingestion (Figure 2, Panel A). It was significantly lower at 0 and 15 min after meal than at other times and was maximal between 45 and 60 min in both treatments. Arterial concentration of glucose was greater in the CTR sows than in the LIG sows at 45 min ($P = 0.02$) and at 60 min ($P = 0.05$), and lower at 150 min ($P = 0.04$). Glucose arteriovenous difference, uptake of glucose by the uterus and glucose uptake per

Figure 1. Effects of litter size on postprandial uterine blood flow before (time = 0) and after meal tests (1.4 kg feed). Values presented are least squares means $\pm$ SEM. Uterine blood flow is presented according to treatment (CTR, control; LIG, left oviduct severed). Treatments did not affect uterine blood flow ($P > 0.10$).

Figure 2. Effects of litter size on postprandial kinetics of arterial blood concentrations of glucose, insulin, and NEFA before (time = 0) and after meal tests (1.4 kg feed). Values presented are least squares means $\pm$ SEM. Concentrations are presented according to treatment (CTR, control; LIG, left oviduct severed). Treatments did not affect blood concentrations of metabolites ($P > 0.05$, Panels A, C). *Sampling times during which metabolite or insulin mean concentrations differed between treatments ($P < 0.05$). Mean concentrations of insulin were greater in LIG than in CTR sows ($P = 0.04$, Panel B).

fetus in the uterine horn did not differ between treatments ($0.15 < P < 0.31$). However, between 30 and 45 min, glucose arteriovenous difference and uptake of glucose by the uterus was lower in the LIG than in the CTR sows ($0.001 < P < 0.05$), whereas glucose uptake per fetus tended to be greater in the LIG sows ($P = 0.07$). Area under the curve for glucose was similar in both groups ($P = 0.64$).

Mean concentrations of insulin increased from 15 min after the meal and were maximal at 45 min in both treatments. Insulin returned to basal concentrations at 90 min in the CTR sows and at 180 min in the LIG sows. Insulinemia was greater in the LIG than in the CTR sows ($P = 0.04$). The difference was significant between 75 and 90 min ($0.003 < P < 0.02$; Figure 2, Panel B). Area under the curve for insulin was greater in the LIG sows ($P = 0.05$).

Arterial concentrations of NEFA were greater before the meal (Figure 2, Panel C), and started to decrease from 30 min up to 60 min later. Treatment did not affect the parameters related to NEFA.

Glucose Tolerance Tests

UBF remained stable during glucose tolerance test and did not differ between treatments. Profiles of glucose and insulin during glucose tolerance tests are presented in Figure 3. Fasting concentrations of glucose and insulin were not affected by treatment (Table 2). Glucose injection induced a similar hyperglycemia in both groups (28.7 ± 0.8 mM; Figure 3, Panel A). Glucose concentration decreased rapidly thereafter and returned to the fasting concentration after 34 ± 1 min on average in both groups of sows ($P = 0.93$). It then continued to decrease under the basal level in the LIG sows only. It was significantly lower than the basal level after 45 min postinjection in the LIG sows ($P = 0.02$), whereas it never differed from the basal level in the CTR sows. Glucose half-life did not differ between treatments (13.6 ± 0.5 vs. 13.1 ± 0.7 min in the LIG and CTR sows, respectively; $P = 0.63$). Glucose half-life calculated from blood concentration in the uterine vein did not differ from those calculated from arterial blood ($P = 0.74$) (Table 2).

Glucose injection caused hyperinsulinemia that differed between treatments ($P = 0.016$). It was maximal between 6 and 21 min for the LIG sows (311 ± 50 μ U insulin/mL) and between 12 and 25 min for the CTR sows (148 ± 21 μ U insulin/mL) (Figure 3, Panel B). It then decreased and returned to the basal concentrations within 35 min in both treatments. Insulin concentrations were greater between 0 and 25 min after glucose injection in the LIG than in the CTR sows ($0.0001 < P < 0.02$). The AUC for insulin was greater in the LIG sows than in the CTR sows (9.61 ± 1.46 vs. 4.82 ± 0.42 mIU·mL⁻¹·min; $P = 0.018$). Time needed to reach

Figure 3. Effects of treatment (CTR, control; LIG, left oviduct severed) on plasma concentrations of glucose (Panel A) and insulin (Panel B), and on glucose arteriovenous difference concentrations across the uterus (Panel C), after an intravenous injection of 0.5 g of glucose/kg of BW. Values presented are least squares means $\pm$ SEM. *Sampling times during which insulin concentrations or glucose arteriovenous differences differed between treatments ($0.0001 < P < 0.03$).

Table 2. Fasting concentrations of glucose and insulin, and characteristics of glucose and insulin profiles during glucose tolerance tests

Treatment group ¹	CTR	LIG	RMSE ²	P-value
Glucose in artery				
Concentration, mM	4.57	4.85	0.39	0.25
Glucose half time, min	13.1	13.6	1.4	0.63
Return to basal ³ , min	34.2	34.4	4.6	0.93
Glucose in uterine vein				
Concentration, mM	4.14	4.57	0.32	0.06
Glucose half time, min	13.4	13.8	1.7	0.74
Return to basal ³ , min	36.9	35.3	5.2	0.64
Insulin				
Concentration, μ IU/mL	10.5	15.1	3.9	0.09
AUC ⁴	4.82	9.61	2.74	0.02
Time 25% AUC ⁵	8.4	5.9	1.2	0.007
Time 50% AUC ⁵	17.0	13.5	1.8	0.01
Time 75% AUC ⁵	26.1	21.7	3.3	0.06
Time insulin max ⁶	17.0	9.0	3.6	0.005
Concentration insulin max ⁷	169.3	339.1	95.8	0.02

¹CTR, control; LIG, left oviduct severed.²Root mean square error.³Time (min) needed to return to basal level.⁴AUC, area under the insulin curve, $\text{mIU}\cdot\text{mL}^{-1}\cdot\text{min}$.⁵Time (min) required to reach 25%, 50%, or 75% of the area under the insulin curve.⁶Time (min) required to reach the maximum insulin concentration during tolerance test.⁷Maximum insulin concentration ($\mu\text{IU}/\text{mL}$) during tolerance test.

25%, 50%, or 75% of this area was longer in the CTR sows ($0.01 < P < 0.05$) (Table 2).

The arteriovenous difference of glucose was greater in the LIG than in the CTR sows when blood glucose levels rose above 15 mM, i.e., between 0 and 9 min ($0.02 < P < 0.03$; Figure 3; Panel C).

Euglycemic Hyperinsulinemic Clamp

Whatever treatment, UBF remained stable during clamp and did not differ between LIG and CTR sows. Hematocrit rate of sows did not vary during clamps. During clamp, insulin infusion increased plasma insulin concentrations to steady-state concentrations from 60 to 150 min after start of infusion (Figure 4). Whatever the time, hyperinsulinemia did not differ between treatments ($292 \pm 4 \mu\text{IU}$ insulin/mL on average between 60 and 150 min, $P = 0.76$). Clamps were conducted so as to maintain plasma glucose concentrations at the average of that observed during fasting. Target concentrations of glucose were reached and maintained stable during the clamps (4.87 ± 0.11 and 4.68 ± 0.09 on average

Figure 4. Effects of treatment (CTR, control; LIG, left oviduct severed) on plasma concentrations of glucose and insulin (Panel A) and on glucose infusion rate (GIR, Panel B) during euglycemic hyperinsulinemic clamps. Values presented are least squares means $\pm$ SEM. Insulin infusion began at time 0. The upper curves in Panel A refer to the plasma insulin data and the lower curves refer to the glycemia data. Panel B, Glucose infusion rate from 30 to 150 min was less in LIG than in CTR sows ($P < 0.01$). *Sampling times during which GIR concentrations differed between treatments ($0.002 < P < 0.05$; Panel B).

between 60 and 150 min, in the LIG and CTR sows, respectively (Figure 4, Panel A). Steady state was achieved 30 to 45 min after the initiation of the glucose clamp; therefore, the quantity of glucose administered was considered only after the first hour of infusion. The GIR increased gradually during the first 90 min, reaching a plateau between 105 and 150 min (Figure 4, Panel B). Glucose infusion rate was always lower for the LIG sows than for the CTR sows (6.1 ± 0.2 vs. $7.8 \pm 0.1 \text{ mg glucose}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ on average between 90 and 150 min, $P = 0.007$; Figure 5).

Irrespective of treatment, hyperinsulinemia under euglycemic conditions decreased plasma NEFA concentrations (Figure 6). Plasma concentrations of NEFA expressed as a percentage of their basal concentrations declined less but not significantly in the LIG sows than in the CTR sows. As an example, at 30 min, NEFA decreased by $41 \pm 3\%$ in the LIG sows and by $49 \pm 6\%$ in the CTR sows; $P = 0.32$). Nevertheless, NEFA were never significantly affected by treatment during the first 60 min of the clamp.

Figure 5. Effects of treatment (CTR, control; LIG, left oviduct severed) on glucose infusion rate (GIR) during the last hour of the euglycemic hyperinsulinemic clamps (times 90 to 150 min). Values presented are least squares means $\pm$ SEM. Glucose infusion rate from 90 to 150 min was lower in LIG than in CTR sows ($P = 0.007$).

DISCUSSION

In agreement with earlier results (Père et al., 1997), mean litter size of the LIG sows was about half that of the CTR sows. In the LIG sows, embryos derived from ova produced by the ovary corresponding to the nonligatured oviduct had migrated and were then distributed among the two uterine horns (Dhindsa et al., 1967). Litter weight did not differ between groups because piglets in the LIG treatment were significantly heavier than piglets of CTR sows at birth. It can be assumed that greater growth rate for LIG than for CTR fetuses mainly occurred during the last third of pregnancy as about two thirds of fetal pig growth are achieved throughout the last month (Etienne and Jemmali, 1982; McPherson et al., 2004). Greater growth rate is supported by the higher UBF per fetus in the LIG group meaning a greater nutrient disposal for the progeny. It was shown that maternal hyperglycemia improves fetal glucose uptake in sows (Père, 1995). In LIG sows, the greater arteriovenous difference of glucose across the uterus during tolerance test also supports that supply of glucose to their gravid uterus is greater than that of CTR sows.

Figure 6. Effects of treatment (CTR, control; LIG, left oviduct severed) on plasma concentrations of NEFA, expressed as absolute values (Panel A) and as percentage of basal concentration (Panel B), during the euglycemic hyperinsulinemic clamps. Values presented are least squares means $\pm$ SEM.

During meal tests, postprandial glycemia did not differ between groups, whereas insulinemia profiles differed. In the LIG sows, postprandial hyperinsulinemia and area under the insulin curve were greater. In addition, insulin production was extended in the LIG sows as indicated by its delayed return to baseline fasting levels. This expresses a lower effectiveness of insulin to stimulate glucose uptake in the LIG sows.

During the glucose tolerance tests, decrease of glycemia after injecting a same amount of glucose per kilogram BW did not differ between the two groups of sows. Half-life time of glucose was similar in both treatments and amounted to 13.4 min as a mean. This value is comparable to those obtained at similar stages in multiparous sows (14.5 min according to Père et al., 2000; 14.7 min according to Père and Etienne, 2007). A similar glucose half-life was also reported in primiparous sows by Le Cozler et al. (1998) at 106 d of pregnancy (13.0 min) and Mosnier et al. (2010) at 103 d of pregnancy (13.6 min). During glucose tolerance

tests, the AUC of insulin was significantly greater in the LIG than in the CTR sows. This shows differences between insulin secretion patterns that occurred rapidly as time needed to reach 25%, 50%, or 75% of AUC was significantly shorter in the LIG group. An increased or prolonged increase in insulin concentration over time was found in late pregnancy in comparison with early gestation in sows by [George et al. \(1978\)](#), [Bouillon-Hausman et al. \(1986\)](#), [Schaefer et al. \(1991\)](#), and [Père et al. \(2000\)](#), in parallel with the increased energy requirements of fetuses. Present results show that these effects are more pronounced in the LIG than in the CTR sows. They agree with those of the meal test and reveal that insulin resistance adaptation is strongly accentuated in the LIG sows in comparison with the CTR sows at the end of gestation. Differences in insulin sensitivity have sometimes been shown between tissues. For instance, [Burnol et al. \(1986, 1987\)](#) reported that in rats, the mammary gland is more sensitive to insulin than other tissues such as white adipose tissue or muscle. In the present study, glucose tolerance of the uterus was the same than that of the whole body of sows.

The numerous blood samples during the euglycemic hyperinsulinemic clamp did not affect hematocrit rate of sows, and then alteration in blood volume. During these tests, the same rate of insulin infusion induced a similar insulinemia and the basal glycemia used as target level for euglycemia was the same in both groups. The amount of glucose perfused needed to maintain basal glycemia was comparable to those obtained at 106 d of pregnancy in primiparous sows ($6.5 \text{ mg glucose} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ according to [Père and Etienne, 2007](#)). The GIR was lower throughout clamps in the LIG sows, and the difference amounted to about 22% during the stable period, between 105 and 150 min. It means that the LIG sows showed a greater insulin resistance at the end of pregnancy than the CTR sows.

In the present experiment, three tests (meal test, glucose tolerance test, and euglycemic hyperinsulinemic clamp) were performed to evaluate the effectiveness of insulin to regulate blood glucose. The meal test assesses the variations of both endogenous glucose and insulin in normal conditions of feed intake (i.e., within a physiological range of values). The glucose tolerance test evaluates the responsiveness of insulin after an intravenous load of glucose ([Weldon et al., 1994](#)). Finally, during the euglycemic hyperinsulinemic clamp, glucose and insulin are infused. Glucose infusion rate measures the ability of insulin to increase glucose uptake and to suppress glucose production. Therefore, the

clamp is the direct method for assessing insulin sensitivity ([De Fronzo et al., 1979](#)). The advantage of the glucose tolerance test is to study the response of endogenous insulin, but the differences observed might be related to changes in insulin secretion or glucose uptake mechanisms. The advantage of the euglycemic hyperinsulinemic clamp is to study the insulin resistance in steady-state conditions of glycemia and insulinemia, whereas insulin and glucose concentrations vary considerably with time during the glucose tolerance test. Each test has then its own advantages and disadvantages. Whatever, in the present experiment, both tests lead to the same conclusion, i.e., the LIG sows resist more to insulin than the CTR sows. During the last third of gestation, a decrease in insulin sensitivity, which is progressive and reversible, has been shown in women, rats, rabbits, and ewes ([Gilbert et al. 1984](#); [Ryan et al., 1985](#); [Leturque et al., 1987](#); [Catalano et al., 1991](#)). This adaptation is thought to favor glucose availability for the pregnant uterus at the expense of muscles and adipose tissue. Its occurrence has also been shown in sows ([Père et al., 2000](#); [Père and Etienne, 2007](#)). In ruminants, insulin resistance that has developed at the end of pregnancy continues at the beginning of lactation and decreases thereafter ([Debras et al., 1989](#); [Bell, 1995](#); [Bell and Bauman, 1997](#)). In sheep, [Vernon et al. \(1990\)](#) suggest that lactation results in insulin resistance in skeletal muscle, at least with respect to glucose utilization. Resistance to insulin in sows is further accentuated during lactation and disappears very quickly after weaning ([Père and Etienne, 2007](#)). This evolution parallels with variation of energy requirements of sows that increase with the important growth of fetuses at the end of gestation, and rise even much more during lactation. This could be related to the increased concentration of NEFA in plasma from early gestation until lactation. Indeed, the increase of plasma NEFA concentrations during pregnancy has been associated with the development of insulin resistance in rabbits ([Gilbert et al., 1991, 1993](#)). After meal ingestion or during clamp, insulin-induced suppression of plasma NEFA levels provides a reasonable estimate for the antilipolytic insulin sensitivity. However, basal concentration of plasma NEFA and their drop after meal or during clamp did not differ between groups in the present work. It may be underlined that in the present experiment, characteristics of the greater insulin resistance of the LIG than the CTR sows differ from those of insulin resistance that develops during gestation. When compared with early gestation, glycemia post-feeding increases in multiparous ([Père et al. 2000](#))

and primiparous sows (Père and Etienne, 2007) at the end of pregnancy, but maximal levels of insulin postfeeding or during glucose injection as well as the AUC of insulin in the meal and glucose tolerance tests are not significantly altered by gestation stage (Père et al., 2000). In the present experiment, glycemia was not altered, whereas levels and AUC of insulin were significantly greater in the LIG than in the CTR sows during meal and tolerance tests. Moreover, glucose half-life increases at the end of pregnancy (Père et al., 2000; Père and Etienne, 2007), but does not differ here between CTR and LIG sows. The variation of glucose half-life with gestation stage in sows contrasts with results obtained in other species: glucose half-life does not change during pregnancy in women (Silverstone et al., 1961; Yen 1973) or in rats (Leturque et al., 1980), whereas their resistance to insulin increases. It seems here that in the LIG sows, insulin is less efficient than in the CTR sows, and LIG sows secrete more insulin to maintain glycemia in the normal range, whereas in the phenomenon of increased insulin resistance in late gestation, insulin becomes less efficient but its secretion changes are not sufficient to prevent higher plasma glucose levels. It seems that in LIG sows, the pancreatic beta cells produce enough insulin to compensate for insulin resistance and blood glucose is maintained in the normal range.

We hypothesized that the intensity in insulin resistance could be related to the amount of energetic substrates required by uterus, and thus to litter size. However, comparison of the two groups of sows does not support this hypothesis. The sows bearing less fetuses resisted more to insulin than those having larger litters. In an earlier experiment where sows fed ad libitum nursed 13 to 14 or seven piglets, we also found that sows with smaller litters resisted more to insulin than the others at d 27 of lactation (Quesnel et al., 2007). This was observed despite basal level of plasma NEFA did not differ between groups, and despite sows with large litters had mobilized much more body reserves. Insulin resistance was also characterized by an increased secretion of insulin without change in glucose half-life. In the present experiment, litter weight did not differ significantly between CTR and LIG sows because the LIG fetuses were heavier and grew most likely faster than the CTR fetuses during the period of measurements. Greater insulin resistance would then be connected to the faster growth rate of fetuses rather than to requirements of a larger litter of fetuses having a slower individual development. Independently of litter size,

sows would adapt glucose metabolism to nutrient intake and nutrient requirements related to fetal growth rate and not to litter weight. Therefore, increase in uterine energetic demand, when litter size increases, is not necessarily associated with an accentuation of sow insulin resistance. More likely, each sow adapts insulin and glucose metabolism to nutrient balance in late pregnancy.

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